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2010 PRIMER ON ALLERGIC AND IMMUNOLOGIC DISEASES

Guest Editors
William T. Shearer, MD, PhD
Baylor College of Medicine
Texas Children's Hospital
Houston, Texas

Donald Y. M. Leung, MD, PhD
National Jewish Health
University of Colorado Health Sciences Center
Denver, Colorado

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2010 Primer on Allergic and Immunologic Diseases

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Preface to the 2010 *Primer on Allergic and Immunologic Diseases*

William T. Shearer, MD, PhD, Editor,[a] and Donald Y. M. Leung, MD, PhD, Associate Editor[b] *Houston, Tex, and Denver, Colo*

A new era for the *Primer on Allergic and Immunologic Diseases* begins with this online-only 6th edition. Despite our long love affair with paper texts, the times they are a changing, and we graciously yield to the new world of advanced technology, education, and communication that prefers to learn from a computer screen, with its versatility and availability at all hours. Indeed, this high-tech Primer 2010 is very new, with well over 50% new topics and authors. The new chapters include those on innate immunity (Turvey and Broide[1]) and adaptive immunity (Bonilla and Oettgen[2]), the structure and function of immunoglobulins (Schroeder and Cavacini[3]), interpretation of tests for autoimmunity (Castro and Gourley[4]), complement disorders and hereditary angioedema (Frank[5]), and anaphylaxis (Simons[6]). All of the chapters have been completely updated, with timely revisions and current references. Readers will note the increased length of text and literature citations made possible by removal of printed page limitations in this new online edition. This has permitted a more thorough presentation of new information and accommodates the requests of authors, peer reviewers, and readers alike.

Looking back to the publication of the 5th edition of the Primer in 2003 and the Mini-Primers of 2006 and 2008, it is gratifying to see the advances in molecular sciences and how these advances have been incorporated into the translational research, leading to new and sophisticated forms of therapy. Molecular targeting by mAbs and fusion proteins is the best example of this sweep of information taken from laboratory to clinic (Lee et al[7]). Discovery of genes that contribute to specific genetic diseases vary from the spectacular monogenic deficiencies causing selective immunodeficiencies (Notarangelo[8]) to the bewildering number of candidate genes for allergy and asthma (Holloway et al[9]). The chapter on cytokines and chemokines has kept pace with the rapid discovery of new messenger molecules of the immune system (Commins et al[10]). Fonacier et al[11] are new authors for the excellent chapter on allergic skin disease as are Atkins and Furuta,[12] who present a chapter on mucosal immune disorders and eosinophilic esophagitis. The chapter on secondary immunodeficiencies includes an update on discoveries in HIV infection and the immunosuppressive hazards of space flight (Chinen and Shearer[13]). New authors of chapters on environmental and occupational allergies (Peden and Reed[14]) and drug allergy (Khan and Solensky[15]) add fresh updates of traditional topics. Several headline authors have given us completely updated versions of previous chapters: Stone et al[16] on IgE, mast cells, basophils, and eosinophils; Lemanske and Busse[17] on adult and childhood asthma; Sicherer and Sampson[18] on food allergy; Dykewicz and Hamilos[19] on rhinitis and sinusitis; Joseph et al[20] on immunologic rheumatic diseases; Langford[21] on vasculitis; Michels and Eisenbarth[22] on endocrine system disorders; Greenberger and Grammer[23] on pulmonary disorders and vocal cord dysfunction; Whiteside[24] on immune response to malignancy; Frew[25] on immunotherapy (including sublingual) of allergic disease; Chinen and Buckley[26] on transplantation immunology (ie, solid organ, bone marrow, and gene therapy); and Brignier and Gewirtz[27] on embryonic and adult stem cell therapy. Undergirding all the chapters of Primer 2010 are essential chapters on the overview of the immune response (Chaplin[28]) and the critical chapters on the clinical laboratory assessment of immediate hypersensitivity (Hamilton[29]) and clinical and laboratory assessment of immune-mediated diseases (Oliveira and Fleisher[30]).

In the 5th edition of the Primer in 2003, we created the concept of the Clinical Immunology Tree of Life (Fig 1), which depicted the wide branches and leaves that represent the spectrum of allergic and immunologic diseases managed by allergists and immunologists. The growth of the tree depends on the diseases we treat (rain) and the research we conduct (sunshine) and on the roots of the tree that gather nourishment from the soil (basic science). In the Mini-Primers of 2006 and 2008, we extended the analogy to seeds of the tree falling to the ground with growth of new trees (education that imparts new knowledge to future generations). We believe that Primer 2010 carries on that age-old tradition.

The Editors thank all of the authors who set aside their other numerous duties to write this magnificent summary of allergic and immunologic diseases, the silent peer reviewers who greatly enhanced the value of each chapter, and the Elsevier Publishing Co and the American Academy of Allergy, Asthma & Immunology who provided the support for Primer 2010. Special thanks are due to Mr George Woodward, Managing Editor, and Ms Dawn Angel, Senior Submission Editor, for the *Journal of Allergy and Clinical Immunology* for too-numerous-to-count inquiries, searches, and general all-around assistance and finally to our editorial assistant Carolyn Jackson for keeping the entire project of Primer 2010 on track from promotion to publication.

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From [a]the Baylor College of Medicine, Texas Children's Hospital, Houston, and [b]National Jewish Medical and Research Center, University of Colorado Health Sciences Center, Denver, Colo.

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Reprint requests: William T. Shearer, MD, PhD, Chief, Allergy and Immunology Service, Texas Children's Hospital, 1102 Bates Street, Suite 330, Houston, TX 77030-2399. E-mail: wtsheare@TexasChildrensHospital.org.

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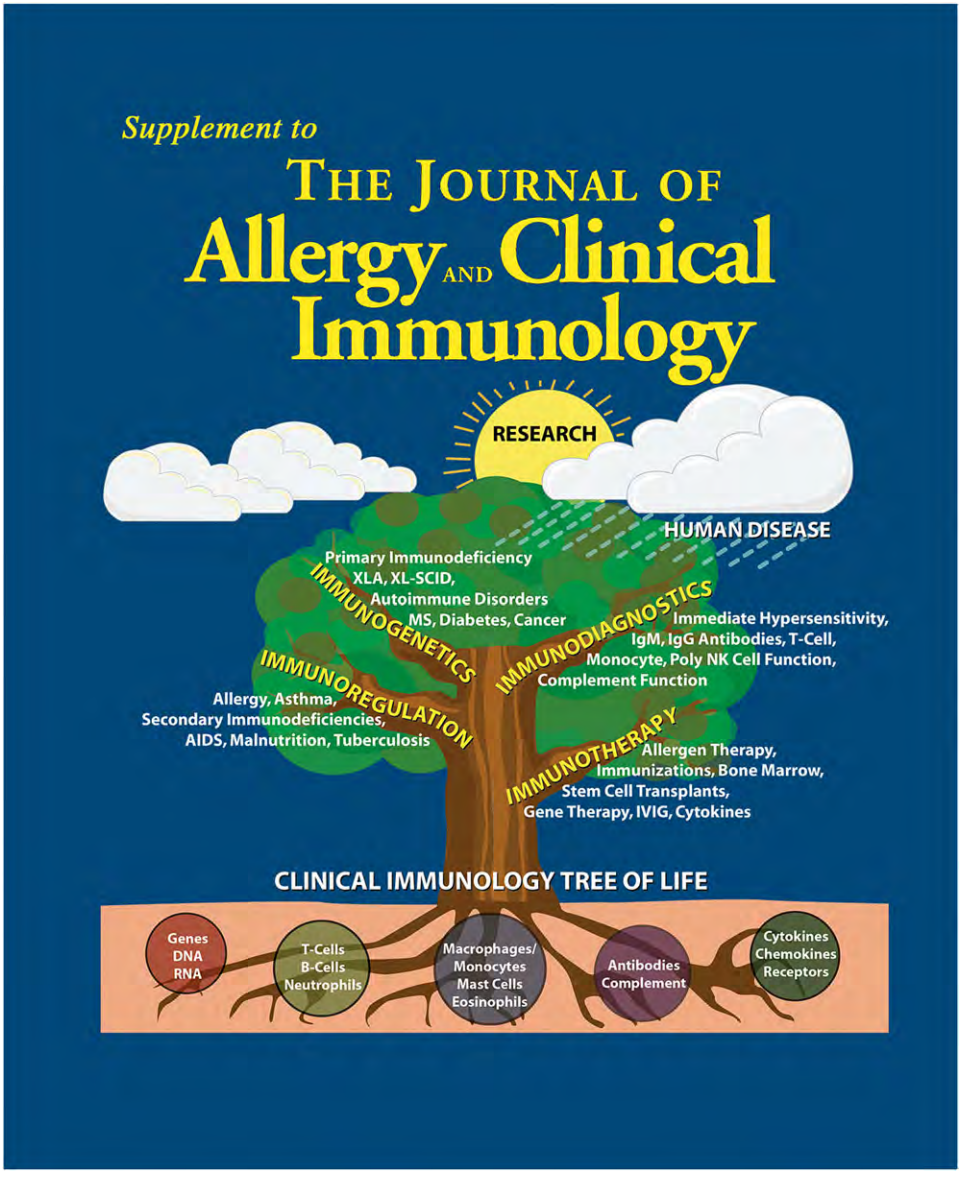

FIG 1. The clinical immunology tree of life. Used with permission from Primer 2003, 5th edition.

Overview of the immune response

David D. Chaplin, MD, PhD *Birmingham, Ala*

The immune system has evolved to protect the host from a universe of pathogenic microbes that are themselves constantly evolving. The immune system also helps the host eliminate toxic or allergenic substances that enter through mucosal surfaces. Central to the immune system's ability to mobilize a response to an invading pathogen, toxin, or allergen is its ability to distinguish self from nonself. The host uses both innate and adaptive mechanisms to detect and eliminate pathogenic microbes, and both of these mechanisms include self-nonself discrimination. This overview identifies key mechanisms used by the immune system to respond to invading microbes and other exogenous threats and identifies settings in which disturbed immune function exacerbates tissue injury. (J Allergy Clin Immunol 2010;125:S3-23.)

Key words: *Adaptive immunity, atopy, B cell, complement, costimulation, inflammation, innate immunity, superantigen, T cell, tolerance*

Abbreviations used

AIRE:	Autoimmune regulator gene
AMC:	Acidic mammalian chitinase
APC:	Antigen-presenting cell
CD:	Cluster of differentiation
CLP:	Chitinase-like protein
DNA-PK:	DNA-dependent protein kinase
DP:	Double positive
ER:	Endoplasmic reticulum
Foxp3:	Forkhead box protein 3
HLDA:	Human leukocyte differentiation antigen
Ii:	Invariant chain
ITAM:	Immunoreceptor tyrosine-based activation motif
Jak:	Janus kinase
LMP:	Low molecular mass polypeptide
MAC:	Membrane attack complex
MBL:	Mannan-binding lectin
MIC:	MHC class I–related chain
MyD88:	Myeloid differentiation primary response gene 88
NALP3:	Nacht domain-, leucine-rich repeat-, and PYD-containing protein 3
NK:	Natural killer
NLR:	Nucleotide-binging domain leucine-rich repeat
RAG:	Recombinase-activating gene
STAT:	Signal transducer and activator of transcription
TCR:	T-cell receptor
TdT:	Terminal deoxynucleotidyl transferase
TLR:	Toll-like receptor
Treg:	Regulatory T

Humans and other mammals live in a world that is heavily populated by both pathogenic and nonpathogenic microbes and contains a vast array of toxic or allergenic substances that threaten normal homeostasis. The community of microbes includes both obligate pathogens and beneficial commensal organisms, which the host must tolerate and hold in check to support normal tissue and organ function. Pathogenic microbes possess a diverse collection of mechanisms by which they replicate, spread, and threaten normal host functions. At the same time that the immune system is eliminating pathologic microbes and toxic or allergenic proteins, it must avoid responses that produce excessive damage of self-tissues or that might eliminate beneficial commensal microbes. Our environment contains a huge range of pathogenic microbes and toxic substances that challenge the host through a very broad selection of pathogenic mechanisms. Therefore it is not surprising that the immune system uses a complex array of protective mechanisms to control and usually eliminate these organisms and toxins. A general feature of the immune system is that these mechanisms rely on detecting structural features of the pathogen or toxin that mark it as distinct from host cells. Such host-pathogen or host-toxin discrimination is essential to permit the host to eliminate the threat without damaging its own tissues.

The mechanisms permitting recognition of microbial, toxic, or allergenic structures can be broken down into 2 general categories: (1) hard-wired responses that are encoded by genes in the host's germ line and that recognize molecular patterns shared by many microbes and toxins that are not present in the mammalian host and (2) responses that are encoded by gene elements that somatically rearrange to assemble antigen-binding molecules with exquisite specificity for individual, unique foreign structures. The first set of responses constitutes the innate immune response. Because the recognition molecules used by the innate system are expressed broadly on a large number of cells, this system is poised to act rapidly after an invading pathogen or toxin is encountered and thus constitutes the initial host response. The second set of responses constitutes the adaptive immune response. Because the adaptive system is composed of small numbers of cells with specificity for any individual pathogen, toxin, or allergen, the responding cells must proliferate after encountering the antigen to attain sufficient numbers to mount an effective response against the microbe or the toxin. Thus the adaptive response generally expresses itself temporally after the innate response in host defense. A key feature of the adaptive response is that it produces long-lived cells that persist in an apparently dormant state but that can re-express effector functions rapidly after another encounter with their specific antigen. This provides the adaptive response with the ability to manifest immune memory, permitting it to contribute prominently to a more effective host response against specific pathogens or toxins when they are encountered a second time, even decades after the initial sensitizing encounter.

From the Departments of Microbiology and Medicine, University of Alabama at Birmingham.

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Reprint requests: David D. Chaplin, MD, PhD, Departments of Microbiology and Medicine, University of Alabama at Birmingham, 845 19th St South, BBRB 276/11, Birmingham, AL 35294-2170. E-mail: dchaplin@uab.edu.

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DISCRIMINATION OF SELF FROM NONSELF

The immune system uses many potent effector mechanisms that have the ability to destroy a broad range of microbial cells and to clear a broad range of both toxic and allergenic substances. Therefore it is critical that the immune response is able to avoid unleashing these destructive mechanisms against the mammalian host's own tissues. The ability of the immune response to avoid damaging self-tissues is referred to as self-tolerance. Because failure of self-tolerance underlies the broad class of autoimmune diseases, this process has been extensively studied. It is now clear that mechanisms to avoid reaction against self-antigens are expressed in many parts of both the innate and the adaptive immune response. The mechanisms that underlie protection of normal self-tissues from immune damage will be discussed as each of the major effector arms of the host immune response is introduced.

Because an important aspect of the T-cell arm of the immune system is to recognize host cells that are infected by viruses, intracellular bacteria, or other intracellular parasites, T cells have evolved an elegant mechanism that recognizes foreign antigens together with self-antigens as a molecular complex (see the "Antigen recognition by T lymphocytes…" section below). This requirement that T cells recognize both self-structures and foreign antigens makes the need for these cells to maintain self-tolerance particularly important.

GENERAL FEATURES OF INNATE AND ADAPTIVE IMMUNITY

Broadly defined, the innate immune system includes all aspects of the host's immune defense mechanisms that are encoded in their mature functional forms by the germline genes of the host. These include physical barriers, such as epithelial cell layers that express tight cell-cell contacts (tight junctions, cadherin-mediated cell interactions, and others); the secreted mucus layer that overlays the epithelium in the respiratory, gastrointestinal, and genitourinary tracts; and the epithelial cilia that sweep away this mucus layer, permitting it to be constantly refreshed after it has been contaminated with inhaled or ingested particles. The innate response also includes soluble proteins and bioactive small molecules that are either constitutively present in biological fluids (eg, the complement proteins, defensins, and ficolins[1-3]) or that are released from cells as they are activated (including cytokines that regulate the function of other cells, chemokines that attract inflammatory leukocytes, lipid mediators of inflammation, reactive free radical species, and bioactive amines and enzymes that also contribute to tissue inflammation). Lastly, the innate immune system includes membrane-bound receptors and cytoplasmic proteins that bind molecular patterns expressed on the surfaces of invading microbes. Some aspects of the innate host defenses are constitutively active (eg, the mucociliary blanket overlying many epithelia), and others are activated after interactions of host cells or host proteins with chemical structures that are characteristic of invading microbes but are absent from host cells.

Unlike the innate mechanisms of host defense, the adaptive immune system manifests exquisite specificity for its target antigens. Adaptive responses are based primarily on the antigen-specific receptors expressed on the surfaces of T and B lymphocytes. Unlike the germline-encoded recognition molecules of the innate immune response, the antigen-specific receptors of the adaptive response are encoded by genes that are assembled by somatic rearrangement of germline gene elements to form intact T-cell receptor (TCR) and immunoglobulin (B-cell antigen receptor) genes. The assembly of antigen receptors from a collection of a few hundred germline-encoded gene elements permits the formation of millions of different antigen receptors, each with potentially unique specificity for a different antigen. The mechanisms governing the assembly of these B- and T-cell antigen receptors and ensuring the selection of a properly functioning repertoire of receptor-bearing cells from the huge, randomly generated potential repertoire will be introduced below and discussed in more detail in chapters 3 and 4 of this Primer.[4,5]

The innate and adaptive immune systems are often described as contrasting separate arms of the host response; however, they usually act together, with the innate response representing the first line of host defense and the adaptive response becoming prominent after several days as antigen-specific T and B cells have undergone clonal expansion. Components of the innate system contribute to activation of the antigen-specific cells. Additionally, the antigen-specific cells amplify their responses by recruiting innate effector mechanisms to bring about the complete control of invading microbes. Thus although the innate and adaptive immune responses are fundamentally different in their mechanisms of action, synergy between them is essential for an intact, fully effective immune response.

CELLULAR ELEMENTS OF THE IMMUNE RESPONSE

An intact immune response includes contributions from many subsets of leukocytes. The different leukocyte subsets can be discriminated morphologically by using a combination of conventional histologic stains and analysis of the spectrum of glycoprotein differentiation antigens that are displayed on their cell membranes. These differentiation antigens are detected by their binding of specific mAbs. These cell phenotype–determining antigens are assigned cluster of differentiation (CD) numbers. There are currently more than 350 defined CD antigens. Updates are issued by Human Cell Differentiation Molecules, an organization that organizes periodic Human Leukocyte Differentiation Antigen (HLDA) workshops at which newly identified cell-surface molecules are defined and registered. The next HLDA workshop (HLDA9) will be held in Barcelona, Spain, and the summary of authorized CD molecules will be published at http://www.hcdm.org/.

Mature circulating leukocytes differentiate from hematopoietic stem cells (Fig 1).[6] These stem cells can be recognized by their own spectrum of defining cell-surface antigens and can be purified from bone marrow, peripheral blood, and the placenta.[7] The recognition that pluripotent hematopoietic stem cells can be purified in substantial quantities has accelerated progress in hematopoietic cell transplantation and provides considerable promise for somatic cell–based gene therapy. Progress in the field of stem cell therapy is described in chapter 30 of this Primer.[8]

Formation of the full complement of immune system cells begins when a pluripotent hematopoietic stem cell differentiates into the myeloid stem cell or the common lymphoid progenitor. The common lymphoid progenitor differentiates further into the 4 major populations of mature lymphocytes: B cells, T cells, natural killer (NK) cells, and NK-T cells. These lymphocyte subsets can be discriminated by surface phenotype. B cells are phenotypically defined by their expression of the B-cell receptor for antigen (membrane-anchored immunoglobulin). Subsets of B cells have

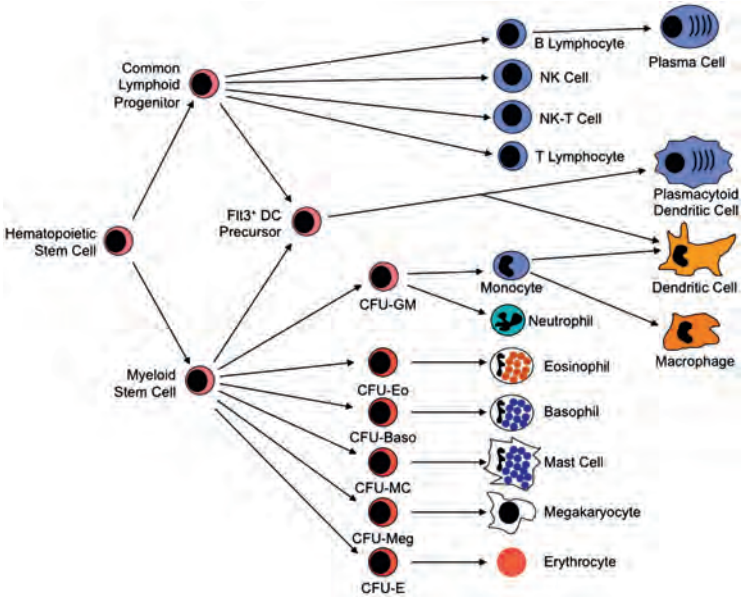

FIG 1. Hematopoietic stem cell–derived cell lineages. Pluripotent hematopoietic stem cells differentiate in bone marrow into common lymphoid progenitor cells or myeloid stem cells. Lymphoid stem cells give rise to B-cell, T-cell, and NK cell lineages. Myeloid stem cells give rise to a second level of lineage-specific colony-forming unit *(CFU)* cells that go on to produce neutrophils, monocytes, eosinophils, basophils, mast cells, megakaryocytes, and erythrocytes. Monocytes differentiate further into macrophages in peripheral tissue compartments. Dendritic cells appear to develop primarily from a dendritic cell precursor that is distinguished by its expression of the Fms-like tyrosine kinase receptor 3 *(Flt3)* receptor. This precursor can derive from either lymphoid or myeloid stem cells and gives rise to both classical and plasmacytoid dendritic cells. Classical dendritic cells can also derive from differentiation of monocytoid precursor cells. Modified with permission from Huston.[6]

been defined that differ in the types of antigen to which they respond and in the types of antibody they produce. T cells are defined by their cell-surface expression of the TCR, a transmembrane heterodimeric protein that binds processed antigen displayed by antigen-presenting cells (APCs). As will be discussed below, T cells exist in several functionally significant subtypes and subsets of those types. NK cells are defined morphologically as large granular lymphocytes. They are distinguished by their lack of either TCR or surface immunoglobulin. They recognize their virus-infected or tumor cell targets using a complex collection of activating and inhibitory cell surface receptors.[9] NK-T cells share characteristics of both NK cells and T cells.[10]

Myeloid stem cells (also termed common myeloid progenitors) give rise to several different forms of granulocytes, to megakaryocytes and platelets, and to erythrocytes. Cells of the granulocyte lineage that play prominent immune functions include neutrophils, monocytes, macrophages, eosinophils, basophils, and mast cells. In some mammals, platelets also release immunologically significant mediators that expand their repertoire beyond their role in hemostasis. The immune functions of the classical granulocytes have been inferred from the immunologically active molecules they produce and from their accumulation in specific pathologic conditions. For example, neutrophils produce large quantities of reactive oxygen species that are cytotoxic to bacterial pathogens. They also produce enzymes that appear to participate in tissue remodeling and repair after injury. Neutrophils accumulate in large quantities at sites of bacterial infection and tissue injury and possess prominent phagocytic capabilities that permit them to sequester microbes and particulate antigens internally, where they can be destroyed and degraded. Thus it is clear that they play a major role in clearance of microbial pathogens and repair of tissue injury.[11] More recently, however, neutrophils have been recognized to produce substantial amounts of the cytokines TNF and IL-12, as well as certain chemokines. This supports an additional immunoregulatory role of neutrophils.

Like neutrophils, monocytes and macrophages are also highly phagocytic for microbes and particles that have been marked for clearance by binding immunoglobulin, complement, or both. They appear to be mobilized shortly after the recruitment of neutrophils, and they persist for long periods at sites of chronic inflammation and infection. In addition to participating in acute inflammatory responses, they are prominent in granulomatous processes throughout the body. They use production of nitric oxide as a major mechanism for killing microbial pathogens and also produce large amounts of cytokines, such as IL-12 and IFN-γ, giving them a regulatory role in adaptive immune responses. Depending on the nature of activating signals that are present when macrophages differentiate from immature precursor cells and when they receive their first activation signal, macrophages can adopt one of several phenotypes.[12] Classically activated macrophages produce large amounts of IFN-γ, IL-6, IL-12, and TNF and express potent proinflammatory and antibacterial activities. The formation of alternatively activated macrophages can be induced by IL-4, IL-10, or IL-13, especially in the presence of glucocorticoid hormones, and express anti-inflammatory functions through their own production of IL-10, the IL-1 receptor antagonist, and TGF-β.[13] It is likely that further study will identify additional functional macrophage subsets, establishing additional ways in which these innate immune system cells serve fundamental immunoregulatory functions.

Eosinophils are readily recognized by their prominent cytoplasmic granules, which contain toxic molecules and enzymes that are particularly active against helminths and other parasites. The production of eosinophils from the bone marrow and their survival in peripheral tissues are enhanced by the cytokine IL-5, making them prominent cells in most allergic responses.[14]

Basophils and mast cells are morphologically similar cells that represent distinct lineages. By virtue of the cell-surface expression of high-affinity receptors for IgE (FcϵRI), they are key initiators of immediate hypersensitivity responses and the host response to helminthic parasites, releasing histamine and other preformed mediators from their granules and producing important quantities of lipid mediators that stimulate tissue inflammation, edema, and smooth muscle contraction. Recent studies have demonstrated that in addition to their role in immediate hypersensitivity responses, mast cells play prominent roles in the host response to bacterial infection as well. Importantly, mast cells and, more prominently, basophils can release substantial amounts of IL-4, suggesting that they can play important roles in the induction of allergic immune responses.[15]

Phagocytic cells of the monocyte/macrophage lineage also play key roles in the adaptive immune response by taking up microbial antigens, processing them by means of proteolysis to peptide fragments, and presenting them in forms that can activate T-cell responses. Additional cells in this lineage include Langerhans cells in the epidermis, Kupffer cells in the liver, and microglial cells in the central nervous system. The most potent types of APCs are the broad class of dendritic cells that are present in most tissues of the body and concentrated in the secondary lymphoid tissues.[16] All of these cells express both class I and class II MHC molecules that are used to permit recognition of processed antigen by the TCR on T cells (see below). All MHC-bearing cells appear to have the potential to express APC function if stimulated appropriately. In addition to the conventional dendritic cells described above, which have been thought to be derived from myeloid precursor cells (Fig 1), a second type of dendritic cell is recognized. These cells are designated plasmacytoid dendritic cells because of their histologic morphology. They can produce very high levels of type I interferon and are thought to play special roles in antiviral host defense and autoimmunity.[17] Recent studies of dendritic cell differentiation indicate that both myeloid stem cells and common lymphoid progenitors can give rise to both conventional dendritic cells and plasmacytoid dendritic cells, most likely through a dendritic cell precursor that is defined by its expression of the Fms-like tyrosine kinase receptor 3 (Flt3).[18,19]

ANTIGEN RECOGNITION BY T LYMPHOCYTES/ MAJOR HISTOCOMPATIBILITY MOLECULES

A major challenge faced by the immune system is to identify host cells that have been infected by microbes that then use the cell to multiply within the host. Simply recognizing and neutralizing the microbe in its extracellular form does not effectively contain this type of infection. The infected cell that serves as a factory for production of progeny microbes must be identified and destroyed. In fact, if the immune system were equally able to recognize extracellular microbes and microbially infected cells, a microbe that managed to generate large amounts of extracellular organisms or antigen might overwhelm the recognition capacity of the immune system, allowing the infected cells to avoid immune recognition. A major role of the T-cell arm of the immune response is to identify and destroy infected cells. T cells can also recognize peptide fragments of antigens that have been taken up by APCs through the process of phagocytosis or pinocytosis. The way the immune system has evolved to permit T cells to recognize infected host cells is to require that the T cell recognize both a self-component and a microbial structure. The elegant solution to the problem of recognizing both a self-structure and a microbial determinant is the family of MHC molecules. MHC molecules (also called HLA antigens) are cell-surface glycoproteins that bind peptide fragments of proteins that either have been synthesized within the cell (class I MHC molecules) or that have been ingested by the cell and proteolytically processed (class II MHC molecules).

Class I MHC molecules

There are 3 major HLA class I molecules, designated HLA-A, HLA-B, and HLA-C, each encoded by a distinct gene. The class I HLA molecules are cell-surface heterodimers consisting of a polymorphic transmembrane 44-kd α chain (also designated the class I heavy chain) associated with the 12-kd nonpolymorphic β_2-microglobulin protein.[20] The α chain determines whether the class I molecule is an HLA-A, HLA-B, or HLA-C molecule. The *HLA-A, HLA-B,* and *HLA-C* α-chain genes are encoded within the MHC on chromosome 6 (Fig 2), and the β_2-microglobulin gene is encoded on chromosome 15. The α-chain gene encodes 3 extracellular domains (designated α_1, α_2, and α_3), a transmembrane domain, and a short intracellular domain that anchors the protein in the cell membrane. The α_3 domain consists of 5 antiparallel β-strands that form an immunoglobulin-type fold (Fig 3).[20] The α_1 and α_2 domains each encode an α-helix and several β-strands. The α_1 and α_2 domains associate with each other, with their β-strands forming a platform on which the 2 α-helices rest. This forms a groove in which antigenic peptides can bind. This complex of class I MHC molecule and antigenic peptide produces a composite structure that is the molecular target of the TCR. The TCR contacts both the antigenic peptide and the flanking α-helices. The TCR has no measurable affinity for the antigenic peptide alone and very low affinity for MHC molecules containing other peptides. These observations form the molecular basis for the phenomenon of "MHC restriction," which was described in studies of Zinkernagel and Doherty[21] in which they recognized that T cells could only recognize their specific antigen when it was presented in association with a specific self-MHC molecule.

A key biological consequence of requiring the T cell to recognize antigenic peptides only when they are bound in the groove of an HLA molecule is that this permits the T cell to ignore free extracellular antigen and to focus rather on cells that contain the antigen. In the case of cells that are infected by a pathogenic microbe, this permits the T cells to focus their response on the infected cells. The α_3 domain of the class I heavy chain interacts with the CD8 molecule on cytolytic T cells. This restricts recognition of antigenic peptides that are presented in class I HLA molecules to $CD8^+$ cytolytic T cells. The binding of CD8 expressed by the T cell to the α_3 domain of the class I molecule expressed by the APC strengthens the interaction of the T cell with the APC and helps ensure that full activation of the T cell occurs.[22]

A prominent characteristic of HLA molecules is their structural polymorphism. As of October 2009, the ImMunoGeneTics HLA Database (http://www.ebi.ac.uk/imgt/hal/atats.html) recognized more than 650 alleles at the *HLA-A* locus, more than 1,000 alleles

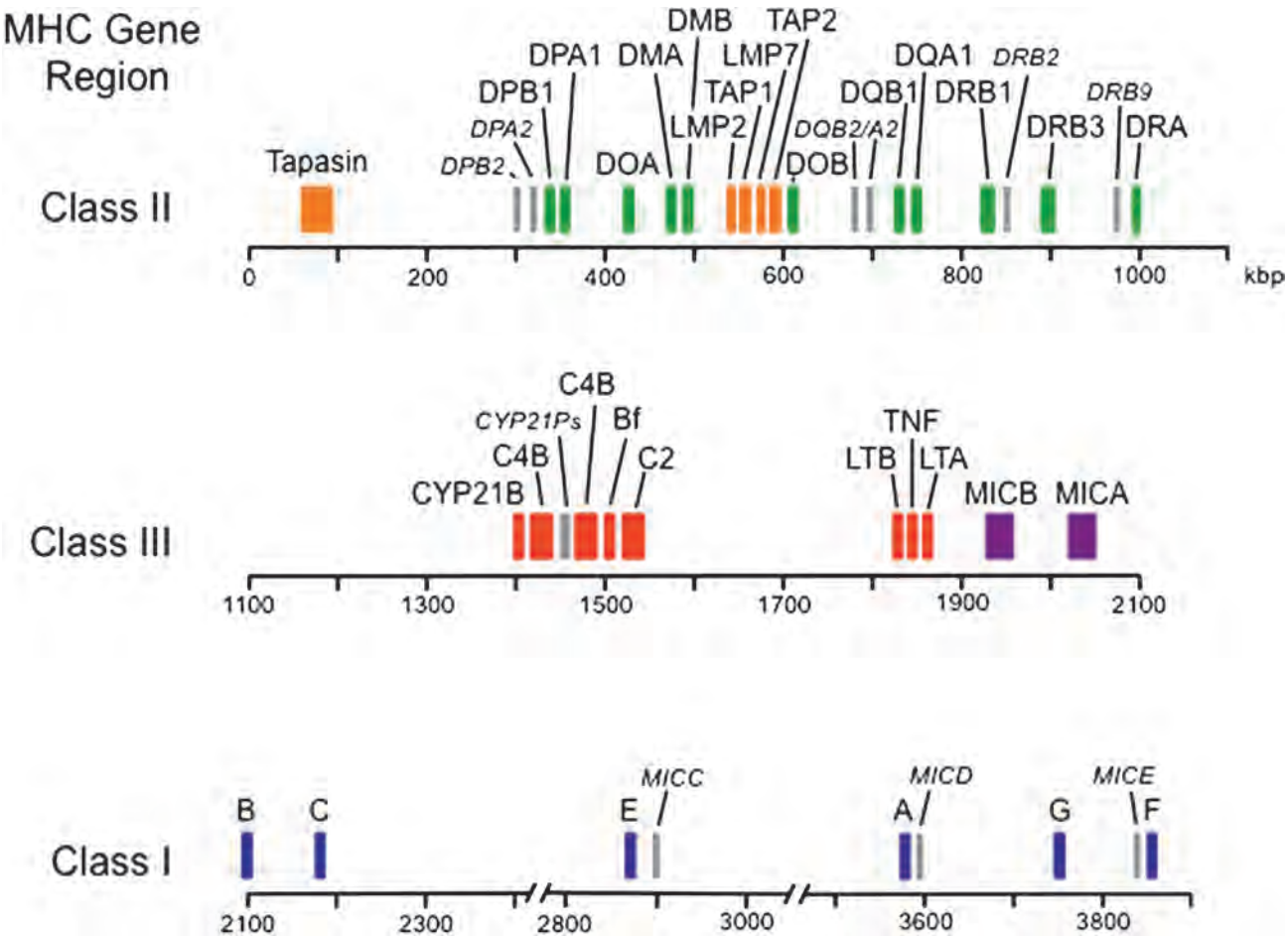

FIG 2. Molecular map of the human MHC. The human MHC, designated HLA, is encoded on the short arm of chromosome 6. The locations of the major HLA and related genes are shown above a scale showing approximate genetic distances in kilobase pairs of DNA *(kbp)*. The genes encoding the class I HLA heavy chains (shown in blue) are clustered at the telomeric end of the complex. The genes encoding the class II HLA α and β chains (shown in green) plus the genes encoding the LMP2/7, transporter associated with antigen presentation *(TAP)* 1/2, and tapasin *(TAPBP)* molecules (shown in orange) are clustered at the centromeric end of the complex. In between the class I and class II genes are additional genes designated class III (shown in red). These include genes encoding the cytochrome P450 21-hydroxylase *(CYP21B)*; an inactive cytochrome P450 pseudogene *(CYP21Ps)*; complement components C4, C2, and factor B; TNF; and the 2 lymphotoxin chains (*LTA* and *LTB*). There are 2 isoforms of complement C4 designated *C4A* and *C4B*. C4A interacts more efficiently with macromolecules containing free amino groups (protein antigens), whereas C4B interacts more efficiently with macromolecules containing free hydroxyl groups (glycoproteins and carbohydrates). There are genes encoding 2 additional HLA class I–like molecules designated *MICA* and *MICB* (shown in purple) located between the class III genes and the classical class I genes. Nonfunctional pseudogenes are shown in gray and further designated by italics.

at the *HLA-B* locus, and more than 350 alleles at the *HLA-C* locus. This polymorphism is largely in amino acids located in the floor and sides of the peptide-binding groove, resulting in different peptide-binding specificities of different class I alleles. The fact that there are 3 distinct HLA class I genes and that each is highly polymorphic means that all subjects in the population who are heterozygous at these loci have 6 distinct peptide-binding grooves. Because each class I protein can bind many different peptides, having 6 peptide-binding molecules results in the ability to bind a very diverse collection of antigenic peptides. Furthermore, on a population level, the diversity of peptide-binding motifs is huge. Mutations in microbial antigens might permit the microbe to avoid binding (and, consequently, recognition) by a few HLA class I alleles, but no mutations will permit the microbe to avoid recognition broadly through the population.

Generally, the antigenic peptides that are found bound in the peptide-binding groove of the HLA class I molecules are derived from proteins synthesized within the cell bearing the class I molecules. They are, consequently, described as "endogenous" antigens. The molecular machinery that generates peptide fragments from intracellular proteins and directs them into the grooves of the class I molecules is increasingly well understood (Fig 4).[6] Peptide fragments are generated from cellular proteins through the action of the proteasome, a proteolytic factory composed of more than 25 subunits.[23] Proteasomes are expressed constitutively in all cell types, where they function in cellular homeostasis. Stimulation of cells with IFN-γ activates them for the production of antigenic peptide fragments that can be presented in HLA class I molecules. This activation induces the production of a variant of the proteasome termed the

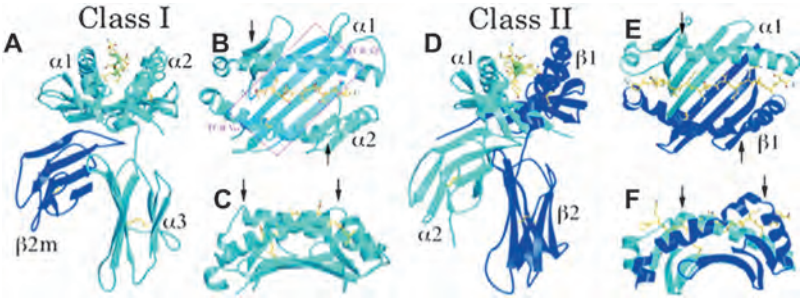

FIG 3. Structure of HLA molecules. Molecular models derived from crystal structures of class I **(A-C)** and class II **(D-F)** HLA molecules. Fig 3, *A*, the class I α_1, α_2, and α_3 domains are shown (light blue) in noncovalent association with the β_2-microglobulin molecule (dark blue). *Coils* represent α-helices, and *broad arrows* represent β-strands. Antiparallel β-strands interact to form β-sheets. The α-helices in the α_1 and α_2 domains form the sides of a groove that binds processed antigenic peptides (yellow). The transmembrane and intracytoplasmic portions of the heavy chain are not shown. Fig 3, *B*, Top view of the α_1 and α_2 domains displaying the antigenic peptide in a molecular complex for recognition by the TCR of a $CD8^+$ T cell (recognition site outlined by pink rectangle). Fig 3, *C*, Side view of the α_1 and α_2 domains highlighting the TCR contact points on both the α-helices and antigenic peptide. Fig 3, *D*, Side view of the HLA class II molecule showing the α chain (light blue) and β chain (dark blue). In the class II protein the peptide-binding groove is made of α helices in both the α_1 and β_1 domains and a β-sheet formed again by both the α_1 and β_1 domains. Fig 3, *E*, Top view of the both the α_1 and β_1 domains and the processed antigenic peptide fragment as they would be seen by the TCR of a $CD4^+$ T cell. Fig 3, *F*, Side view highlighting the α_1 and β_1 domains and the antigenic peptide. Modified with permission from Bjorkman.[20]

immunoproteasome. Two of the subunits of the constitutively expressed proteasome are replaced in the immunoproteasome by the IFN-γ–induced low molecular mass polypeptide 2 (LMP2) and LMP7 protein, both of which are encoded within the HLA complex in the interval between the *HLA-DP* and the *HLA-DQ* gene loci (Fig 2). The LMP2 and LMP7 proteins alter the proteolytic specificity of the proteasome, enhancing the production of peptide fragments of appropriate length and charge for binding in the groove of the HLA class I proteins. The addition of another IFN-γ–induced protein termed the PA28 proteasome activator also enhances the generation of antigenic peptides that are favorable for presentation in HLA class I molecules.[24] After exiting from the immunoproteasome, peptide fragments are transported into the endoplasmic reticulum (ER) by the action of a specific multisubunit transmembrane transporter. This transporter contains 2 ATP-binding cassette subunits designated transporter associated with antigen presentation 1 and 2 encoded by genes that are located within the MHC gene complex in the same region that encodes LMP2 and LMP7 (Fig 2). Once in the ER, the peptides are loaded into the class I protein-binding groove under the direction of the ER protein tapasin with the help of the calcium-binding chaperone protein calreticulin and the oxidoreductase Erp57.[25,26] Before its interaction with β_2-microglobulin, the class I protein is maintained in a conformation that favors interaction with peptide fragments by association with the chaperone protein calnexin. Interaction with β_2-microglobulin stabilizes the complex, causing dissociation of calnexin and permitting transport of the peptide-loaded class I molecule through the Golgi complex into exocytic vesicles that release the intact complexes onto the cell surface. This pathway is well adapted to delivering viral peptides produced in a virus-infected cell to the cell surface bound to class I HLA molecules in a form that can be recognized by cytotoxic $CD8^+$ T cells. It can also be used to present tumor-specific protein fragments that might be useful targets for antitumor immunotherapy.

Studies over the past several years have shown that under certain circumstances, exogenous antigens (synthesized outside of the APC) can also be internalized by means of endocytosis and presented in HLA class I molecules. This uptake of exogenous antigens and display to T cells in HLA class I proteins is known as "cross-presentation."[27] Cross-presentation is particularly important in antiviral immunity, in which it helps the host to overcome the ability of some viruses to suppress antigen processing through the endogenous pathway.[28]

Class II MHC molecules

Like the class I molecules, the class II HLA molecules consist of 2 polypeptide chains, but in this case both are MHC-encoded transmembrane proteins and are designated α and β. There are 3 major class II proteins designated HLA-DR, HLA-DQ, and HLA-DP.[20] Molecules encoded in this region were initially defined serologically and by using cellular immune assays, and consequently, their nomenclature does not always reflect the underlying genes encoding the molecules. This is particularly true for HLA-DR, in which the genes in the HLA-DR subregion encode 1 minimally polymorphic (1 common and 2 very rare alleles) α chain (designated DRA) and 2 polymorphic β chains (designated DRB1 and DRB3, Fig 2). Pairing of the common α chain with the DRB1 chain produces the HLA-DRB1 protein. More than 500 HLA-DRB1 alleles have been defined. Pairing of the common α chain with the DRB3 chain produces molecules designated HLA-DRB2 through HLA-DRB9. There are a total of 60 HLA-DRB2 through HLA-DRB9 alleles. The HLA-DQ subregion encodes 1 polymorphic α chain (25 alleles) and 1 polymorphic β chain (72 alleles). The HLA-DP subregion encodes 1 polymorphic α chain (16 alleles) and 1 polymorphic β chain (118 alleles). Because both the α and β chains of the HLA-DQ and HLA-DP proteins are polymorphic, each person can express 4 different HLA-DQ and 4 different HLA-DP proteins based on pairing between the gene products of both the maternal and paternal chromosomes. Furthermore, because the minimally polymorphic HLA-DR α chain can pair with an HLA-DRB1 and an HLA-DRB3 chain from both the maternal and paternal chromosomes, each person can express 4

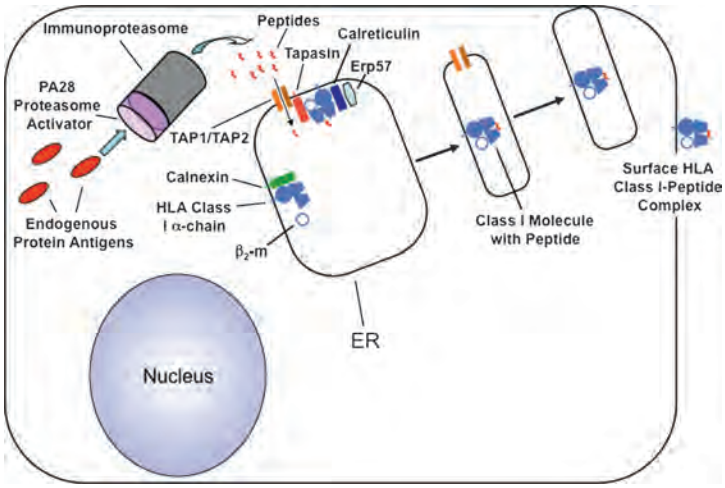

FIG 4. Cellular pathway for processing and presentation of endogenous antigens. Endogenous proteins are digested by the immunoproteasome to small peptide fragments. Production of the immunoproteasome is induced by IFN-γ, which leads to expression of LMP2 and LMP7 (which replace certain components of the conventional cellular proteasome) and the PA28 proteasome activator that modifies the proteasome so that it produces antigenic peptide fragments that are optimal for loading into class I molecules. Peptides are transferred from the immunoproteasome to the ER through the transporter associated with antigen presentation (TAP). There the peptides are loaded, with the help of tapasin, calreticulin, and the chaperone Erp57, into a class I heavy chain that associates with a β_2-microglobulin subunit before transport to the cell surface, where it can be recognized by $CD8^+$ T cells. The association of β_2-microglobulin with the class I heavy chain is facilitated by an additional chaperone protein, calnexin. Modified with permission from Huston.[6]

distinct HLA-DR proteins as well. Each of these has the potential to bind a large repertoire of antigenic peptides, making it difficult for a pathogenic microbe to mutate its structure to a form that cannot be recognized by binding in an HLA class II protein.

Each chain of the class II proteins contains a short cytoplasmic anchor, a transmembrane domain, and 2 extracellular domains designated for the α chain, α_1 and α_2, and for the β chain, β_1 and β_2.[20] When the α and β chains pair, the α_1 and β_1 domains combine to form a peptide-binding groove very similar in structure to that formed by the association of the α_1 and α_2 domains of the class I proteins. The α_2 and β_2 domains of the proteins provide a support for this peptide-binding domain, and the β_2 domain also interacts with the CD4 molecule. This provides a mechanism by which CD4 expressed on T_H cells can enhance the interaction between these T cells and the class II–expressing APCs in a fashion similar to the way binding of the HLA class I molecule by CD8 enhances cytotoxic T-cell activation.[29]

The class II proteins are expressed constitutively on B cells, dendritic cells, monocytes, and macrophages, all cells that present antigens to $CD4^+$ T cells. Expression of MHC class II proteins can also be induced on many additional cell types, including epithelial and endothelial cells after stimulation with IFN-γ, permitting these cells to present antigens to $CD4^+$ T cells at sites of inflammation.

Antigens that are presented by class II proteins are loaded into the class II peptide-binding groove through the "exogenous" pathway that starts by endocytosis or phagocytosis of extracellular proteins (Fig 5).[6] The exogenous antigens include antigenic proteins of extracellular pathogens, such as most bacteria, parasites, and virus particles that have been released from infected cells and taken up by phagocytosis, as well as environmental proteins and glycoproteins, such as pollens and venoms, and alloantigens. The ingested antigens are processed to linear peptide fragments by means of proteolysis after fusion of lysosomes with the phagocytic vacuoles or endosomes to form an acidic compartment.[30] The peptide fragments then accumulate in the MHC II loading compartment, where they encounter nascent class II proteins. The α and β chains of the class II molecules are synthesized in the ER. To protect the class II molecule's peptide-binding groove so that it can later accommodate an antigenic peptide, the α and β chains associate with the nonpolymorphic invariant chain (Ii), assisted by the chaperone protein calnexin. A portion of the Ii chain designated class II–associated invariant-chain peptide lies in the peptide-binding groove of the class II heterodimer, preventing binding of antigenic peptides. Once the class II–Ii complex has formed, it dissociates from calnexin and is transported to the class II loading compartment.[31] In the class II loading compartment, the bulk of the Ii is degraded by acid proteases, such as cathepsins, and exchange of the class II–associated invariant-chain peptide for an antigenic peptide is catalyzed by the action of the HLA-DM molecule, resulting in the formation of a mature class II protein.[32] The class II proteins loaded with antigenic peptide are then delivered to the cell surface by means of fusion of the class II^+ endosome to the plasma membrane.

Association of HLA types and disease susceptibility

Epidemiologic studies have demonstrated that more than 40 diseases are found more frequently in subjects carrying certain HLA class I or II alleles than in the general population.[33] The magnitude of these effects can be quite large but are probably never absolute. For example, they range from the finding that between 90% and 95% of white patients with ankylosing spondylitis are HLA-B27[34] to the observation that between 30% and 50% of white patients with type I diabetes mellitus are heterozygous for HLA-DQ2/DQ8.[35] Interestingly, HLA-DQ6 appears to provide

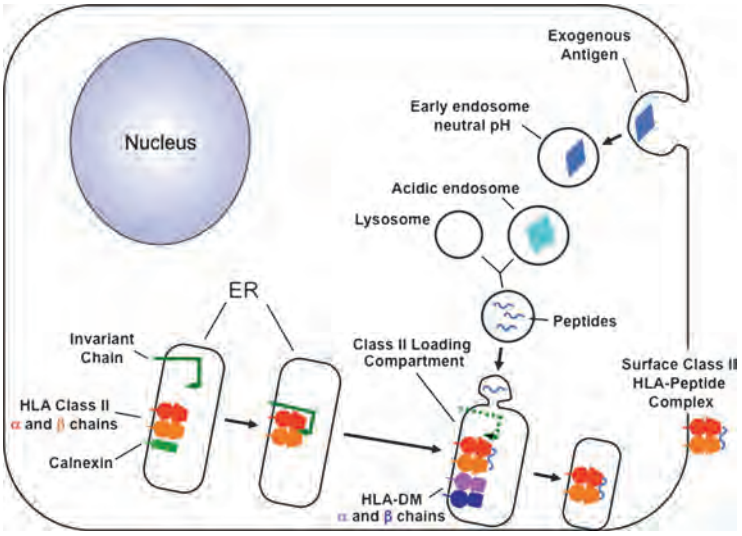

FIG 5. Cellular pathway for processing and presentation of exogenous antigens. In the ER newly synthesized class II proteins associate with the help of calnexin with an Ii protein that protects the antigen-binding groove of the class II molecule until it is transported to the class II^+ endosomal protein–loading compartment. Exogenous antigens are taken up by phagocytosis or endocytosis, digested by the action of lysosomal enzymes, and transported to the class II^+ peptide loading compartment for loading into a class II protein. There the Ii is proteolytically degraded and replaced by antigenic peptide with the help of the HLA-DM protein. The assembled class II protein–peptide complex is then delivered to the plasma membrane for recognition by $CD4^+$ T cells. Modified with permission from Huston.[6]

dominant protection from development of type I diabetes. Most diseases that show linkage of susceptibility to particular HLA genes have a prominent autoimmune character. Although the mechanisms by which HLA genotypes control susceptibility to these diseases remains imprecisely defined, it is likely that the participation of HLA molecules in the establishment of immune tolerance or permitting immune recognition of environmental antigens underlies this phenomenon.[36,37] Protective HLA gene alleles might mediate the elimination in the thymus of potentially pathogenic T cells, whereas susceptibility HLA gene alleles might fail to contribute appropriately to elimination of pathogenic T cells. HLA genotypes can also underlie responsiveness or nonresponsiveness to certain vaccines. For example, subjects who are HLA-DR3 have a substantially increased incidence of nonresponsiveness to vaccination with hepatitis B surface antigen,[38] and subjects who are HLA-DRB1*03 or HLA-DQA1*0201 have an increased incidence of seronegativity after measles vaccination.[39]

HLA-independent presentation of antigen

Antigen presentation by class I and class II HLA molecules to $CD8^+$ and $CD4^+$ T lymphocytes is limited to protein antigens. Initially, it was thought that responses to polysaccharide antigens and lipid antigens was restricted to T cell–independent responses that resulted in direct activation of B cells by an antigen with a repeating structure; however, recently it has become clear that there is a class of T cells that recognizes antigens presented by molecules that are not classical HLA class I or class II antigens. One of these classes of T cells uses an antigen receptor composed of α and β chains and recognizes lipid antigens that are presented bound to CD1 molecules.[10] CD1 molecules are structurally related to class I HLA molecules, being transmembrane proteins with 3 extracellular domains and associating with β_2-microglobulin. There are 5 human CD1 isoforms designated CD1a to CD1e and encoded by linked genes that are not associated with the MHC. X-ray crystallography shows that the α_1 and α_2 domains of CD1 molecules associate like class I MHC molecules to form a binding groove that can accommodate glycolipid components of microbial pathogens.[40] CD1-glycolipid complexes can also serve as targets for recognition by T cells that use the γδ TCR (see below). This presentation of microbial glycolipids by CD1 molecules appears to underlie the MHC-independent recognition of mycobacteria by both αβ and γδ T cells. Glycosphingolipids, a class of carbohydrate-containing lipids that are found in both eukaryotic and prokaryotic cells, can also be presented by the CD1d molecule to NK-T cells, leading to their release of large quantities of immunoregulatory cytokines.[41] Human γδ T cells can also recognize target cells by virtue of their expression of the stress-inducible MHC class I–related chains A and B (MICA and MICB). MICA and MICB are encoded by genes that lie between the *TNF* gene cluster in the class III region of the MHC and the *HLA-B* locus in the class I region (Fig 2). They share structural characteristics with the class I protein heavy chains but appear not to associate with β_2-microglobulin and not to bind antigenic peptides. Rather, they act as stress-induced molecules that are targets for intestinal γδ T cells, further expanding the repertoire of molecules that can contribute to activation of responding T lymphocytes. In addition to the 2 functional *MICA* and *MICB* genes, there are at least 3 inactive *MIC* pseudogenes encoded within the class I region of the MHC (Fig 2).[42]

T LYMPHOCYTES

The major class of T cells is defined by its surface expression of the αβ TCR. This receptor has evolved primarily to recognize

peptide antigens presented in a complex with class I or class II MHC proteins. αβ T cells differentiate into several different subsets, some of which ($CD8^+$ T cells) act primarily to kill cells infected with intracellular microbes and others ($CD4^+$ T cells) that act primarily to regulate the cellular and humoral immune responses. A small subset of αβ T cells that expresses the NK1.1 (CD161) NK cell antigen (NK-T cells) are usually CD4 and CD8 double negative, recognize glycolipid antigens presented by the CD1d molecule, and appear to be immunoregulatory based on their ability to release rapidly large quantities of the cytokines IFN-γ, IL-4, GM-CSF, TNF, and others.[43] Details of the mechanisms by which T cells develop, acquiring their antigen specificity, and then are regulated as they encounter antigen in the peripheral tissues are discussed in chapter 3 of this Primer.[4] An introductory overview is presented here.

T-cell development

Each individual T cell bears antigen receptors of a single specificity. A repertoire of T cells that can protect against the vast universe of microbial pathogens must therefore include a very large number of cells encoding a huge array of discrete TCRs. These receptors are somatically assembled from variable, diversity, and joining gene elements to generate mature $V_\alpha J_\alpha$ chains and $V_\beta D_\beta J_\beta$ chains (see chapter 3 of this Primer).[4] The assembly of these gene elements is initiated by the lymphoid-specific recombinase-activating gene (RAG) 1 and RAG2 proteins, which cleave the DNA near the V, D, and J segments, and the gene segments are rejoined by a collection of non–lymphoid-specific DNA repair enzymes, including DNA-dependent protein kinase (DNA-PK), Ku, XRCC4, XLF, DNA ligase IV, and the Artemis nuclease.[44] XRCC4, XLF, and DNA-PK help recruit the enzyme terminal deoxynucleotidyl transferase (TdT), which adds deoxynucleotides into some of the VDJ junctions, providing extra junctional diversity to the recombined gene sequences.[45] The action of these recombinase enzymes results in the V, D, and J gene elements assembling in an apparently random process, producing a huge diversity of receptor sequences but also frequently producing nonfunctional genes. Selection of cells carrying functional TCR genes occurs in the thymus (Fig 6),[6] a complex lymphoid organ located in the anterior mediastinum at the base of the neck.[46] The thymus contains 3 compartments. The first, the subcapsular zone, is where bone marrow–derived prothymocytes begin to differentiate, proliferate, and rearrange their TCR β chains. The cells then move to the thymic cortex, where the α chain gene elements rearrange, potentially forming a functional, mature αβ TCR. In the cortex cells test whether their receptors have sufficient affinity for self-MHC molecules to permit them ultimately to recognize antigen-MHC complexes. This involves interactions between the developing lymphocyte and the specialized cortical epithelium.[47] If the lymphocyte fails this positive selection, then it undergoes apoptosis and is cleared by thymic cortical macrophages. Finally, in the thymic medulla cells are screened for potential autoreactivity. This screening includes testing for reactivity for an extensive array of tissue-specific proteins that are expressed by a population of thymic medullary epithelial cells under the control of a gene called autoimmune regulator *(AIRE)*. Defective expression of *AIRE* gives rise to the severe autoimmune syndrome called autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy.[48] Cells that recognize self-peptides expressed by these epithelial cells are removed by means of apoptosis, and cells that have survived this negative selection are exported to the circulation. Fewer than 5% of the developing T cells survive positive and negative selection.

Approximately 90% to 95% of circulating T cells use the αβ TCR described above. The other 5% to 10% use an alternate heterodimeric TCR composed of γ and δ chains. The γ and δ chains also assemble by means of RAG1/RAG2-mediated rearrangement of V, D (for the δ chain only), and J elements. A portion of the γδ T cells is generated in the thymus, but a major fraction appears to be generated in an extrathymic compartment, resulting in cells that largely populate the gastrointestinal tract.[49]

T cell–antigen receptor complex

The antigen-specific α and β chains of the TCR associate with invariant accessory chains that serve to transduce signals when the TCR binds to antigen-MHC complexes.[50] These accessory chains make up the CD3 complex, consisting of the transmembrane CD3γ, CD3δ, and CD3ϵ chains plus a largely intracytoplasmic homodimer of 2 CD3ζ chains. Although the stoichiometry of the CD3 complex is not definitively established, it appears that each TCR αβ pair associates with a CD3γϵ heterodimer, a CD3δϵ heterodimer, and a CD3ζ homodimer (Fig 7).

Interaction of the TCR/CD3 complex with antigenic peptide presented in an HLA molecule provides only a partial signal for cell activation. Full activation requires the additional participation of a costimulatory molecule, such as CD28 on the T cell and CD80 (also designated B7.1) or CD86 (B7.2) on the APC (Fig 7).[51] In fact, interaction of peptide-MHC with the TCR without a costimulator can lead to an anergic state of prolonged T-cell nonresponsiveness.

The cytoplasmic portions of each of the CD3 chains contain sequence motifs designated immunoreceptor tyrosine-based activation motifs (ITAM). When key tyrosines in these ITAMs are phosphorylated by the receptor-associated kinases Lck and Fyn, this initiates an activation cascade involving the proteins zeta-chain-associated protein kinase 70 (ZAP-70), and, farther downstream, Linker of Activated T cells (LAT) and SH2 domain containing leukocyte protein of 76kDa (SLP-76). Activation of these proteins leads to stimulation of phospholipase C, activation of the G proteins Ras and Rac, and both protein kinase C and the mitogen-associated protein kinases. Together, this complex of activation events leads to activation of genes that control lymphocyte proliferation and differentiation.

The pathways that downregulate this activation pathway are becoming increasingly well defined. The membrane molecule CD45 is a key tyrosine phosphatase that occupies a central position in this deactivating process. In addition, a specific receptor-ligand pair, programmed death 1 and programmed death ligand 1, transduces signals to the activated lymphocyte to inhibit its proliferation and effector functions, thus extinguishing the T-cell response.[52] Mutations affecting the function of many of the molecules involved in intracellular lymphoid cell-signal transduction processes underlie congenital primary immunodeficiency syndromes (see chapter 15 of this Primer).[53]

T-cell subpopulations

During their progress through the thymus, αβ T cells differentiate into discrete subpopulations, each with defined repertoires of effector functions. The major subsets are defined by their selective surface expression of CD4 or CD8. In the thymus most developing T cells follow a developmental program in which in the cortex they first express neither CD4 nor CD8 (double

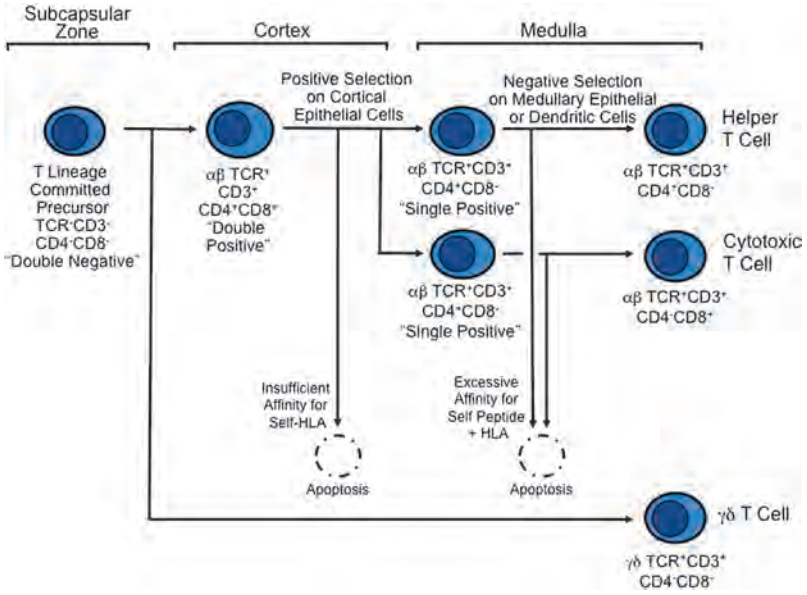

FIG 6. Differentiation and maturation of T cells in the thymus. Hematopoietic stem cells, which do not express CD3, CD4, or CD8 but are committed to T-cell differentiation, move from the bone marrow to the thymic subcapsular zone. There they begin rearrangement of the TCR genes. Once a productive TCR β chain has been produced, they move to the thymic cortex, where TCR α chain rearrangement occurs and surface expression of the CD3, CD4, and CD8 proteins is induced. These $CD4^+CD8^+$ (double-positive) cells are positively selected on cortical epithelial cells for their ability to recognize self class I or class II HLA proteins. If the developing T cell has adequate affinity to recognize a self class I protein, then it retains expression of CD8 and extinguishes expression of CD4. If the cell recognizes a self class II protein, then it retains expression of CD4 and extinguishes expression of CD8. Selected CD4 or CD8 single-positive cells then move to the thymic medulla, where they are negatively selected on medullary epithelial cells to remove cells with excessive affinity for self-antigens presented in HLA molecules. Cells emerge from positive selection single positive for CD4 or CD8 expression and then are exported to the periphery. Cells that fail positive or negative selection are removed by apoptosis. A small fraction of cells differentiate to rearrange their TCR γ and δ chains, rather than their TCR α and β chains. Modified with permission from Huston.[6]

negative) and then express both CD4 and CD8 (double positive).[54] Double-positive cells are tested by means of positive selection in the thymic cortex, and those that are selected on class I MHC molecules become $CD4^-CD8^+$ and those that are selected on class II MHC molecules become $CD4^+CD8^-$. The fact that the CD4 molecule contributes to a stable interaction of the developing T cell with class II MHC molecules on the selecting APC and that CD8 contributes to interactions with class I molecules is central to the association of CD4 with class II MHC–restricted antigen recognition and of CD8 with class I–restricted antigen recognition. Cells that survive positive selection then move to the thymic medulla for negative selection and export to the periphery. In the blood and secondary lymphoid organs, 60% to 70% of T cells are $CD4^+CD8^-$ ($CD4^+$) and 30% to 40% are $CD4^-CD8^+$ ($CD8^+$). $CD4^+$ T cells are generally designated helper cells and activate both humoral immune responses (B-cell help) and cellular responses (delayed-type hypersensitivity responses and others). $CD8^+$ cells show a major cytotoxic activity against cells infected with intracellular microbes and against tumor cells but also contain regulatory cells that downregulate immune responses (suppressor cells). A portion of the circulating $CD4^+$ T cells play an important regulatory role that acts to down modulate immune responses. These regulatory T (Treg) cells fall into 2 groups. The first group develops its regulatory function in the thymus and is known as natural Treg cells. These cells are characterized by surface expression of the CD4 and CD25 antigens and by nuclear expression of the forkhead box protein 3 (Foxp3) transcription factor that is essential for their development. A major portion of this population's regulatory activity is due to its secretion of the immunomodulatory cytokines TGF-β and IL-10.[55] Under some conditions, suppression of effector T-cell proliferation by Treg cells requires cell-cell contact. In this situation it has been reported that TGF-β acts in a membrane-associated form.[56] The second group of Treg cells is thought to differentiate in the periphery from naive $CD4^+$ T cells. Because they appear to develop in response to stimulation with specific antigen, they are called adaptive or induced Treg cells. Their differentiation appears to depend on the presence of IL-10 during their initial activation. Expression of Foxp3 is variable in this subset, and IL-10 is a prominent secreted product, with TGF-β also participating.[57] The phenotype of these cells can be unstable, with Foxp3 expression disappearing soon after withdrawal of the inductive IL-10 or TGF-β. Recent studies have indicated that epigenetic modification of the Foxp3 locus, in the form of both histone acetylation and altered DNA methylation in the area around the Foxp3 promoter, are essential for establishment of stable expression of Foxp3 and maintenance of the Treg cell phenotype.[58]

Approximately 5% to 10% of T cells in the peripheral blood, lymph nodes, and spleen are $CD4^-CD8^-$. Some of these cells use αβ TCRs, and others use γδ TCRs. Double-negative cells do not recognize antigen in the context of MHC class I or II. Some of these cells recognize antigen in the class I–related protein CD1

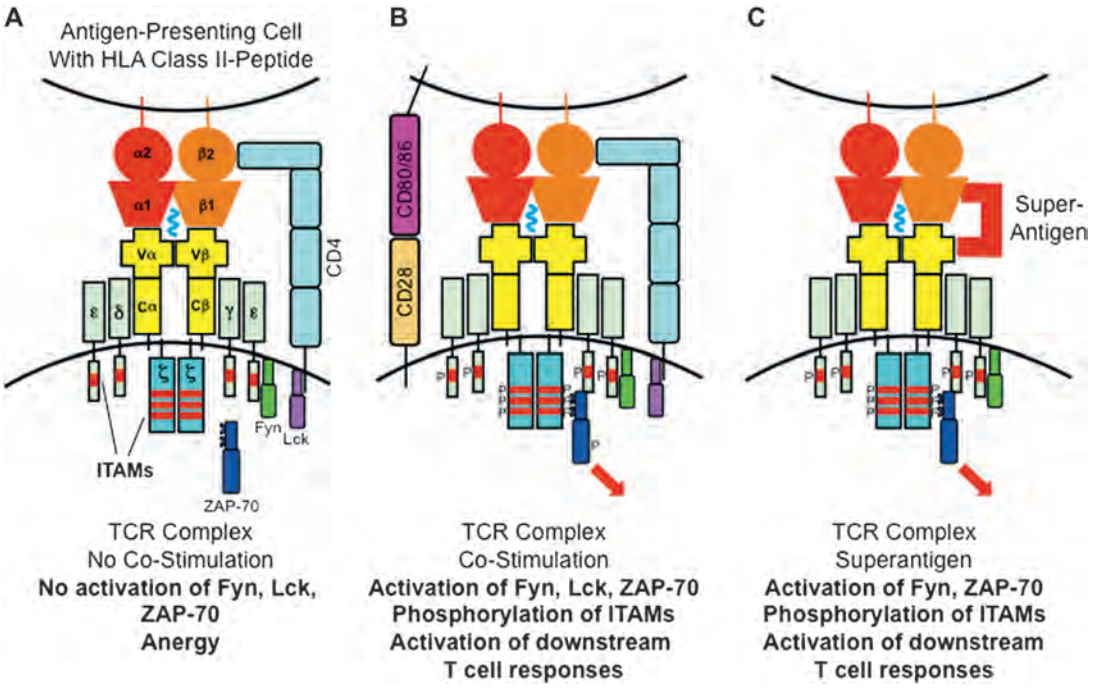

FIG 7. The TCR complex and T-cell activation. **A**, the complete TCR complex includes the rearranged TCR α and β chains and also the CD3γ, CD3δ, CD3ϵ, and CD3ζ chains. The CD3 chains contain ITAMs in their cytoplasmic domains that can be phosphorylated to activate the intracellular signaling cascade for T-cell activation. The signaling protein tyrosine kinases Lck and Fyn associate with the intracellular portions of the CD4 and CD3 chains, respectively. TCR engagement by MHC plus peptide without the presence of costimulatory proteins fails to activate phosphorylation of the CD3 ITAMs and results in anergy. **B**, TCR engagement by MHC plus peptide with costimulatory interactions between CD28 on the T cell and CD80 or CD86 (B7.1 or B7.2) on the APC results in Lck- and Fyn-dependent phosphorylation of the CD3 chains and recruitment of the adapter protein zeta-chain-associated protein kinase 70 *(ZAP-70)*, to the CD3 complex. This leads to phosphorylation of ZAP-70, which induces the downstream program of T-cell activation. **C**, polyclonal activation of T cells can be elicited by superantigens, which interact outside the peptide-binding groove with the β_1 chain of the class II molecule and with all Vβ chains of a particular subclass. This activates CD4-independent but Fyn-dependent phosphorylation of the CD3 chains, recruitment and phosphorylation of ZAP-70, and cell activation.

that is adapted to presentation of glycolipid components of mycobacteria and other microbes.[40] A subset of double-negative γδ T cells recognizes the MHC class I chain–related proteins designated MIC.[42]

Both $CD4^+$ and $CD8^+$ T cells differentiate into functionally distinct subsets after exposure to antigen. This is best described for the transition of $CD4^+$ T cells from naive to effector populations. Resting naive $CD4^+$ T cells (designated T_H cells) release very low levels of cytokines. Early after stimulation by antigen and APCs, the T_H cells begin to produce IL-2 and are designated T_H0. As the T_H cells continue to respond to the activating signal, they progress toward polar extremes of differentiation designated T_H1, T_H2, and T_H17 depending on the nature of the cytokines present at the site of activation.[59] IL-12 produced by macrophages or NK cells induces differentiation toward T_H1; IL-4 produced by $NK1.1^+$ T cells, basophils, or mast cells induces differentiation toward T_H2; and TGF-β and IL-6 produced by yet to be defined cells induce differentiation toward T_H17. T_H1 cells are characterized by their expression of the T-box transcription factor (T-bet) and by the production of IL-2, IFN-γ, and lymphotoxin. T_H2 cells are characterized by their expression of the transcription factor GATA3 and produce IL-4, IL-5, IL-9, IL-13, and GM-CSF, and T_H17 cells express the transcription factor Retinoic-acid-related Orphan Receptor C isoform 2 (RORC2) and produce the cytokines IL-6 and IL-17 (see chapter 3 of this Primer).[4,60] T_H17 cells are induced early in the adaptive response to extracellular bacteria and help to recruit the neutrophil response that eliminates these pathogens. They also direct the destructive inflammatory responses that are part of many autoimmune diseases. T_H1 and T_H2 cells often participate together in immune responses; however, after prolonged immunization, the response can become dominantly T_H1 or T_H2 like. Generally, T_H1 cells support cell-mediated immune responses, and T_H2 cells support humoral and allergic responses. $CD8^+$ T cells also can manifest type 1 and type 2 cytokine responses, in which case the cells are designated cytotoxic T cell type 1 and cytotoxic T cell type 2.[61] Understanding the factors that govern whether a T_H response adopts a predominantly T_H1-type, T_H2-type, or T_H17-type response is crucial to the allergist/clinical immunologist. Recent progress using immunization with different types of adjuvants (eg, CpG DNA) demonstrates the feasibility of reprogramming, in atopic patients, allergic T_H2-type responses to nonallergic T_H1-type responses.[62]

Superantigens

Conventional antigens bind to a subset of MHC molecules and to a very small fraction of the huge array of TCRs. Thus a

TABLE I. Structure, function, and distribution of antibody isotypes

	IgM	IgD	IgG1	IgG2	IgG3	IgG4	IgA1	IgA2	IgE
Subunit form*	5	1	1	1	1	1	1, 2	1, 2	1
Molecular weight (kd)	950	175	150	150	150	150	160, 400	160, 400	190
Concentration in serum (mg/mL)	2	0.03	10	4	1	0.5	2	0.5	0.003
Complement-activating C/A†	+/−	−/+	++/+	+/+	++/+	−/+	−/+	−/+	−/−
Macrophage FcR binding	+	−	++	++	++	−	++	++	−
Mast cell sensitizing	−	−	−	−	+	−	−	−	+++
Placental transport	−	−	++	+	++	+/−	−	−	−
Mucosal transport‡	−	−	−	−	−	−	+++	+++	−

*5, Pentamer; 2, dimer; 1, monomer.
†C, Classical pathway; A, alternative pathway.
‡Dimer only.

conventional peptide antigen activates only a very small fraction of the total pool of T cells. Superantigens, in contrast, are microbial products that bind to large subsets of TCR proteins and MHC molecules, so that a single superantigen can activate up to 20% or more of the total T cells in the body. The superantigen does this by binding without proteolytic processing to the MHC molecule outside of the antigen-binding groove and to TCR proteins outside of their antigen-MHC binding site (Fig 7). For example, the toxic shock syndrome toxin 1 produced by *Staphylococcus aureus* can activate all T cells with TCRs that use the Vβ2 and Vβ5.1 chains. The activation of large numbers of T cells induced by superantigens results in the massive release of cytokines producing clinical conditions, such as toxic shock syndrome.[63]

B LYMPHOCYTES

B-cell development and the B-cell antigen receptor

B cells constitute approximately 15% of peripheral blood leukocytes. They are defined by their production of immunoglobulin. Except as noted below, immunoglobulin molecules are composed of 2 identical 50-kd heavy chains and 2 identical 25-kd κ or λ light chains (see chapter 3 of this Primer).[4] The amino terminal portions of the heavy and light chains vary in amino acid sequence from one antibody molecule to another. These variable portions are designated V_H and V_κ or V_λ, respectively. The juxtaposition of one V_H segment and one V_κ or V_λ segment creates the antigen-binding portion of the intact immunoglobulin molecule. The variable regions of both the heavy and light chains contain 3 subregions that are highly variable between different antibody molecules. These hypervariable sequences are brought together in the immunoglobulin protein to form the antigen-binding domain of the molecule. Thus each immunoglobulin has 2 identical antigen-binding sites. The carboxyl terminal portions of the heavy and light chains are constant in each subclass of antibody. The heavy chain constant regions pair to form the Fc domain of the molecule that is responsible for most of the effector functions of the immunoglobulin molecule, including binding to Fc receptors and activating the complement system.

The genes encoding the κ light chain are encoded on chromosome 2, and the genes encoding the λ light chain are on chromosome 22. The complex heavy chain locus is encoded on chromosome 14. The light chain and heavy chain loci are each composed of a series of V (variable) gene elements, followed by several D (diversity) segments (for the heavy chain gene only), some J (joining) segments, and C (constant region) exons. The constant regions of both the κ and λ light chain genes are encoded as single exons. The heavy chain gene, in contrast, contains exons that encode 9 different constant regions that are used to produce the different classes and subclasses of immunoglobulins (Table I).

B cells differentiate from hematopoietic stem cells in the bone marrow. It is here that their antigen receptors (surface immunoglobulin) are assembled from genetic building blocks in a RAG1/RAG2-mediated process similar to that used for the production of functional TCRs.[64] The amino terminal portion of each heavy chain is created by somatic joining of genes encoding a variable (V_H), diversity (D_H), and joining (J_H) region. Joining of genes encoding variable and constant light chain elements generates the amino terminal portion of the light chain. The VDJ junctions formed by this recombination make up the third hypervariable region that contributes to the antigen-binding site. The amino acid sequence diversity of the third hypervariable region is the result of combinatorial V-D-J joining and also of non–gene-encoded sequences added into the junction sites by the action of the enzyme TdT that is expressed in developing B cells during the time this gene rearrangement is occurring.

Establishment of the B-cell repertoire

Differentiation of stem cells to the B lineage depends on bone marrow stromal cells that produce IL-7. The developing B cells follow a program of differential surface antigen expression and sequential heavy and light chain gene rearrangement (Fig 8).[6] First, the recombinase enzyme complex catalyzes the fusion of one of the D_H region genes to a J_H region gene with the deletion of the intervening DNA sequences. This D_HJ_H recombination occurs on both chromosomes. Next, the recombinase joins one of the V_H region genes to the rearranged D_HJ_H gene. TdT is expressed during this period, resulting in the addition of random nucleotides into the sites of D_H-J_H and V_H-D_HJ_H joining, adding to the potential diversity of amino acid sequences encoded by the rearranged $V_HD_HJ_H$ gene. The rearranged $V_HD_HJ_H$ element forms the most 5′ exon of this rearranged heavy chain gene and is followed downstream by exons encoding the constant region of the μ chain that pairs with a light chain to produce IgM and farther downstream by exons encoding the constant region of the δ chain that is used to make IgD. μ Chains and δ chains are produced as a result of alternative RNA splicing of the $V_HD_HJ_H$ exon to either the μ or δ exons. If the rearrangements of the V_H, D_H, and J_H elements yields a heavy chain transcript that is in frame and encodes a functional heavy chain protein, this heavy chain is synthesized

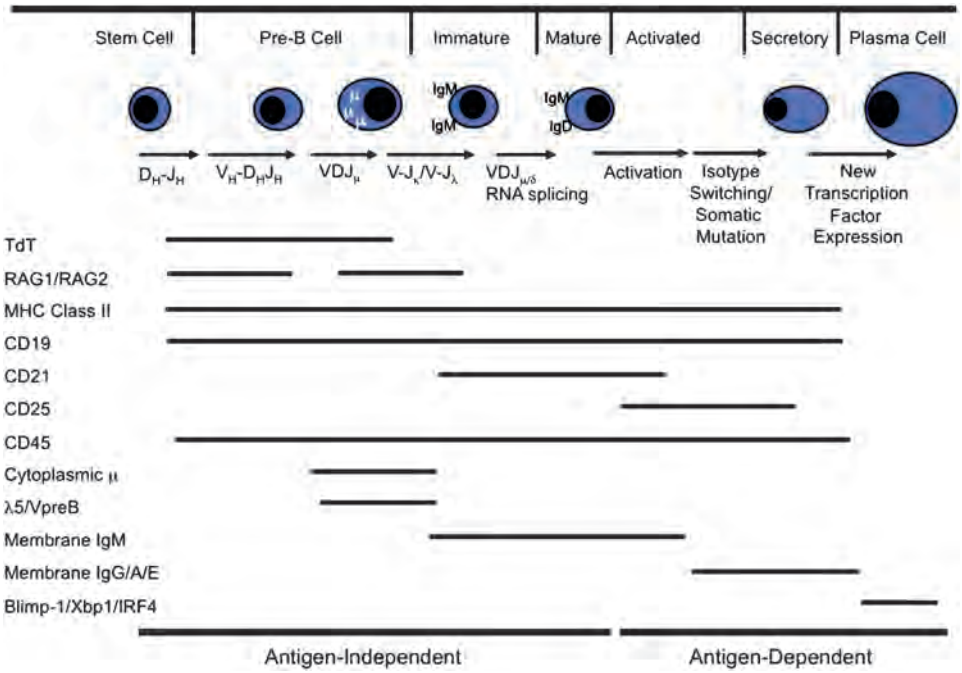

FIG 8. B-cell differentiation and development. B cells differentiate in the bone marrow from stem cells to become mature surface IgM- and IgD-expressing cells. This occurs in the absence of antigen. In peripheral lymphoid tissues the B cell can then mature further under the influence of antigen and T-cell help to undergo isotype switching and affinity maturation by means of somatic mutation. The factors controlling the final differentiation from antibody-secreting B cell to plasma cell are incompletely characterized but require the participation of the transcription factors B-lymphocyte-induced maturation protein 1 (Blimp-1), X-box binding protein 1 (Xbp1), and interferon regulatory factor 4 (IRF4). Correlations are show between the stage of cell differentiation and the expression of key molecules in the cell (TdT, RAG1/RAG2, and cytoplasmic μ) and on the cell surface (class II, CD19, CD21, CD25, CD45, and surface immunoglobulin). Modified with permission from Huston.[6]

and pairs in the cell with 2 proteins, λ5 and VpreB, which act as a surrogate light chain (Fig 8). Expression of this pre–B-cell receptor on the cell surface prevents V_H to D_HJ_H rearrangement on the other chromosome, assuring that the developing B cell produces only 1 antigenic specificity. This process is called allelic exclusion. If the first $V_HD_HJ_H$ rearrangement is out of frame and does not produce a functional heavy chain protein, then a V_H gene proceeds to rearrange on the other chromosome in a second attempt to generate a successful heavy chain rearrangement. If this second rearrangement is unsuccessful, the cell undergoes apoptosis and is removed.

Once a functional heavy chain is produced, the cell downregulates its TdT gene and initiates light chain rearrangement. First, a V_κ element rearranges to a J_κ element. If this forms a functional light chain, then the κ light chain pairs with the heavy chain to form a functional immunoglobulin protein and further light chain rearrangement stops. If the first κ rearrangement fails, then rearrangement proceeds on the other chromosome. If that fails, then rearrangement of the λ chains proceeds. The *RAG1* and *RAG2* genes are only expressed during times of heavy and light chain rearrangement, except that some B cells that express autoreactive receptors appear able to re-express their *RAG* genes and undergo receptor editing by secondary rearrangements of their already rearranged immunoglobulin genes.[65] These processes result in the assembly of the antigen-binding component of the B-cell receptor. Like the TCR, the fully mature B-cell receptor also includes additional transmembrane proteins designated Igα and Igβ that activate intracellular signals after receptor binding to antigen.[66,67] B cells also have a coreceptor complex consisting of CD19, CD81, and CD21 (complement receptor 2) that is activated by binding to the activated complement protein C3d.[68] Both Igα and Igβ have ITAM domains in their cytoplasmic regions and use similar signal transduction pathways compared with those for T cells. The B-cell pathway includes the Src family of kinases, Blk, Fyn, and Lyn, which phosphorylate the ITAMs on the Igα and Igβ chains. The activation signal is then passed through the tyrosine kinase Syk and the B-cell linker protein (BLNK) to the downstream signaling components phospholipase C and guanine nucleotide exchange factors. Ultimately, as in T cells, activation of protein kinase C, calcium mobilization, and Ras/Rac-dependent activation of mitogen-associated protein kinases leads to activation of new gene transcription that causes cell proliferation and maturation.

Isotype switching and affinity maturation

Naive B cells express IgM and IgD on their cell surfaces. As described above, these 2 immunoglobulin isotypes are expressed by alternative splicing of the same $V_HD_HJ_H$ exon to the μ and δ heavy chain exons. For all heavy chain genes, alternative splicing also permits expression of both membrane-bound (splicing in a transmembrane exon) and secreted (transmembrane exon spliced out) antibody. As B cells mature under the influence of T_H cells, T cell–derived cytokines induce isotype switching. Isotype

switching is a process of DNA rearrangement mediated in part by the RNA-editing enzyme activation-induced cytidine deaminase, uracil DNA glycoslyase, the endonuclease APE1, and the DNA repair enzyme DNA-PK. Switching moves the rearranged $V_HD_HJ_H$ exon into a position immediately upstream of alternative heavy chain exons. This permits a functionally rearranged $V_HD_HJ_H$ exon to be used to produce antibodies of different isotypes but the same antigenic specificity.[69] T cell–derived IL-10 causes switching to IgG1 and IgG3. IL-4 and IL-13 cause switching to IgE, and TGF-β causes switching to IgA. IFN-γ or some other undefined product of T_H1 cells appears to induce switching to IgG2.

At the same time as B cells undergo isotype switching, an active process produces mutations, apparently randomly, in the antigen-binding portions of the heavy and light chains. This process, designated somatic mutation, also appears to require activation-induced cytidine deaminase, uracil DNA glycoslyase, APE1, and DNA repair enzymes.[70] If these mutations result in loss of affinity for the antigen, the cell loses important receptor-mediated growth signals and dies. If, however, the mutations result in increased affinity for the antigen, then the cell producing that antibody has a proliferative advantage in response to antigen and grows to dominate the pool of responding cells. Somatic mutation and clonal expansion of mutated cells occurs in the germinal centers of secondary lymphoid tissues.[71]

T cell–dependent B-cell responses

Antigens that activate T cells and B cells establish immunoglobulin responses in which T cells provide "help" for the B cells to mature. This maturation includes both induction of isotype switching, in which the T-cell cytokines control the isotype of immunoglobulin produced, and activation of somatic mutation. The cellular interactions underlying T-cell help are driven by the specific antigen and take advantage of the ability of B cells to serve as APCs. B cells that capture their cognate antigen through their membrane immunoglobulins can internalize the antigen and process it intracellularly for presentation on the cell surface in the B cell's class II HLA proteins. Uptake of antigen induces increased class II expression and expression of CD80 and CD86. T cells activated by this combination of costimulator and antigen–class II complex on the B cell then signal reciprocally to the B cell through the interaction of the T-cell CD40 ligand with B-cell CD40. Signaling through CD40 is essential for induction of isotype switching, and human patients with defects in the X chromosome–encoded *CD40L* gene manifest X-linked hyper-IgM syndrome, and patients with mutant *CD40* show autosomal recessive hyper-IgM syndrome.[72]

Isotype switching and somatic mutations are strongly associated with the development of B-cell memory. Memory responses, defined as rapid induction of high levels of high-affinity antibody after secondary antigen challenge, are characterized by production of IgG, IgA, or IgE antibodies and by somatic mutations in the antigen-binding domains of the heavy and light chains of these antibodies.[73] The development of B-cell memory is critical to the success of vaccination against pathogens and also perpetuates the pathology of many autoimmune and allergic syndromes. Understanding how to enhance or reduce memory responses will provide important new therapeutic opportunities to the clinical immunologist.

T cell–independent B-cell responses

B cells can also be activated successfully without T-cell help. T cell–independent B-cell activation occurs without the assistance of T-cell costimulatory proteins. In the absence of costimulators, monomeric antigens are unable to activate B cells. Polymeric antigens with a repeating structure, in contrast, are able to activate B cells, probably because they can cross-link and cluster immunoglobulin molecules on the B-cell surface. T cell–independent antigens include bacterial LPS, certain other polymeric polysaccharides, and certain polymeric proteins. Somatic mutation does not occur in most T cell–independent antibody responses. Consequently, immune memory to T cell–independent antigens is generally weak. This is why it is difficult to create fully protective vaccines directed against polysaccharide components of microbes. Covalent attachment of the polysaccharide component to a carrier protein to recruit T-cell help as part of the response can induce a beneficial memory response. The value of coupling a polysaccharide antigen to a carrier protein was observed in the *Haemophilus influenzae* type B vaccine. The original polysaccharide vaccine provided low antibody titers and no protection for children less than 18 months of age. The current conjugate vaccine generally provides higher antibody titers and protection beginning at 2 to 4 months of age.

LYMPHOID TISSUES

Cellular interactions are essential for a normally regulated, protective immune response. In particular, T-cell help is needed to generate high-affinity antibody with memory against most protein antigens. A major challenge for the immune system of a naive subject is to bring rare antigen-specific B cells together with rare antigen-specific T cells and antigen-charged APCs. The primary role of the secondary lymphoid tissues is to facilitate these interactions. Generally, the secondary lymphoid organs contain zones enriched for B cells (follicles) and other zones enriched for T cells.[74] The B-cell zones contain clusters of follicular dendritic cells that bind antigen-antibody complexes and provide sites adapted to efficient B-cell maturation, somatic mutation, and selection of high-affinity B cells. The T-cell zones contain large numbers of dendritic cells that are potent APCs for T-cell activation. The tissues also contain specialized vascular structures for recruitment of cells into the tissue. High endothelial venules in lymph nodes, Peyer patches, and mucosa-associated lymphoid tissues are vascular sites that efficiently extract naive T and B cells from the circulation into the lymphoid organ. The marginal sinus probably serves a similar function in the spleen. Afferent lymphatic vessels provide efficient entry of antigen-charged antigen-transporting cells (eg, epidermal Langerhans cells) from peripheral tissues into lymph nodes. Efferent lymphatic vessels permit efficient export of antigen-experienced cells back into the circulation. Programmed release of distinct chemokines within the lymphoid tissues orchestrate the coming together of antigen-responsive B and T cells and then migration of the activated B cells and selected T cells to the follicular dendritic cell clusters, where they can form a germinal center.[75] In addition to chemokine signals that control leukocyte entry into and migration within secondary lymphoid tissues, it is now understood that specific signals, especially those provided by the lysophospholipid sphingosine-1-phosphate, regulate the egress of cells out of the lymphoid tissues and into the circulation.[76]

Although potent adjuvants can induce some degree of affinity maturation in the setting of congenital absence of lymph nodes and Peyer patches, these secondary lymphoid organs are generally essential for the induction of an efficient, protective immune response. Ectopic lymph node–like structures designated tertiary lymphoid tissues can form at sites of chronic inflammation, such as the synovial membrane of a joint affected by rheumatoid arthritis. Immune reactions ongoing in these tertiary lymphoid tissues can contribute importantly to the pathogenesis of the inflammatory disease.

SIGNALING BY CYTOKINES

Cytokines act on cells through transmembrane cell-surface receptors. Binding of the cytokine to the receptor elicits its cellular response by activating an intracellular signal transduction pathway, which ultimately leads to induction of new gene transcription and synthesis of new cellular proteins. Most cytokine receptors signal by using one of the Janus kinase (Jak) family of molecules that then acts on the signal transducer and activator of transcription (STAT) family of proteins. Specific Jak proteins associate with the cytoplasmic domain of the cytokine receptor. When the receptor is activated by binding the cytokine, the Jak phosphorylates its respective STAT protein, causing the STAT to dimerize and translocate into the nucleus, where it then initiates new gene transcription. The essential role of Jak and STAT proteins in immune regulation is seen in subjects with inherited deficiency of these molecules (see chapter 12 of this Primer).[77] Jak3 interacts with the γc protein, a subunit of several cytokine receptors, including the receptors for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21. Deficiency of the autosomally encoded Jak3 protein causes autosomal recessive severe combined immunodeficiency.[78] Deficiency of the X chromosome–encoded γc protein causes X-linked severe combined immunodeficiency.[79] Mutation of STAT1 causes susceptibility to infection with mycobacteria and a variable increase in susceptibility to viral infections because of impaired ability to respond to signals from either type I or type II interferons.[80] Homozygous deficiency of STAT3 in mice is embryonic lethal, but heterozygous deficiency of STAT3 in human subjects causes autosomal dominant hyper-IgE syndrome associated with deficiency of T_H17 cell differentiation.[81] Deficiency of STAT4 blocks IL-12 signal transduction, resulting in impaired development of T_H1 cells. And STAT6-deficient mice showed impaired signaling through the IL-4 receptor and inability to generate T_H2 cell–dependent responses.[82]

EFFECTORS OF INNATE IMMUNITY

Although the adaptive T- and B-cell immune responses provide important protection for the host and permit the development of immune memory, mutations in elements of the innate immune response demonstrate that innate immune effectors are critical for effective host defense. Initially, the innate and adaptive immune responses were thought to act independently, with the innate response providing the first line of defense against invading microbes and the adaptive response being activated later to sterilize the infection. It is now apparent that the adaptive response has co-opted many of the innate effector mechanisms to enhance its effectiveness. Additionally, the adaptive immune system requires innate signals for its activation. By using innate signals to help initiate its responses, the adaptive immune system takes advantage of the innate system's ability to discriminate between contact with dangerous pathogens and innocuous or even beneficial microbes and environmental factors. This ability of the innate immune system to sense danger is essential for well-regulated immune responses. Thus the innate and adaptive arms of the immune response should be viewed as complementary and cooperating.

Toll-like receptors

Toll was first identified in *Drosophila* species, where it was found to control the polarity of the developing embryo and later was recognized to participate in the fly's antifungal immunity. Cloning of the *Drosophila* species' *Toll* showed that it encoded a transmembrane receptor whose extracellular domain contained leucine-rich repeating units, whereas its cytoplasmic domain had homology to the cytoplasmic domain of the IL-1 receptor of mammals (designated the Toll/IL-1 receptor domain [TIR]). This suggested that there might be *Toll* homologues in mammals. Indeed, 10 human Toll-like receptors (TLRs) have now been defined. The TLRs appear largely to recognize pathogen-associated molecular patterns.[83] These include LPS from gram-negative bacteria, peptidoglycan, lipoteichoic acid, lipoarabinomannan, bacterial flagellar proteins, viral double-stranded RNA, and unmethylated DNA with CpG motifs characteristic of microbial DNA. TLRs are particularly found on macrophages and dendritic cells but also are expressed on neutrophils, eosinophils, epithelial cells, and keratinocytes. Although activation of some TLRs can activate or potentiate an allergic T_H2-type response, activation of most TLRs elicits mediators that program CD4 T cells toward a nonatopic T_H1 response. TLR9, activated by interaction with CpG DNA, provides the molecular basis for efforts to divert T_H2-driven atopic responses to nonatopic T_H1-dominated responses.[84] Downstream signal transduction through most TLRs is dependent on myeloid differentiation primary response gene 88 (MyD88), a cytoplasmic adapter protein. MyD88 also mediates signaling through the IL-1 receptor. MyD88 deficiency leads to life-threatening, recurrent pyogenic infections.[85]

Nucleotide-binding domain leucine-rich repeat proteins and the inflammasome

All of the TLR proteins are transmembrane molecules, some of which are expressed on the plasma membrane of the cell where they can interact with extracellular triggering molecules, and some of which are expressed on intracellular membranes where they can interact with structures on intracellular microbes and viruses. Another set of pattern-recognition molecules, designated nucleotide-binding domain leucine-rich repeat (NLR) proteins, has also been identified. These molecules are cytosolic and appear to interact with soluble intracellular ligands. Like the TLRs, the NLR proteins are characterized by the presence of leucine-rich repeat structures that are thought to contribute to their ability to bind to conserved microbial structures. The NLR proteins can also recognize endogenous signals of cellular damage, such as uric acid crystals. More than 20 NLR-encoding genes have been identified in the human genome. Most are characterized by the presence of a C-terminal leucine-rich repeat domain that is thought to interact with microbial structures, a central nucleotide-binding oligomerization domain that is used to form multimeric

complexes of the NLR, and an N-terminal effector domain that allows the NLR to recruit a class of intracellular cysteine proteinases (caspases) that activate the cellular apoptosis pathways or that activate the nuclear factor κB transcription factor to induce a broad proinflammatory response.[86] One of the NLR proteins, designated Nacht domain-, leucine-rich repeat-, and PYD-containing Protein 3 (NALP3), has a special function in the innate immune response. Activation of NALP3 leads to its association with the intracellular adapter protein that is designated apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), which combines with and activates caspase-1, leading to an active enzyme complex termed the inflammasome. The inflammasome functions to activate the potent proinflammatory molecules IL-1, IL-18, and IL-33.[87]Recent studies have shown that alum, the most common adjuvant in vaccines administered to human subjects, is taken up by phagocytic cells, where it activates NALP3, activating the inflammasome. This is crucial for its adjuvant activity. If any one of NALP3, ASC, or caspase-1 is absent or defective, then alum can no longer serve to augment the antibody response.[88]

Dectin-1, collectins, pentraxins, and ficolins

Additional pattern-recognition proteins that contribute to the innate response to microbes include dectin-1, the collectins, certain of the pentraxins, and the ficolins.

Dectin-1 is a transmembrane receptor that is activated when it binds β-glucans that are major components of the cell walls of yeast.[89]

The 3 major collectins in human subjects, mannan-binding lectin (MBL) and surfactant proteins A and D, are all expressed at substantial levels in the human airway and recognize microbial carbohydrates through their carbohydrate recognition domains. Activation of the collectins opsonizes the microbe for phagocytosis and activates the expression of proinflammatory cytokines and the production of antimicrobial reactive oxygen free radicals.[90]

The pentraxins are a group of homopentameric proteins that also recognize microbial molecular patterns. The best known are the short pentraxins, C-reactive protein and serum amyloid P-component. C-reactive protein binds to bacterial low-density lipoproteins, a variety of bacterial polysaccharides, apoptotic host cells, and nuclear material and induces activation of the complement system (see below) and phagocytosis. Serum amyloid P-component recognizes microbial carbohydrates, nuclear substances, and amyloid fibrils and thus contributes to the host response to clear infections, autoimmunity, and amyloidosis.[91]

The ficolins contain carbohydrate recognition domains that share structure with fibrinogen.[2] After binding to carbohydrates on a microbe, they activate complement through the lectin pathway (see below) and thus contribute importantly to clearance of the microbe.

Chitinases

Chitin is a biopolymer of N-acetyl-β-D-glucosamine, which is the major constituent of the cell walls of fungi and the exoskeletons of helminths, insects, and crustaceans. It is thought to be the second most abundant glycopolymer in the world. Chitinase designates a group of enzymes that digest chitin, both for the purpose of cellular and tissue remodeling during homeostasis in these organisms and for digestion of these organisms by the mammalian innate immune response. Because infestation with helminths and some of the chitin-expressing insects leads to the induction of high levels of IgE antibodies and eosinophil-predominant inflammation, scientists have investigated chitinase in human allergic disease. These studies have shown that chitin is a potent inducer of the production of T_H2 cytokines and leads to the accumulation of eosinophils and basophils in chitin-challenged tissues. Although mammals do not synthesize chitin, they do express both enzymatically active chitinases and enzymatically inactive chitinase-like proteins (CLPs).[92] The acidic mammalian chitinase (AMC) rapidly degrades chitin, contributing importantly to host defense against chitin-expressing organisms, dramatically reducing the allergic inflammatory response these organisms induce.[93] AMC, which is expressed in epithelial cells, as well as tissue leukocytes, and the related chitin-digesting enzyme chitotriosidase are anti-inflammatory in settings of chitin challenge and thus form part of the innate host defense mechanisms. The biologic functions of the enzymatically inactive CLPs are not known; however, the fact that many of them, including the human YKL-40 protein, avidly bind and sequester chitin and its degradation products suggests that they might have immunoregulatory functions.[93] Levels of AMC and CLP are both dramatically increased in the lungs of asthmatic subjects, suggesting that these molecules might contribute to the immunopathology of these disorders and might be appropriate targets for new drug therapy of this important clinical disorder.[94,95]

Phagocytic cells

The major phagocytic cells are neutrophils, macrophages, and monocytes. These cells engulf pathogenic microbes and localize them in intracellular vacuoles, where they are exposed to toxic effector molecules, such as nitric oxide, superoxide, and degradative enzymes in an effort to destroy the organism. Phagocytic cells use a variety of Fc and complement receptors to enhance uptake of particles that have been marked by the adaptive and innate immune systems for destruction.

Natural killer cells

Natural killer (NK) cells are thought to represent a third lineage of lymphoid cells. When activated, they have the morphology of a large granular lymphocyte. They develop in the bone marrow under the influence of IL-2, IL-15, and bone marrow stromal cells. They represent only a small fraction of peripheral blood cells and a small fraction of lymphoid cells in the spleen and other secondary lymphoid tissues. NK cells have no antigen-specific receptors. Their cytotoxic activity is inhibited by encounter with self-MHC molecules through inhibitory receptors on their surface that recognize class I HLA molecules. They thus kill self cells that have downregulated class I molecule expression. This is important in host defense because several viruses have developed mechanisms to downregulate class I expression in infected cells as a strategy to avoid $CD8^+$ cell killing. NK cells, however, also possess activating receptors. The nature of the ligands for these receptors and the mechanisms by which they contribute to identifying proper targets for NK cell cytotoxicity are currently under investigation. NK cells can destroy target cells through antibody-dependent cell-

mediated cytotoxicity. They have prominent antitumor effects and are potent killers of virally infected cells.[96]

Complement

The complement system is a very important effector component of both adaptive and innate immunity. The complement system is composed of more than 25 plasma and cell-surface proteins that include 3 activation pathways (Fig 9) and soluble and membrane-bound downmodulating regulatory pathways.[97,98] Many of the proteins of the activation pathway are proteinases, and activation occurs in a cascade by means of proteolytic activation of one zymogen that then activates the next zymogen in the pathway. The main goal of the activation pathway is to mark targets permanently for destruction, to recruit other proteins and cells that facilitate target destruction, and, in the case of some bacteria and viruses, to participate directly in the destructive process through osmotic lysis of the pathogen. Antigen-antibody complexes provide the activating signal for the classical pathway of complement activation. Sequential activation of complement components C1, C4, and C2 produces the key enzyme in the pathway, the C3 convertase, which acts to cleave and activate C3. The cleavage results in release of the small C3a fragment, a potent anaphylatoxin that induces mast cell degranulation, creates edema and recruits phagocytic cells, and the larger C3b fragment, which covalently attaches to the activating antigen, marking it for destruction. C3b serves both as a site for activation of C5 that becomes a site for attack of the complement membrane attack complex (MAC), a self-assembling pore-forming complex of serum proteins that kills targets by osmotic lysis. C3b also acts as an opsonin, enhancing phagocytosis through its binding to complement receptors on the surfaces of neutrophils and macrophages.[99]

The second activation pathway, the alternative pathway of complement activation, is activated without antibody by microbial structures that neutralize inhibitors of spontaneous complement activation. This activation pathway can deposit more than 10^5 molecules of C3b on a single bacterium in less than 5 minutes. C3b deposited in this way then triggers the MAC and also enhances phagocytosis and killing.[100]

The third activation pathway is triggered by microbial cell-wall components containing mannans and is called the lectin pathway of complement activation.[101] The interaction of mannan-containing microbes with plasma MBL activates the zymogenic plasma proteases MBL-associated serine protease 1 and 2. These form a protease analogous to the activated C1 of the classical pathway that then goes on to activate C4, C2, and the remainder of the pathway. The lectin pathway can also be activated by complexes of microbes and host pentraxins and ficolins. Together, these 3 activation pathways permit complement to participate in the destruction and clearance of a wide variety of pathogens and macromolecules.

The effector mechanism of complement is potent and recruits intense local inflammation. There are several plasma proteins (factor H and C4 binding protein) and membrane proteins (complement receptors 1-4, decay-accelerating factor, and membrane cofactor protein) that inhibit the complement activation pathways to prevent unwanted damage to host tissues.[101]

The importance of the activation and regulatory pathways of complement are underscored by the dramatic phenotype of inherited deficiencies of individual components.[97,101] Deficiencies of components of the MAC lead to increased susceptibility to infection with *Neisseria* species. Deficiency of C3 results in life-threatening susceptibility to pyogenic infections, which are often fatal during childhood. Deficiency of C4 or C2 causes a lupus-like immune complex disease, indicating that one of the roles of the classical pathway is to participate in the host response to and clearance of immune complexes. Deficiency of the serum inhibitor of C1 (an inhibitor of spontaneous activation of C1 and also of several components of the fibrinolytic pathway) leads to episodic mast cell–independent episodes of angioedema. Clonal hematopoietic lineage deficiency of the regulatory protein decay-accelerating factor (expressed on erythrocytes, leukocytes, and endothelial cells) causes paroxysmal nocturnal hemoglobinuria.[102]

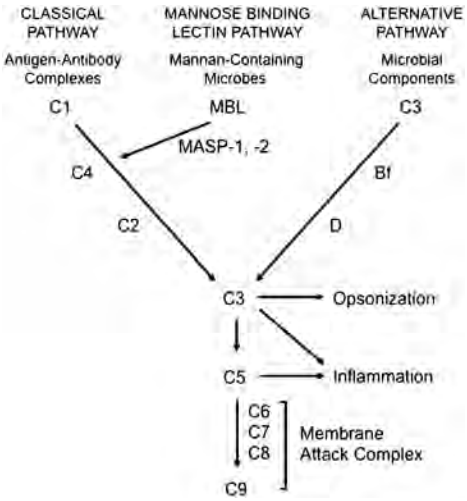

FIG 9. The activation pathways of complement. Three pathways lead to activation of complement. The classical pathway is initiated by complexes of IgM, IgG1, or IgG3 with antigens. This activates proteolysis of C1 that cleaves C4 and C2 to form the classical pathway C3 convertase. The mannose lectin pathway is activated by interaction of mannan-containing microbes with MBL, which activates MBL–associated serine protease *(MASP)* 1 and 2 to cleave C4 and C2, again forming a C3 convertase. The alternative pathway is initiated by interactions between microbial antigens and inhibitory complement regulatory proteins. This permits autoactivation of the pathway in which C3 interacts with factor B and factor D to generate the alternative pathway C3 convertase. These convertases all cleave C3 to generate the anaphylatoxic C3a fragment and depositing C3b on the activating microbial particle or immune complex. This opsonizes the particle for phagocytosis and initiates the activation of the MAC. The C5a fragment that is proteolytically released from C5 also is a highly anaphylatoxic molecule that induces intense local inflammation.

LEUKOCYTE ADHESION AND TISSUE INFLAMMATION

Recruitment of leukocytes both to secondary lymphoid tissues and to peripheral tissue sites of microbial invasion is essential for intact host defense. Cellular adhesion molecules and chemotactic proteins both contribute importantly to this process.[103] There are 3 main families of cell adhesion proteins: selectins, integrins, and immunoglobulin domain cell adhesion molecules. In addition to mediating recruitment to tissues, these molecules contribute to cell-cell interactions between leukocyte subsets and can contribute to intercellular and intracellular signaling.[104]

There are 3 selectin glycoproteins, designated L-selectin, E-selectin, and P-selectin. Selectins are present on the surfaces of all

leukocytes and on endothelial cells. Leukocytes also express ligands for selectins. The interactions between selectin ligands on leukocytes and selectins on vascular endothelial are low affinity and lead to rolling of cells along the vessel wall.[103]

Rolling cells can then be induced to arrest and adhere firmly to the endothelium through interactions between integrins on the leukocyte surface and immunoglobulin domain cell adhesion molecules on the endothelial cells. Integrins are heterodimers of one α and one β chain. Key integrins for leukocyte adhesion are lymphocyte function–associated antigen 1 (CD11a/CD18, $\alpha_L\beta_2$), very late antigen 4 (CD49d/CD29, $\alpha_4\beta_1$), and Mac-1 (CD11b/CD18, $\alpha_M\beta_2$), which bind to the immunoglobulin domain cell adhesion molecules intercellular adhesion molecule 1, vascular cell adhesion molecule 1, and intercellular adhesion molecule 1/C3b, respectively. Binding of leukocytes to endothelial cells is enhanced by the expression of chemokines by the endothelial cells or by underlying damaged cells and tissues (see chapter 5 of this Primer).[105]

CELLULAR HOMEOSTASIS

After an immune response is completed, the majority of antigen-responsive cells must be removed to prepare for the next immune challenge faced by the organism. Removal of effector cells without causing inflammation and tissue damage is best achieved by inducing the unwanted cells to undergo apoptosis. Molecules of the TNF family provide strong signals for the apoptotic programmed cell death pathway. TNF, signaling through the type I TNF receptor, induces death in tumor cells and at sites of ongoing inflammation. An alternative apoptosis-inducing receptor, Fas, is more specifically involved in regulatory apoptotic events. Fas, for example, transmits important apoptotic signals during thymic T-cell selection.[55,106] It also contributes to the regulation of autoreactive cells in the periphery.[107] Defects in Fas or in its ligand, FasL, result in autoimmune disorders with prominent lymphoproliferation.[108] Thus deregulated Fas or its ligand might contribute importantly to autoimmune diseases.

TOLERANCE, IMMUNOPATHOLOGY, AND ATOPY

The goal of a properly regulated immune response is to protect the host from pathogens and other environmental challenges without causing unnecessary damage to self-tissues. In the case of infection with viruses or intracellular bacteria and parasites, it is often impossible to eradicate the pathogen without destroying the infected cells. In cases like this, the use of cellular apoptosis as a mechanism for removing infected cells provides an elegant way to reduce damage to nearby uninfected cells. Infected cells that undergo apoptosis are generally fragmented into membrane-enclosed vesicles that can be taken up by healthy phagocytic cells and digested so as to eliminate both the potentially inflammatory contents of the infected cell and also the microbe that was multiplying inside the cell.

Some degree of local inflammation is, however, often an important part of an effective host immune response. The key elements of inflammation are part and parcel of the host's mobilization of its defense and repair responses. When inflammation is modest and controlled, normal tissue architecture and function can be restored after the pathogen or toxin has been eliminated. If the inflammatory response is excessively severe, however, there is danger of lasting tissue damage and the development of fibrosis during the resolution of the inflammatory state.[109] Mild fibrosis is physiologic and generally does not interfere with normal tissue function; however, when inflammation is either very severe or becomes chronic, the resulting fibrosis can lead to profound organ dysfunction. There has been important progress over the last several years in understanding the mechanisms that control the transition from physiologically appropriate inflammation and tissue repair to damaging fibrosis. A common theme underlying the fibrotic process is the local production of activated fibroblasts through the action of selected cytokines and other mediators on tissue epithelial cells. Through the process of epithelial to mesenchymal transition, epithelial cells are thought to be converted to activated fibroblasts and myofibroblasts that are then responsible for the tissue changes that lead to fibrosis.[110] Development of therapeutics that target the mediators of tissue fibrosis might prevent many of the long-term complications of chronic inflammation.

Perhaps more puzzling are conditions in which tissue inflammation appears to develop without any underlying infectious or noxious stimulus. Prominent in these are autoimmune diseases and atopic illnesses. These disorders appear to represent a fundamental misdirection of the immune response, resulting in tissue damage when no real danger was present. The growing spectrum of autoimmune diseases appears to represent a breakdown in self-tolerance. This leads to the induction of both cellular and humoral immune responses against components of self-tissues. Usually, both the cellular and humoral aspects of these pathologic responses have features of a T_H1-type or T_H17-type CD4 T-cell response, suggesting that defective regulation of either T-cell differentiation or activation underlies the response.[111] Atopic diseases rarely manifest autoimmune character (although some forms of chronic urticaria are thought to have an autoimmune cause; see chapters 12 and 17 of this Primer).[77,112] Rather, they appear to represent an overly aggressive T_H2-type response, leading to hypersensitivity to a broad spectrum of normally encountered environmental antigens. Epidemiologic studies have demonstrated that there is an inherited component to both the autoimmune and atopic diseases.[113] There also appears to be a strong interplay with environmental factors, perhaps including unrecognized infectious microbes or toxic agents in the environment. The central role of Treg cells in controlling all aspects of the CD4 T-cell response and the observation that congenital absence of Treg cells (as in the immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome) leads to development of an aggressive autoimmune state[114] suggest that disturbed Treg cell function might underlie all autoimmune and atopic diseases. Although disordered T_H1, T_H17, and T_H2 responsiveness is a major manifestation of these illnesses, the disorders do not simply represent a predisposition to overpolarization of the $CD4^+$ T-cell response. Epidemiologic studies have shown that the presence of atopy shows little protection against development of the T_H1/T_H17-predominant illness rheumatoid arthritis.[115] In fact, other studies have suggested that patients with an autoimmune illness are more likely to have an atopic disorder, suggesting that they have a common underlying cause.[116] Development of a thorough understanding of the mechanisms underlying these 2 types of T cell–mediated inflammation will lead to important new therapeutic options for successful treatment of these common diseases.[117]

A special situation in which tolerance is modulated in a physiologic way concerns the suppression of the maternal

immune response to permit the maintenance of the semiallogeneic fetus and placenta in the setting of normal pregnancy. Recent studies have demonstrated that in midgestation human fetuses 20% to 25% of the CD4 T cells in the lymph nodes and spleen had a Treg cell phenotype, and levels of TGF-β were remarkably high in these lymphoid organs.[118] Additionally, lymphocytes in these secondary lymphoid tissues were poorly activated when exposed to an allogeneic stimulus.[119] These high numbers of Treg cells returned to normal shortly after delivery. Interestingly, spontaneous abortion has been associated with loss of normal pregnancy-associated immune suppression.[118] Alterations in Treg cell function do not constitute the entire mechanism underlying the tolerance of pregnancy. Other studies have shown very high levels of expression of galectin-1, an immunoregulatory glycan-binding protein, in fetal tissues and loss of galectin-1 in failing pregnancies.[120] Additionally, levels of thymic stromal lymphopoietin are increased in pregnancy, and this induces placental dendritic cells to drive the differentiation of T_H cells that produce abundant levels of IL-10, an immunomodulating cytokine well adapted to help maintain the fetal allograft.[121] Understanding the mechanisms that control tolerance to the fetal allograft might provide new insights into the regulatory systems that have failed in autoimmunity and atopy.

CONCLUSION

The immune system uses many mechanisms to combat infection by microbes and to avoid coincidental damage to self-tissues. These mechanisms work together, and the fully integrated immune response draws elements from many effector systems to tailor a response to the specific invading pathogen or toxic agent. Abnormal regulation of the various effector mechanisms can lead to chronic or acute tissue damage. Understanding the relationships between the different immune effector pathways will permit improved immunomodulatory therapeutics, development of improved vaccines, and avoidance of unintended tissue injury.

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Innate immunity

Stuart E. Turvey, MB, BS, DPhil,[a] and David H. Broide, MB, ChB[b] *Vancouver, British Columbia, Canada, and La Jolla, Calif*

Recent years have witnessed an explosion of interest in the innate immune system. Questions about how the innate immune system senses infection and empowers a protective immune response are being answered at the molecular level. These basic science discoveries are being translated into a more complete understanding of the central role innate immunity plays in the pathogenesis of many human infectious and inflammatory diseases. It is particularly exciting that we are already seeing a return on these scientific investments with the emergence of novel therapies to harness the power of the innate immune system. In this review we explore the defining characteristics of the innate immune system, and through more detailed examples, we highlight recent breakthroughs that have advanced our understanding of the role of innate immunity in human health and disease. (J Allergy Clin Immunol 2010;125:S24-32.)

Key words: *Host defense, innate immunity, Toll-like receptors, nucleotide oligomerization domain–like receptors*

Abbreviations used

DAMP:	Damage-associated molecular pattern
IPAF:	IL-1β-converting enzyme (ICE) protease-activating factor
IRAK4:	IL-1 receptor–associated kinase 4
MAL:	MyD88 adapter-like
MPL:	Monophosphoryl lipid A
MyD88:	Myeloid differentiation primary response gene 88
NK:	Natural killer
NLR:	Nucleotide oligomerization domain–like receptor
NLRP3:	NLR family, pyrin domain-containing 3
NOD:	Nucleotide oligomerization domain
SNP:	Single nucleotide polymorphism
TIR:	Toll/IL-1 receptor–like domain
TIRAP:	Toll/IL-1 receptor–like domain–containing adaptor protein
TLR:	Toll-like receptor

THE "NEW" SCIENCE OF INNATE IMMUNITY

The integrated human immune response has traditionally been divided into 2 branches: innate and adaptive (or acquired) immunity. Although appreciation of innate immunity dates back to at least the 1908 Nobel Prize–winning efforts of Ilya Mechnikov, until the last decade, study of innate immunity has been eclipsed by dramatic discoveries in the field of adaptive immunity. However, the recent molecular definition of how the innate immune system senses infection to empower protective immune responses has precipitated a renaissance in the field of innate immunity. Innate immunity has shed its older, disparaging title of "nonspecific immunity" and now stands as a proud partner with the adaptive immune system in protecting human hosts from infectious insults. For any who doubt the impressive protective capacity of the innate immune system, it is instructive to consider that only vertebrates boast the added benefits of an adaptive immune system, leaving most organisms on our planet to survive on innate immunity alone!

Although innate immunity is critical for host defense against infectious challenges, the innate immune system is emerging as a critical regulator of human inflammatory disease. Indeed, innate immune responses have been implicated in the development of asthma and atopy, as well as a variety of autoimmune disorders, including type 1 diabetes, inflammatory bowel disease, and systemic lupus erythematosus.

In this review we examine the basic structure of the innate immune system and how innate immunity interfaces with adaptive immune responses. We explore the role of innate immunity in human health and disease, and we outline how novel therapies can harness the beneficial capacity of the innate immune system. Rather than attempting to comprehensively review this enormously broad topic, our focus is on highlighting common defining mechanisms of innate immunity and illustrating the clinical relevance of innate immunity to human health. We have deliberately avoided a detailed exploration of the complement system because a separate Primer chapter is devoted to this important aspect of innate immunity.[1]

ORGANIZATION OF THE HUMAN IMMUNE SYSTEM: THREE LEVELS OF HOST DEFENSE

The human microbial defense system can be simplistically viewed as consisting of 3 levels: (1) anatomic and physiologic barriers; (2) innate immunity; and (3) adaptive immunity (Fig 1 and Table I). Failure in any of these systems will greatly increase susceptibility to infection.

Anatomic and physiologic barriers provide the crucial first line of defense against pathogens. These barriers include intact skin, vigorous mucociliary clearance mechanisms, low stomach pH, and bacteriolytic lysozyme in tears, saliva, and other secretions. The extreme susceptibility to infections observed in subjects with severe cutaneous burns or primary ciliary dyskinesia demonstrates that intact innate and adaptive immune systems are not able to compensate for failure of essential anatomic and physiologic barriers.

From [a]the Department of Paediatrics, BC Children's Hospital and Child & Family Research Institute, University of British Columbia; and [b]the Department of Medicine, University of California San Diego, La Jolla.

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Reprint requests: Stuart Turvey, MB, BS, DPhil, FRCPC, Division of Infectious and Immunological Diseases, BC Children's Hospital and Child & Family Research Institute, University of British Columbia, 950 West 28 Ave, Vancouver BC V5Z 4H4, Canada. E-mail: sturvey@cw.bc.ca.

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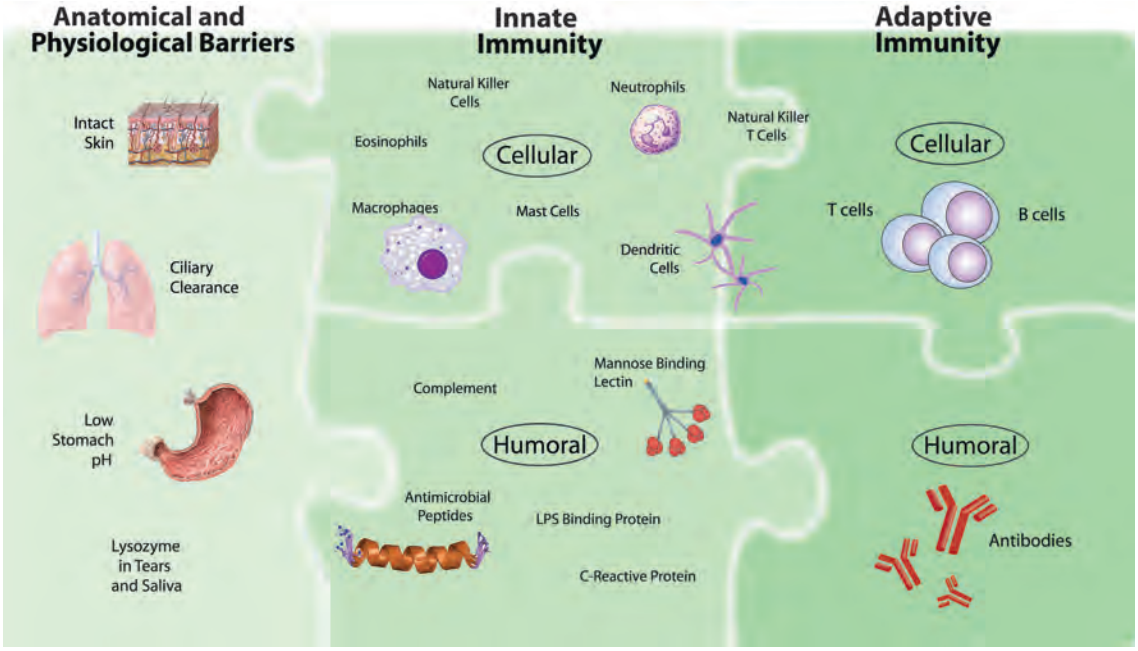

FIG 1. Integrated human immune system. The human microbial defense system can be simplistically viewed as consisting of 3 levels: (1) anatomic and physiologic barriers; (2) innate immunity; and (3) adaptive immunity. In common with many classification systems, some elements are difficult to categorize. For example, NK T cells and dendritic cells could be classified as being on the cusp of innate and adaptive immunity rather than being firmly in one camp.

Innate immunity augments the protection offered by anatomic and physiologic barriers.[2] The innate immune system relies on a limited repertoire of receptors to detect invading pathogens but compensates for this limited number of invariant receptors by targeting conserved microbial components that are shared by large groups of pathogens. Speed is a defining characteristic of the innate immune system: within minutes of pathogen exposure, the innate immune system starts generating a protective inflammatory response. Moreover, innate immunity plays a central role in activating the subsequent adaptive immune response.

T and B lymphocytes are the main self-defensive weapons of the adaptive immune system, so-named because this system is shaped by antigen exposure. In contrast to the limited number of pathogen receptors used by the innate immune system, the adaptive immune system boasts an extremely diverse, randomly generated repertoire of receptors. The benefit of this receptor diversity is that the adaptive immune system can recognize virtually any antigen, but there is a price for this diversity.

First is the risk of autoimmune disease. Receptors specific for self-proteins (eg, insulin and myelin) are created by means of the random process of gene rearrangement that generates receptors expressed by T and B cells. Consequently, elaborate tolerance mechanisms have evolved to eliminate or regulate self-reactive cells.

Second is the time delay required to generate a protective adaptive immune response after the first exposure to a pathogen. Adaptive immunity relies on a clonal system, with each T and B cell expressing its own unique receptor, and after the initial encounter with a pathogen, it takes up to 5 days for clonal expansion of these rare antigen-specific T and B cells to occur before the adaptive immune response is sufficiently robust to clear the pathogen.

ELEMENTS OF THE INNATE IMMUNE SYSTEM

In contrast to the adaptive immune system, which depends on T and B lymphocytes, innate immune protection is a task performed by cells of both hematopoietic and nonhematopoietic origin (Fig 1 and Table I). Hematopoietic cells involved in innate immune responses include macrophages, dendritic cells, mast cells, neutrophils, eosinophils, natural killer (NK) cells, and NK T cells. In addition to hematopoietic cells, innate immune responsiveness is a property of the skin and the epithelial cells lining the respiratory, gastrointestinal, and genitourinary tracts.

To augment these cellular defenses, innate immunity also has a humoral component that includes well-characterized components, such as complement proteins, LPS binding protein, C-reactive protein and other pentraxins, collectins, and antimicrobial peptides, including defensins. Circulating innate immune proteins are involved in both sensing of microbes and effector mechanisms to facilitate clearance of the infection. For example, mannose-binding lectin, a member of the collectin family of receptors, binds mannose-containing carbohydrates on microbes, triggering activation of the complement cascade, which enhances clearance of the pathogen.

HOST DEFENSE IS ACHIEVED THROUGH INTEGRATION OF INNATE AND ADAPTIVE IMMUNITY

Innate immunity, an evolutionarily ancient component of host defense, is present in all multicellular organisms, whereas adaptive immunity evolved much later and is only found in jawed fish and all "higher" vertebrates.[3] During evolution, adaptive immunity developed in the context of a functioning innate immune

TABLE I. Overview of defining features of innate and adaptive immunity: Comparing and contrasting some of the defining features of the innate and adaptive immune systems

	Innate immune system	Adaptive immune system
Cellular elements	Hematopoietic cells: macrophages, dendritic cells, mast cells, neutrophils, eosinophils, NK cells, and NK T cells Nonhematopoietic cells: epithelial cells (eg, skin, airways, and gastrointestinal tract)	Hematopoietic cells: T and B lymphocytes
Humoral elements	Large arsenal of components: complement proteins, LPS binding protein, C-reactive protein and other acute-phase reactants, antimicrobial peptides, and mannose-binding lectin	Immunoglobulins secreted by B cells
Receptor characteristics	Invariant, germline encoded All cells of a class express identical receptors (ie, nonclonal).	Generated by random somatic gene segment rearrangement All cells of a class express a single type of receptor with unique specificity (ie, clonal).
Ligands recognized	Conserved microbial components Common metabolic or biologic consequences of infection (eg, uric acid, K^+ efflux, and MHC class I downregulation)	Specific details or epitopes of macromolecules (eg, proteins, peptides, and carbohydrates)
Types of receptors	Activating: TLR, NLR, and complement Inhibitory: killer cell immunoglobulin-like receptors	B-cell receptor and T-cell receptor
Response time	Immediate	Delayed by hours to days
Immunologic memory	None: responses are the same with each exposure. Nonanticipatory immunity	Responsiveness enhanced by repeated antigen exposure. Anticipatory immunity
Risk of autoreactivity	Low: self-tolerant receptors are selected during evolution.	High: random gene rearrangement generates autoreactive receptors requiring the presence of multiple tolerance mechanisms.

Adapted with permission from Janeway and Medzhitov.[1]

system. Consequently, the classic demarcation between innate and adaptive immunity is overly simplistic because many adaptive immune responses build on the foundation of innate immunity. For example, the capacity of neutrophils to kill bacteria is enhanced when the bacteria are opsonized by antibodies produced through the coordinated efforts of T and B cells. In a similar fashion, the C3d fragment that is generated in the course of complement activation acts as a molecular adjuvant to profoundly influence the subsequent adaptive immune response. Specifically, C3d fragments act to bridge innate and adaptive immunity because covalent binding of single or multiple copies of C3d to a foreign antigen generally enhances B-cell effector and memory function.[4] Another illustrative example of the interdependence of innate and adaptive immunity is the critical role played by antigen-presenting cells of the innate immune system (eg, dendritic cells) to empower full activation of the T and B cells of the adaptive immune system. Further blurring of the distinction between innate and adaptive immunity is highlighted by the fact that cells of the adaptive immune system, including regulatory T lymphocytes, express Toll-like receptors (TLRs) and other innate immune receptors.[5] The interrelatedness of innate and adaptive immunity is most eloquently articulated by Beutler in his observation that "…the roots of adaptive immunity are buried deep in the soil of the innate immune system."[6]

INNATE IMMUNE RECOGNITION STRATEGIES

The innate immune system serves as the initial immune defense against foreign and dangerous material. In the most simplistic view, the innate immune system is hardwired with germline-encoded receptors for immediate responsiveness. In contrast to adaptive immunity, innate immune responses do not require genetic recombination events or a developmental phase to mediate function.

The strategy used for immune recognition is the main feature distinguishing innate and adaptive immunity. In contrast to the massive, randomly generated repertoire of antigen receptors expressed by T and B lymphocytes, the innate immune system relies on a limited number of genetically predetermined germline-encoded receptors that recognize either highly conserved structures expressed by large groups of microbes or common biologic consequences of infection. Pathogens can rapidly evolve and, in principle, could avoid detection by the innate immune system by simply altering the targeted microbial molecules. However, the innate immune system has evolved to recognize either microbial components that are essential for the viability and virulence of microbes and are thus less prone to modifications or common biologic consequences of infection.

At least 3 broad strategies are used by the innate immune system to recognize invading microorganisms (Table II). In the first innate immunity relies on a limited repertoire of germline-encoded receptors to recognize "microbial nonself," conserved molecular structures that are expressed by a large variety of microbes. Charles Janeway coined the terms *pattern recognition receptors* to collectively describe these receptors and *pathogen-associated molecular patterns* (PAMPs) to denote the microbial structures recognized by the pattern recognition receptos.[7] However, this terminology has been criticized as being vague[6]; therefore in this review we will focus on naming specific receptors and their microbial ligands.

A second approach used by the innate immune system is to detect immunologic danger in the form of damage-associated molecular patterns (DAMPs). DAMPs represent common metabolic consequences of infection and inflammation.[8] DAMPs are

TABLE II. Common innate immune recognition strategies

Innate immune recognition strategy	Receptor families	Specific examples	
		Receptor	Ligand
1. Detecting "microbial nonself" (ie, pathogen-associated molecular patterns)	TLRs	TLR4 TLR5	LPS Flagellin (extracellular)
	NOD-like receptors	NOD2 IPAF	Muramyl dipeptide Flagellin (intracellular)
	Collectin family	MBP	Microbial terminal mannose residues
2. Detecting common metabolic consequences of cell infection or injury (ie, DAMPs)	NOD-like receptors	NLRP3 (or NALP3)	Uric acid, K^+ efflux, ATP
	RAGE family	RAGE	HMGB1, S100
3. Detecting "missing self"	MHC class I–specific inhibitory receptors	KIR CD94-NKG2A heterodimers	Self MHC class I (inhibitory signal) Self MHC class I (inhibitory signal)

RAGE, Receptor of advance glycation end product; *HMGB1*, high mobility group box 1.

molecules that are upregulated and released during the cell lysis and tissue damage that occurs in the context of both infectious and sterile inflammation. Well-characterized DAMPs include high mobility group box 1 protein and other endogenous alarmins, heat shock proteins, and uric acid.

In the third innate immune recognition strategy, innate immune receptors detect "missing self," molecules expressed by normal healthy cells but not expressed by infected cells or microbes. Recognition of these signals indicates that all is well, and an inhibitory signal is delivered to prevent activation of the immune response against host tissues. This inhibitory system is well illustrated by NK cells. Inhibitory receptors specific for self–MHC class I molecules play a central role in missing-self recognition by NK cells, ensuring NK cells preferentially attack infected cells that downregulate their MHC class I proteins.[9]

ROLE OF THE INNATE IMMUNE SYSTEM IN HEALTH AND DISEASE

We will now turn our attention to specific components of the innate immune system. We deliberately selected 2 illustrative examples, TLRs and nucleotide oligomerization domain (NOD)–like receptor (NLRs), for which our mechanistic understanding has increased considerably in the past 5 years and for which the clinical relevance of these systems is beginning to emerge.

TLRs

Overview of TLR structure and function. The recent explosion of interest in innate immunity was catalyzed in the mid-1990s when the *Drosophila* species protein Toll was shown to be critical for defending fruit flies against fungal infections.[10] This observation opened the way for the subsequent description of similar proteins, called TLRs, in mammalian cells. The human TLR family consists of 10 receptors that are critically important for innate immunity.[11,12] TLRs allow for recognition and response to diverse microbial epitopes on pathogens, enabling the innate immune system to discriminate among groups of pathogens and to induce an appropriate cascade of effector adaptive responses.

TLRs exist as dimeric proteins (either heterodimers or homodimers). The ectodomains of TLRs are composed of leucine-rich repeat motifs, whereas the cytosolic component, called a Toll/IL-1 receptor–like domain (TIR), is involved in signaling. Individual TLRs recognize a distinct but limited repertoire of conserved microbial products; for example, well-characterized receptor-ligand pairs include TLR4 and LPS, TLR5 and flagellin, and TLR1/TLR2/TLR6 and lipoproteins. Collectively, the complete TLR family allows the host to detect infection by most (if not all) types of microbial pathogens. For example, gram-positive organisms, such as *Streptococcus pneumoniae*, are initially recognized by TLR1, TLR2, TLR4, TLR6, and TLR9, which in turn interact with a range of downstream signaling molecules to activate an inflammatory cascade. TLR signaling pathways have been the focus of considerable attention (Fig 2).[12,13] The emerging model has ligation of microbial products by TLRs culminating in the activation of nuclear factor κB, activator protein 1, interferon regulatory factor 3, and other transcription factors, driving the production of proinflammatory cytokines, maturation of dendritic cells, and other immunologic responses.

Human disease resulting from TLR defects. Naturally occurring genetic mutations in human subjects causing extreme immunodeficiency phenotypes present powerful opportunities to determine the relationship between specific immunologic defects and human disease processes *in vivo*. Recent description of human primary immunodeficiencies associated with abnormal TLR signaling demonstrates that this pathway is critical for human defense against infection. Empowered by technologic advances in genotyping and bioinformatics, we are now beginning to appreciate how common genetic variation and polymorphisms in genes controlling the innate immune response alter infectious susceptibility in a subtle but specific fashion. Importantly, human primary immunodeficiencies associated with abnormal TLR signaling provide unique insights into the immunologic pathways vital for host defense and identify candidate genes that might cause subtle immunodeficiencies in the broader population of apparently healthy persons.[14]

Monogenic primary immunodeficiencies. IL-1 receptor–associated kinase 4 (IRAK4) deficiency (OMIM #607676)[15] and myeloid differentiation primary response gene 88 (MyD88) deficiency (OMIM #612260)[16] are novel primary immunodeficiencies specifically affecting TLR function. MyD88 and IRAK4 are binding partners involved in downstream signaling from most TLRs (Fig 2); hence the clinical and laboratory phenotypes of IRAK4 and MyD88 deficiencies are identical. The narrow spectrum of infections experienced by affected individuals is striking in light of their profound impairment of TLR function and pathogen sensing. IRAK4- and MyD88-deficient patients predominantly experience recurrent infections caused by pyogenic gram-positive

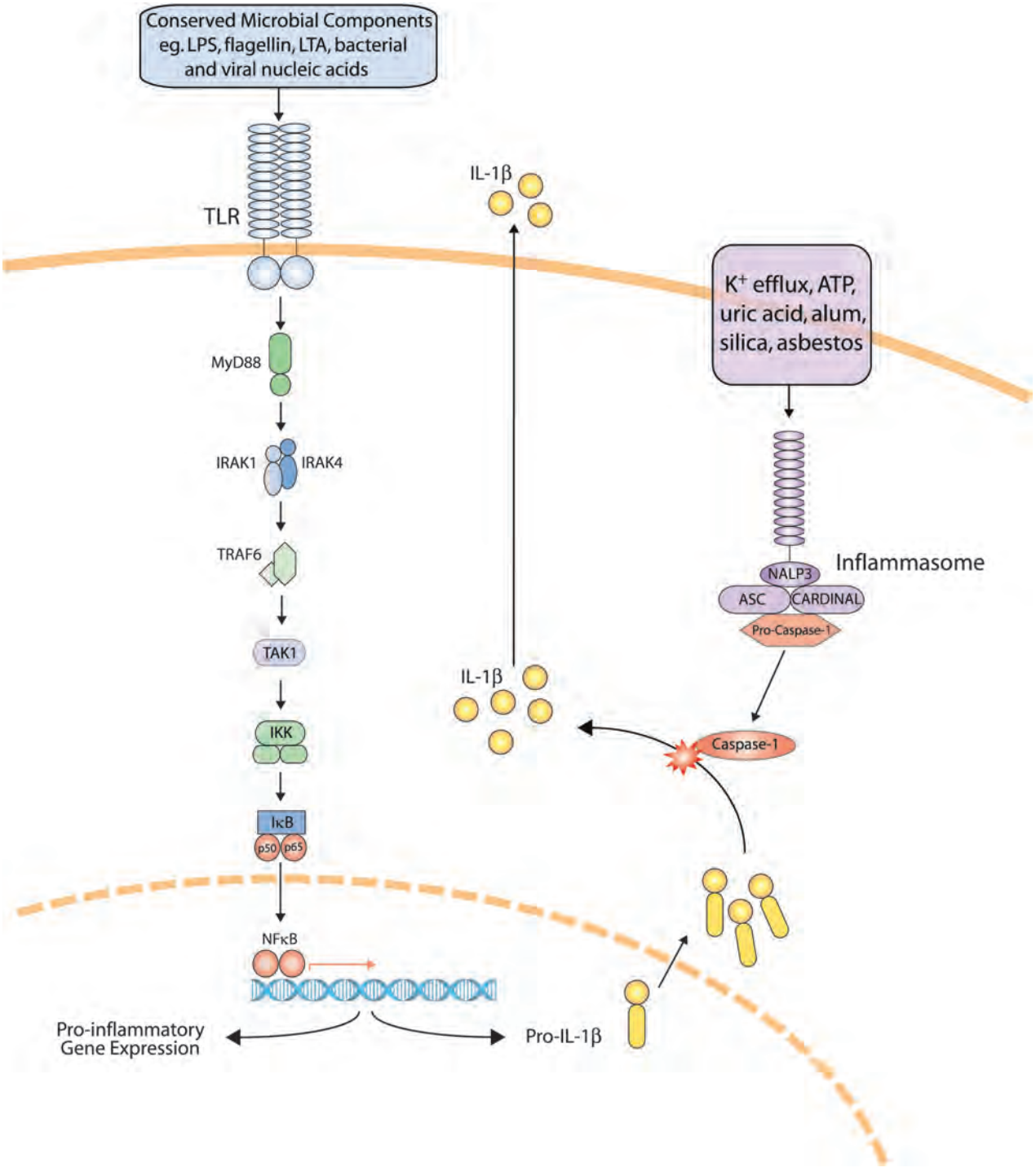

FIG 2. Overview of TLR signaling and the NLRP3 inflammasome. TLR ligation initiates a signaling cascade that culminates in the translocation of the transcription factor nuclear factor κB *(NF-κB)* and others to the nucleus, generating an acute inflammatory response. The NLRP3 (or NALP3) inflammasome is triggered by a wide variety of stimuli, culminating in the activation of caspase 1, which will then cleave pro–IL-1β and pro–IL-18 to drive an inflammatory response. Human mutations and polymorphisms in many of the genes encoding elements of these pathways appear to alter susceptibility to infectious and inflammatory diseases. *TRAF6,* TNF receptor-associated factor 6; *TAK1,* Transforming growth factor-beta-activated kinase 1; *IKK,* I-kappa-B kinase; *ASC,* Apoptosis-associated speck-like protein containing a card.

bacteria, with *Streptococcus pneumoniae* causing invasive infection in all reported cases and *Staphylococcus aureus* and *Pseudomonas aeruginosa* causing infections in about half the patients. The surprising clinical observation that IRAK4-deficient patients are resistant to viral infections was recently explained at a molecular level because IRAK4-deficient patients are able to control viral infections by means of TLR3- and TLR4-dependent production of interferons.[17]

Arguably one of the most powerful messages to arise from the recognition of IRAK4 and MyD88 deficiency is the value of studying human subjects to understand human immune function. Although MyD88-deficient patients are susceptible to *S pneumoniae* and a limited number of pyogenic bacteria, they are able to resist infection by most common bacteria, viruses, fungi, and parasites. In contrast, MyD88 deficiency renders mice profoundly susceptible to most pathogens tested.

Contribution of TLR polymorphisms to human disease. At the population level, susceptibility to common diseases, such as infections, seldom follows the simple pattern of Mendelian inheritance seen in IRAK4 and MyD88 deficiency.[18] Most infections follow a complex mode of inheritance, with disease arising from an intricate interplay between environmental and genetic factors. The complexity of common infectious diseases has made them, until very recently, largely impervious to genetic analysis. However, advances in high-throughput genotyping techniques and bioinformatics are now allowing us to understand how common genetic variants alter human susceptibility to infection.

Although human subjects are identical at most of the 3 billion base pairs in their genome, interindividual variation is present in approximately 3 million nucleotides (ie, 0.1% of the genome).[19] A common type of human genetic variation is the single nucleotide polymorphism (SNP), in which 2 alternative bases occur at appreciable frequency (>1%) in the population. There is convincing evidence that common TLR SNPs regulate cellular signaling events, cytokine production, and susceptibility to infection based on the specific pathogens recognized by the TLR. Arguably the best evidence implicates amino acid–changing (ie, nonsynonymous) SNPs in TLR1, TLR2, and TLR5, as well as variants in the adaptor molecule TIR-containing adaptor protein (TIRAP, also know as MyD88 adaptor-like [MAL]). This genetic variation in the population results in some individuals having a subtle but specific immunodeficiency. For example, a common *TLR5* polymorphism in the ligand-binding domain of TLR5 (392STOP) abolishes flagellin signaling and is associated with increased susceptibility to Legionnaire disease caused by the flagellated bacterium *Legionella pneumophila*.[20] In a similar fashion, polymorphisms in the adaptor molecule MAL/TIRAP, which mediates signaling through TLR1, TLR2, TLR4, and TLR6, have been associated with susceptibility to tuberculosis, malaria, and pneumococcal disease.[21]

Given the role of TLRs in sensing the extracellular environment and shaping the inflammatory response, the TLR pathway has been hypothesized to influence the development of atopy and asthma. The best-studied example is CD14. CD14 is encoded on chromosome 5q31.1 in a region linked to atopy and asthma, and CD14 partners with TLR4 to recognize LPS. Therefore a SNP in this gene (*CD14*/−159 C to T), which appeared to alter the functional production of CD14, made an excellent candidate to influence susceptibility to asthma and atopy. Initial investigations showed remarkable variation, with some studies indicating the T allele as a risk factor, others indicating the C allele, and others finding no association.[22] However, when the level of LPS (or endotoxin) exposure was considered, a biologically plausible gene-environment interaction was revealed, with data suggesting that the C allele is a risk factor for allergic phenotypes at low levels of exposure, whereas the T allele is a risk factor at high levels of exposure.[23] Through this informative example, it is clear that complex interactions between genes and the environment determine asthma-related outcomes. Consequently, if we fail to integrate genetic and environmental factors in our study of asthma and allergy, we will only generate an impoverished appreciation of the cause of atopic disease.

Although a rapidly growing number of genetic association studies suggest that *TLR* polymorphisms might be associated with susceptibility to different infectious and immunologically mediated diseases, very few of these studies have been replicated in a convincing fashion. For example, the initial association reported between MAL/TIRAP and susceptibility to tuberculosis was not replicated in another large study.[24] As this field advances and expands to include genome-wide association studies, it is essential to appreciate that the best studies will include large sample sizes, statistical adjustments for multiple comparison, replication of findings with independent cohorts, multiple study designs (including case-control and family-based studies), adjustment of the analysis for population admixture, consideration of environmental variables, and detailed molecular and cellular analyses to determine whether a polymorphism alters function.

NLRs

Overview of NLR structure and function. Although TLRs are outward-looking innate immune receptors detecting microbial signatures either in the extracellular milieu or engulfed in the lumen of endocytic vesicles, NLRs are a recently appreciated family of receptors that survey the intracellular environment.[25,26] In common with other innate immune receptor systems, the NLRs have ancient origins, being structurally reminiscent of plant R-proteins that mediate plant cell defense against pathogenic bacteria. NLRs sense microbial products and metabolic stress, driving inflammation through the formation of an inflammasome: a large cytoplasmic complex that activates inflammatory caspases and the production of the cytokines IL-1β and IL-18.[27]

The human NLR family consists of at least 23 members and can be structurally divided into 4 subfamiles based on N-terminal effector domains.[28] The first NLRs reported to have a direct function as intracellular pathogen detectors were NOD1 and NOD2.[26] Both NOD proteins detect distinct substructures generated during the synthesis, degradation, and remodeling of bacterial peptidoglycan, ensuring the recognition of peptidoglycan from both gram-positive and gram-negative bacteria. IL-1β-converting enzyme (ICE) protease-activating factor (IPAF) is another member of the NLR family known to detect bacterial pathogens.[29] IPAF partners with TLR5 to detect infection by flagellated bacteria: TLR5 senses extracellular flagellin, whereas IPAF focuses on intracellular flagellin. In addition to sensing microbial products, NLRs can sense metabolic stress related to infection and sterile inflammation. This sensing capacity is best demonstrated by NLRP3 (NLR family, pyrin domain-containing 3).[30] When triggered, NLRP3 (also called NALP3 or cryopyrin) activates the caspase 1 inflammasome, leading to IL-1β and IL-18 processing (Fig 2). The NLRP3 inflammasome appears to be activated by common metabolic danger signals, such as potassium efflux, which occurs during inflammation because of disruption of the plasma membrane or increased extracellular ATP released by injured cells. Other clinically relevant NLRP3 activators include uric acid, asbestos, silica, and alum.

Role of NLRs in human health and disease. Although our molecular appreciation of NLRs is very recent, this class of innate immune receptors plays a central role in several human

inflammatory diseases and mediates the adjuvant effect of a common vaccine component, alum.

NLR defects associated with inflammatory diseases. The convergence of clinically defined autoinflammatory disease with the biology of innate immunity and NLRs came with the discovery that 3 well-established autoinflammatory diseases are all caused by activating, gain-of-function mutations in *NLRP3*.[31] These diseases, collectively known as the cryopyrinopathies, are (1) familial cold autoinflammatory syndrome (OMIM #120100), which presents with cold-induced fevers, urticaria-like rash, and constitutional symptoms; (2) Muckle-Wells syndrome (OMIM #191900), which is characterized by fevers, hives, sensorineural hearing loss, and arthritis unrelated to cold exposure; and (3) neonatal-onset multisystem inflammatory disease (NOMID) (or chronic infantile neurologic, cutaneous, and articular syndrome [CINCA]; OMIM #607115), which is a devastating neonatal disease presenting with fever, urticaria, and chronic aseptic meningitis. In these disorders *NLRP3* mutations affect IL-1β production, and IL-1β is upregulated in these diseases.[32] Appreciation of the role of the IL-1β axis in these diseases associated with *NLRP3* mutations has allowed the rational use of targeted anti-inflammatory therapy.[33] Strikingly, even the most clinically severe cryopyrinopathy, NOMID/CINCA, appears to respond well to the IL-1 receptor antagonist anakinra.[34]

More insight into the clinical relevance of NLRs arose when it was recognized that 30% to 50% of patients with Crohn disease in the Western hemisphere carry *NOD2* mutations on at least 1 allele.[35,36] The most common mutations are located in or near the leucine-rich repeat domain of *NOD2*, and patients homozygous for the *3020insC* mutation, resulting in partial truncation of the leucine-rich repeat, demonstrate a much more severe disease phenotype. It seems paradoxical that although Crohn disease results in overt inflammation that probably is triggered by normal bacterial flora, the *NOD2* mutations associated with Crohn disease result in a protein product less capable of responding to the bacterial ligand muramyl dipeptide, which is a component of peptidoglycan. A unifying paradigm addressing this paradox is that NOD2 appears to provide homeostatic signals to maintain the gut environment in a state that is tolerant of its flora and cells with *NOD2* mutations are deficient in their production of IL-10, an immunomodulatory and tolerogenic cytokine.[37] Other evidence suggests that *NOD2* variants are associated with Crohn disease because they lead to a decrease in the negative regulation of TLR responses occurring in the normal gut and thus a pathologic increase in responses to the normal flora.[38] Nevertheless, the genetic polymorphisms that show a well-established association with Crohn disease (including *NOD2*) account for only approximately 20% of the genetic variance observed in patients with Crohn disease, suggesting that significant additional genetic contributions have yet to be discovered.

NLR contribution to vaccine responsiveness. Increased understanding of NLRs has allowed us to shed light on the mechanism of action of vaccine adjuvants.[7] Aluminum-containing adjuvants (alum) have historically served as immunopotentiators in vaccines and continue to be the most widely used clinical adjuvants. Despite the fact that most persons reading this review have received vaccines containing alum, it is only very recently that we have begun to fully appreciate the molecular mechanism of alum adjuvancy. Studies published in 2008 demonstrated that the NLRP3 (NALP3) inflammasome is involved in mediating the adjuvant effects of alum.[39-41] This adjuvancy might occur directly through the triggering of the NALP3 inflammasome by alum crystals or indirectly through release of the endogenous danger signal uric acid, which subsequently activates NLRP3.

THERAPEUTIC MODULATION OF INNATE IMMUNITY

With increased appreciation of the contribution of innate immunity to human health and disease, attention quickly shifted to the possibility of therapeutic modulation of innate immunity. This is an area of active investigation, and therefore rather than attempting to survey the field broadly, we will focus our review on recent attempts to harness the TLR system to modulate infectious and allergic diseases.

Activation of TLRs and modulation of allergic immune response

The interaction of 2 fields of research in the 1990s, epidemiologic investigations of the hygiene hypothesis in allergy and asthma and basic research in the field of TLRs, provided the impetus to investigate whether activating TLRs might represent a novel therapeutic option for the treatment and prevention of allergy and asthma.[42] TLR-based therapies in patients with allergy target in particular the dendritic cell interaction with T cells, which is a critical component in shaping the T_H2 immune response associated with allergic inflammation. Because TLRs are highly expressed on dendritic cells but not on T cells, the goal of TLR-based therapies in allergy and asthma is to activate dendritic cells to produce a cytokine milieu (eg, IL-12 and interferons) that favors inhibition of the T_H2 immune response. Thus TLR-based therapies target the innate immune response to consequently inhibit the adaptive T_H2 immune response and do not directly target T cells.

Studies have examined whether activation of TLRs can modulate allergic immune responses in preclinical animal models of allergy and asthma, as well as in more limited studies in human subjects. The majority of studies have evaluated TLR9 agonists, but additional studies have also examined TLR4 agonists and a TLR7/8 agonist. Studies of the TLR9 agonist CpG DNA have demonstrated that it inhibits eosinophilic airway inflammation, T_H2 cytokine responses, mucus expression, airway remodeling, and airway responsiveness in a murine model.[42,43] Administration of an inhaled TLR9 agonist for approximately 8 months to monkeys allergic to dust mite demonstrated that they had reduced eosinophilic airway inflammation, mucus, airway remodeling, and reduced airway responsiveness.[44] The only published studies in human asthmatic subjects were performed in patients with mild asymptomatic asthma treated with an inhaled TLR9 agonist before allergen challenge.[45] Although treatment with the inhaled TLR9 agonist increased expression of interferon-inducible genes, there was no inhibition of the early- or late-phase decrease in FEV_1 or a reduction in sputum eosinophil counts. These studies suggest that either TLR9-based therapies will not be effective in human subjects with asthma or that different doses, routes of administration (ie, systemic vs local), or study populations (symptomatic asthmatic subjects as opposed to allergen-challenged asymptomatic asthmatic subjects) need to be evaluated.

In addition to TLR9 agonists, studies predominantly in murine models have also evaluated the ability of TLR4- and TLR7/8-

based therapies to modulate allergic responses. In murine models of asthma, TLR4 ligands either inhibit or potentiate allergic responses depending on the timing of administration of the TLR4 ligand and associated allergen sensitization or challenge. In human studies in subjects with ragweed-induced allergic rhinitis, administration of a topical intranasal TLR4 ligand was safe but did not inhibit allergic responses in asymptomatic subjects challenged intranasally with ragweed allergen.[46] Studies have also investigated whether administration of a TLR7/8 agonist, imiquimod, would inhibit asthma responses in preclinical models. Imiquimod is a US Food and Drug Administration–approved therapy that is used as a topical treatment for genital warts, actinic keratoses, and superficial basal cell cancer. In preclinical murine models the TLR7/8 agonist inhibits asthma responses. At present, no human studies in patients with allergy or asthma have been reported with the TLR7/8 agonist.

TLR-based vaccine adjuvants in allergic disease

Studies have also examined whether administering a TLR9 agonist conjugated to an allergen would enhance the immunogenicity of the allergen when used as a TLR9-conjugated allergen vaccine in patients with allergic rhinitis or asthma. Studies in murine models have demonstrated that a conjugate of a TLR9 agonist and an allergen had a 100-fold enhanced uptake by antigen-presenting cells compared with TLR9 ligand alone.[42,47] The ability of a TLR9 ligand to induce a T_H1 immune response is also approximately 100-fold greater than that induced by equivalent amounts of a nonconjugated mixture of the TLR9 ligand and allergen. In murine models the TLR9 allergen conjugate significantly reduces rhinitic and asthmatic responses.[42]

Thus based on this enhanced immunogenicity of the TLR9 allergen conjugate, studies have examined whether a TLR9 ragweed allergen conjugate would reduce allergic responses in human subjects with allergic rhinitis. Studies in human subjects have demonstrated mixed results in terms of the effectiveness of the TLR9 ragweed allergen vaccine. Studies in subjects with ragweed-induced allergic rhinitis in Canada demonstrated that administration of the TLR9 ragweed allergen vaccine reduced nasal mucosal biopsy eosinophil counts and T_H2 cytokine levels but did not reduce nasal symptom scores during the ragweed season.[48] A second study in Baltimore demonstrated that administration of the same TLR9 ragweed allergen vaccine significantly reduced rhinitis symptom scores in subjects with ragweed-induced allergic rhinitis during the ragweed season.[49] Subjects treated with the TLR9 ragweed allergy vaccine also used fewer doses of allergy rescue medications during the ragweed season compared with the placebo-treated subjects. Interestingly, although the study subjects immunized with the TLR9 ragweed vaccine only received 6 injections of the vaccine before the first ragweed season, the beneficial reduction in symptoms persisted through the second ragweed season without administration of additional vaccine.

At present, there are limited numbers of published human studies with either administration of TLRs alone or with TLRs conjugated to allergens. Further studies are thus needed to determine whether the interesting observations regarding TLRs in preclinical models will translate into safe and effective therapeutic advances in allergy and asthma. Potential safety concerns of TLR-based therapies in allergy and asthma include the induction of autoimmune disease. However, induction of autoimmune disease has not been observed in the limited number of clinical trials with TLR9-based therapies.

TLR-based vaccine adjuvants in infectious disease

Vaccination has proved extremely effective in preventing infectious diseases, but knowledge of the immunologic mechanisms that allow vaccines to be so successful is rather limited. In contrast to live vaccines, subunit vaccines, which consist of specific components of pathogens, have little inherent immunogenicity and need to be supplemented with adjuvants to promote a protective immune response. However, there is a paucity of licensed adjuvants for clinical use, and thus there is a critical need to develop safe and effective adjuvants. The renaissance in innate immune biology is facilitating the rational design of novel vaccine adjuvants.[50] Characterization of the NLR system has shed light on the mechanism of action of alum adjuvancy, and our understanding of TLR function is accelerating the discovery of safe and effective vaccine adjuvants.

An illustrative example is the development of the novel adjuvant monophosphoryl lipid A (MPL).[51] The TLR4 ligand LPS is a potent adjuvant, but its toxicity prevents its use in human subjects. However, MPL comes from the cell-wall LPS of gram-negative *Salmonella minnesota* R595 and is detoxified by mild hydrolytic treatment and purification. MPL lacks the toxicity of LPS but retains the beneficial adjuvant properties. MPL combined with aluminum salt (referred to as the AS04 adjuvant system) shows efficacy in a vaccine against human papilloma virus[52] and as a hepatitis B vaccine for patients with advanced renal disease.[53] Interestingly, this adjuvant combination likely benefits from the immune-enhancing capacity of both the TLR pathway (triggered by MPL) and the NALP3 inflammasome (triggered by alum crystals). Further advances in this area are almost certain because many other TLR ligands are being developed as potential vaccine adjuvants.

CONCLUSIONS

In the last decade, we have witnessed exhilarating advances in our understanding of the molecular mechanisms used by the innate immune system to sense infection and trigger a protective immune response. For clinicians and scientists alike, the challenge is to translate this basic mechanistic understanding into a more complete appreciation of the role of innate immunity in health and disease.

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Adaptive immunity

Francisco A. Bonilla, MD, PhD, and Hans C. Oettgen, MD, PhD *Boston, Mass*

The innate immune system provides critical mechanisms for the rapid sensing and elimination of pathogens. Adaptive immunity has evolved to provide a broader and more finely tuned repertoire of recognition for both self- and nonself-antigens. Adaptive immunity involves a tightly regulated interplay between antigen-presenting cells and T and B lymphocytes, which facilitate pathogen-specific immunologic effector pathways, generation of immunologic memory, and regulation of host immune homeostasis. Lymphocytes develop and are activated within a series of lymphoid organs comprising the lymphatic system. During development, sets of gene segments are rearranged and assembled to create genes encoding the specific antigen receptors of T and B lymphocytes. The rearrangement mechanism generates a tremendously diverse repertoire of receptor specificities capable of recognizing components of all potential pathogens. In addition to specificity, another principal feature of adaptive immunity is the generation of immunologic memory. During the first encounter with an antigen (pathogen), sets of long-lived memory T and B cells are established. In subsequent encounters with the same pathogen, the memory cells are quickly activated to yield a more rapid and robust protective response. (J Allergy Clin Immunol 2010;125:S33-40.)

Key words: *Adaptive immunity, antibody, B cell, lymphocytes, T cell*

Abbreviations used

APC:	Antigen-presenting cell
CTL:	Cytolytic T lymphocyte
NK:	Natural killer
NKT:	Natural killer T
PLCγ1:	Phospholipase Cγ1
RAG:	Recombinase activating gene
SCID:	Severe combined immunodeficiency
SHM:	Somatic hypermutation
TACI:	Transmembrane activator and CamL interactor
TCR:	T-cell receptor
TI:	T independent
TLR:	Toll-like receptor
TREC:	T-cell receptor excision circle
ZAP-70:	ζ-Associated protein, 70 kd

Although the innate immune system has evolved to rapidly sense and effect the elimination of a wide range of pathogens, the range of common pathogenic molecular patterns it can recognize is limited. The overwhelming variability of antigenic structures, as well as the ability of pathogens to mutate to avoid host detection, has driven the evolution of the adaptive immune system.[1] In contrast to the recognition receptors of the innate immune system, which are all encoded in their fully functional form in the germline genome, adaptive immune responses depend on receptors that are custom tailored and selected through a process of somatic recombination of a large array of gene segments. These arose by means of gene duplication early in the evolution of vertebrates to generate highly specific and flexible immune responses. After initial pathogen encounters, cells expressing these immune receptors can persist in the host for life, providing immunologic memory and the capacity for rapid response in the event of re-exposure.

Cells of the adaptive immune system include the effectors of cellular immune responses, the T lymphocytes, which mature in the thymus, and antibody-producing cells, the B lymphocytes, which arise in the bone marrow. Lymphocytes are highly mobile. After developing in the primary lymphoid organs (thymus and bone marrow), they traffic to secondary lymphoid organs, including lymph nodes and the spleen, which serve to capture circulating antigens from lymph and blood, respectively. Adaptive immune responses originate in these areas, often under the influence of innate immune system signals provided either directly by circulating pathogens or indirectly by pathogen-activated cutaneous or mucosal antigen-presenting cells (APCs) migrating to the secondary lymphoid organs. Lymphocytes emigrating from the spleen and lymph nodes can then travel to many sites in the body to exert effector functions. This trafficking is regulated by an array of adhesion molecules and chemokine receptors; CLA-1^+ CCR4-bearing lymphocytes traffic to skin, whereas cells bearing the $\alpha 4\beta 7$ integrin which binds to mucosal addressin cellular adhesion molecule-1 (MadCAM-1) on gut endothelial cells preferentially home to the gastrointestinal tract.

T CELLS AND CELLULAR IMMUNITY

T-cell development

T cells develop in the thymus from common lymphoid progenitors coming from the bone marrow or fetal liver.[2-4] Seeding of the thymus is promoted by the interaction of platelet selectin glycoprotein 1 on the progenitors with the adhesion molecule P-selectin on thymic epithelium. Recently arrived cells rapidly expand under the influence of IL-7, the receptor of which signals through the common γ chain, which is encoded on the X-chromosome, and is shared by a number of other cytokine receptors (IL-2, IL-4, IL-9, IL-15, and IL-21). Mutations in this polypeptide underlie X-linked severe combined immunodeficiency (SCID), which is characterized by absent T cells. This early thymocyte expansion is accompanied by induction of Notch-1 and other

From [a]the Division of Immunology, Children's Hospital Boston, and [b]the Department of Pediatrics, Harvard Medical School.

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Reprint requests: Francisco A. Bonilla, MD, PhD, Children's Hospital Boston, 300 Longwood Ave, Boston, MA 02115. E-mail: Francisco.bonilla@childrens.harvard.edu.

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transcription factors, which commit precursors to the T-cell lineage and induce the expression of genes important in T-cell receptor (TCR) assembly. Subsequent differentiation of the expanded pool of T-cell progenitors or pro-T cells in the thymus involves an antigen-independent process in which a coordinated series of genomic rearrangements leads to the creation of functional genes encoding the α and β or γ and δ chains of the TCR.

In their germline configuration the TCR loci contain arrays of V (variable), D (diversity) and J (joining) segments. V and J segments are present at all TCR loci, whereas only the β and δ TCR loci contain D segments. In a spatially and sequentially ordered process, one V, one D (for β and δ) and one J segment are randomly spliced together (Fig 1). This is mediated by an enzymatic complex, the V(D)J recombinase composed of 2 proteins encoded by the recombinase-activating genes 1 and 2 (*RAG1* and *RAG2*). RAG1 and RAG2 bind to recombinase signal sequences flanking the borders of V-D-J segments. Recombination signal sequence accessibility is regulated by chromatin structure.[5] The V(D)J recombinase cleaves the DNA at these sites to give rise to hairpin structures. These, in turn, are substrates for cleavage by the nuclear enzyme Artemis, which is activated by DNA-dependent protein kinase catalytic subunit and exerts endonuclease activity on 5′ and 3′ overhangs and hairpins. Repair of the DNA breaks with resultant genomic juxtaposition of V, D, and J segments is effected by ubiquitous DNA repair enzymes including XRCC4 (X-ray repair cross-complementing protein 4) and Ligase IV in a process called nonhomologous end-joining. As would be predicted, null mutations in *RAG*, Artemis (DCLRE1C), DNA Ligase IV, and other enzymes involved in V(D)J recombination (including the XRCC4-like enzyme Cernunnos) give rise to SCID.

Each assembled V-D-J cassette represents one of a huge number of possible permutations of recombinations of the component V, D, and J segments, and the resulting structure dictates the amino acid sequence and binding specificity of the TCR. This is referred to as combinatorial diversity. Additional diversity, known as junctional diversity, is conferred by some inherent imprecision in the DNA-joining reactions involved in ligation of double-strand DNA breaks, resulting in some addition or removal of bases. Furthermore, the enzyme terminal deoxyribonucleotidyl transferase catalyzes the template-independent addition of several (generally 1-5) nucleotides at the joints. These junctional areas encode the third complementarity determining region of the antigen-binding pocket of the TCR, and this is the site of greatest variability.

In their germline configuration the component gene segments of the TCR are separated by large amounts of DNA. These intervening stretches of DNA are excised in the process of recombination but remain in the nucleus, where they circularize and are stable in an episomal form known as T-cell receptor excision circles (TRECs). TRECs are not duplicated during cell division, and therefore they dilute as newly formed T-cell clones expand. Measurement of TRECs in peripheral blood by means of PCR can be used to examine T-cell emigration from the thymus, and this approach is now in used in several states to analyze newborn blood spots in pilot screening programs for SCID.[6]

Gene-segment rearrangements are termed productive if they do not introduce stop codons and give rise to a gene encoding a full-length TCR protein. Sequential productive rearrangements of 2 TCR genes leading to surface expression of an αβ or γδ TCR marks the transition from a pre-T to a double-positive T cell; these

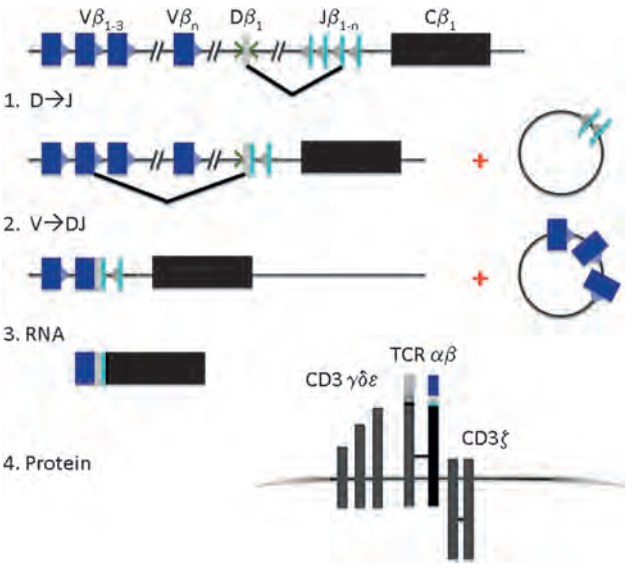

FIG 1. Sequential recombination of a random assortment of gene fragments dictates TCR structure and specificity. This schematic depiction of the TCR $V\beta_1$ locus indicates the relative locations of the Vβ, Dβ, and Jβ segments upstream of $C\beta_1$. *1,* The V(D)J recombinase recognizes signal sequences *(triangles)* upstream of one of many possible Jβ segments and introduces DNA breaks. The same process occurs at an upstream Dβ segment. Double-stranded DNA breaks are generated, and the 2 broken DNA ends are brought together and ligated by means of cellular DNA repair mechanisms (nonhomologous end-joining). The excised intervening DNA (the stretch between Dβ and $J\beta_n$) circularizes and remains in the nucleus as an episome known as a TREC. Such DNA circles are stable but are not replicated during cell division and dilute out during clonal expansion after T cells exit the thymus. *2,* By using the same mechanism, one of approximately 70 possible Vβ segments is brought into juxtaposition with the DJβ segment. A second excision product is generated. *3,* Transcripts of the rearranged TCRβ locus contain Vβ, Dβ, Jβ, and C cassettes. *4,* If this series of events has not introduced any stop codons, the rearrangement is termed productive, and a full functional TCRβ protein is translated. This event is permissive for subsequent TCRα rearrangement followed by expression of the complete TCR complex, including TCRαβ and CD3γδϵζ chains at the T-cell surface. Rearrangement of α genes is the same as for β genes, except that the α gene is assembled only from Vα, Jα, and Cα. The γ chain of the TCR is similar to α and is also assembled from V, J, and C segments. The TCR δ chain is similar to the β chain and is comprised of V, D, J, and C segments. The α and δ gene loci are on chromosome 14. The β and γ loci are on chromosome 7.

cells express both CD4 and CD8. The TCR chains are assembled at the cell surface as a complex with the proteins constituting CD3, including the γ, δ, ϵ and ζ chains.

Further differentiation of these double-positive cells, which reside in the thymic cortex, to single-positive T cells, which are found in the medulla, is regulated by both positive and negative selection events involving antigens and molecules of the MHC. Positive selection occurs when the TCR of double-positive T cells binds with low avidity to self-MHC (complexed with self-peptides) on thymic epithelium. Double-positive cells bearing a TCR, which does not bind to self-MHC, are eliminated. Conversely, negative selection is exerted on double-positive T cells, the TCR of which binds with very high avidity to self-MHC/peptide, ensuring that autoreactive T-cell precursors are not permitted to mature (central tolerance). Deletion of T-cell clones interacting with peptides normally expressed in distant organs is facilitated by the function of the gene *AIRE* (autoimmune regulator), which stimulates expression of genes with wide tissue

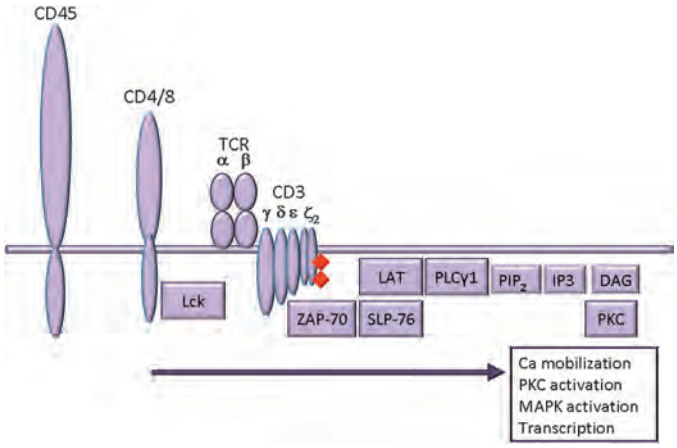

FIG 2. Signaling molecules in T-cell activation. The TCR α and β chains recognize peptide/MHC complexes expressed on APCs, an interaction that is stabilized by the simultaneous binding of T-cell CD8 to MHC class I or CD4 to MHC class II. Signaling is initiated by the CD3 chains (γ, δ, ϵ, and ζ) through cytoplasmic ITAMs *(red diamonds)*, which are phosphorylated by Src family kinases, including CD4/8-associated Lck, leading to recruitment of signaling molecules, including ZAP-70. The tyrosine phosphatase CD45 dephosphorylates inhibitory phosphotyrosines in Lck and is important for initiation of signaling. ZAP-70–mediated phosphorylation of downstream molecules, including the adapter proteins linker of activated T cells *(LAT)* and SH2-containing leukocyte protein, 76 kd *(SLP-76)*, drives the recruitment of PLCγ1, which hydrolyzes the membrane lipid phosphatidylinositol bisphosphate *(PIP_2)*, generating inositol-trisphosphate *(IP_3)* and diacylglycerol *(DAG)*. IP_3 increases intracellular calcium (Ca^{2+}) levels, and DAG activates protein kinase C, leading to the induction of nuclear factor κB *(NF-κB)*–mediated and mitogen-activated protein kinase *(MAPK)*–mediated gene transcription.

specificity in thymic epithelium.[7] Dysfunction of this gene is permissive for the escape of some self-reactive T cells and can give rise to autoimmune polyendocrine syndromes. Double-positive thymocytes that pass both positive and negative selection mature to $CD8^+$ single-positive T cells by means of further interaction with thymic epithelial MHC class I molecules, whereas those selected on MHC class II acquire a $CD4^+$ single-positive phenotype. Both $CD4^+$ and $CD8^+$ single-positive cells are found in the thymic medulla from which they exit to the circulation as fully differentiated but antigen-naive T cells.

T-cell activation

Mature T cells are activated on interaction of their TCRs with antigenic peptides complexed with MHC molecules. $CD8^+$ T cells can interact with peptides (9-11 amino acids in length) on almost any cell expressing MHC class I (HLA-A, HLA-B, and HLA-C). These MHC class I–restricted peptides are generally produced from proteins translated within the cell (endogenous antigens) encoded either in the host genome or by infecting viruses or other pathogens replicating intracellularly. In contrast, the TCRs of $CD4^+$ T cells engage peptides bearing MHC class II (HLA-DR, HLA-DQ, and HLA-DP). Unlike MHC class I expression, which is constitutive in all nucleated cells, MHC class II molecules are present on APCs and are inducible by innate immune stimuli, including ligands for Toll-like receptors (TLRs). APCs are specialized samplers of environmental antigens and danger signals (ligands for TLR and other systems of pattern-recognition receptors). They are present in large numbers in the skin and mucosal sites, where pathogen encounter is most likely, and they actively sample exogenous proteins by means of phagocytosis or endocytosis. Activation of these cells leads not only to induction of MHC class II expression but also to emigration from skin and mucosal sites to regional lymph nodes, where interaction with T cells can occur, leading to initiation of immune responses.

T-cell activation is initiated when the TCR and associated proteins recognize a peptide/MHC complex on an APC, leading to a rapid clustering of TCR-associated molecules at the physical interface between T cells and APCs and the formation of a so-called immunologic synapse.[8] This is also called a supramolecular activation complex. The T-cell side of the synapse is focused around a central cluster of CD3 (γ, δ, ϵ, and ζ) and TCR (α and β), which bind specifically to the peptide/MHC complex, as well as CD4/CD8 molecules, which stabilize this interaction by binding to nonpolymorphic regions of MHC class I or MHC class II, respectively. The synapse is stabilized by adhesion molecules known as integrins. The aggregation of these molecules in the synapse facilitates the early events in TCR signaling (Fig 2). Simultaneous binding to MHC/peptide on the APCs by TCRs and CD4/CD8 in the synapse brings the cytosolic domains of these molecules into proximity. As a result, the CD4- and CD8-associated Src family protein tyrosine kinase Lck is able to phosphorylate tyrosine residues contained in cytoplasmic immunoreceptor tyrosine-based activation motifs of the TCR-associated CD3 chains. This results in the recruitment of the critical adaptor molecule, ζ-associated protein, 70 kd (ZAP-70), which binds to immunoreceptor tyrosine-based activation motif phosphotyrosines and phosphorylates a number of cytosolic proteins triggering the assembly of an intracellular complex of scaffolding and activated signaling proteins, including linker of activated T cells and SH2-containing leukocyte protein, 76 kd. The CD45 transmembrane protein, which contains 2 tyrosine phosphatase domains and is ubiquitous in lymphoid cells, might play a critical role in TCR-triggered activation of this kinase cascade by dephosphorylating inhibitory phosphotyrosine residues in Src family kinases, such as Lck. Mutations in *CD45* give rise to a SCID phenotype.

One of the active signaling enzymes recruited to linker of activated T cells and phosphorylated by ZAP-70 is phospholipase Cγ1 (PLCγ1). PLCγ1 mediates hydrolysis of the membrane inositol phospholipid phosphatidylinositol bisphosphate, generating inositol-trisphosphate and diacylglycerol. Inositol-trisphosphate induces a rapid increase in intracellular calcium (Ca^{2+}) levels by means of activation of stores contained within the endoplasmic reticulum. This calcium flux activates a calcium release–activated calcium channel facilitating the influx of extracellular calcium.[9] Calcium entering the cytosol from the endoplasmic reticulum or extracellular space binds to the regulatory protein calmodulin, which in turn activates the phosphatase calcineurin, which dephosphorylates nuclear factor of activated T cells in the cytosol, generating the active form of this critical transcription factor, which then translocates to the nucleus.

In a simultaneous, parallel pathway triggered by diacylglycerol, the other product of PLCγ1-mediated hydrolysis of phosphatidylinositol bisphosphate, protein kinase C is activated. This leads, through intermediates, to the activation of nuclear factor κB, another critical transcription factor in T-cell activation. Activation of the mitogen-activated protein kinase pathway, which is initiated by recruitment of RasGTP to the supramolecular activation complex, leads to the generation of the activator protein 1 transcription factor. The coordinated action of this series of transcription factors (nuclear factor of activated T cells, nuclear factor κB, and activator protein 1), as well as others, induces a constellation of gene expression important for the function of activated T cells.

T-cell effector subsets

Although the basic principles of thymic development and the mechanisms of activation are shared by all T cells, there is a remarkable diversity of effector functions that are elicited in response to activation. T cells can play direct roles in elimination of pathogens by killing infected target cells. They can function as helper cells, providing cognate (involving direct cellular contact) or cytokine signals to enhance both B- and T-cell responses, as well as causing activation of mononuclear phagocytes. Finally, T cells regulate immune responses, limiting tissue damage incurred by means of autoreactive or overly inflammatory immune responses.

The largest group of T cells in the body is the $CD4^+$ αβ TCR population. Most of these cells serve a helper function and have been designated T_H cells. On activation, T_H cells produce a range of cytokines. About 20 years ago, immunologists Robert Coffman and Tim Mossman first discovered that not every individual $CD4^+$ T_H cell has the capacity to produce the full range of cytokines known to be in the T-cell repertoire.[10] Instead, by means of analysis of T-cell clones, they demonstrated 2 main categories of T_H cells, both T_H1 and T_H2 cells, each producing (mostly) mutually exclusive panels of cytokines. T_H1 cells were characterized by their capacity to make IFN-γ and IL-2 and were shown to differentiate from naive T_H0 precursors under the influence of IL-12 and IFN-γ and the T-box expressed in T cells transcription factor (T-bet) (Fig 3). In contrast, T_H2 cells are producers of IL-4, IL-5, IL-10, and IL-13, and their development is driven by IL-4 and the transcription factor GATA-3. T_H1 cell cytokines drive cell-mediated responses, activating mononuclear phagocytes, natural killer (NK) cells, and cytolytic T cells for killing of intracellular microbes and virally infected targets. The T_H2 cytokine profile enhances antibody production, as well as a number of aspects of hypersensitivity and parasite-induced immune responses, including eosinophilopoiesis. In some cases there is more plasticity to

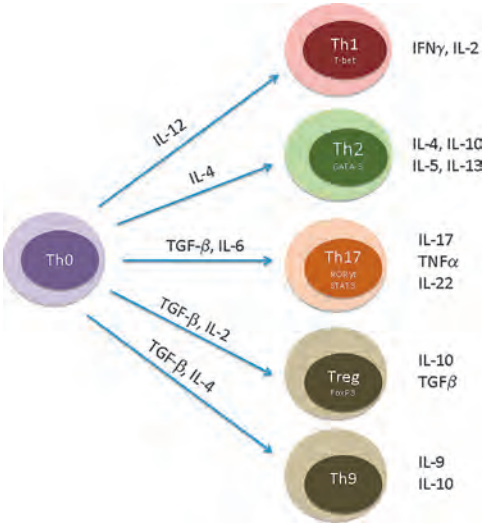

FIG 3. $CD4^+$ T_H cell subsets. Antigen-specific naive T_H0 T cells are stimulated to expand on interaction with APCs expressing MHC class II/peptide complexes. Depending on the type of APC and the cytokine milieu *(arrows)* at the site of antigen encounter, T_H0 cells can be driven down one of several differentiation pathways. The T_H populations that arise retain the TCR specificity of the parent T_H0 cell but secrete unique constellations of cytokine products that mediate distinct effector functions, including activation for killing of microbes (T_H1), production of antibodies and expulsion of helminths (T_H2), induction of inflammatory responses (T_H17), and dampening of immune activation (regulatory T *[Treg]* cells). Specific transcription factors (indicated in the nuclei) stabilize lineage commitments and dictate the specific cytokine secretion profiles. *FoxP3,* Forkhead box protein 3; *RORγt,* (retinoic acid receptor related orphan receptor γt); *STAT3,* signal transducer and activator of transcription 3; *T-bet,* T-box expressed in T cells.

T-cell production of T_H1 and T_H2 cytokine production than the constraints of the T_H1/T_H2 paradigm would suggest; overlapping cytokine expression profiles are possible. For example, it was recently shown that T-box transcription factor expression, along with IFN-γ production, can be induced in some T_H2 cells.[11]

Over the 2 decades since their discovery, the relationship between T_H1 and T_H2 cells has been viewed as a Yin-Yang paradigm, and immune responses to pathogens or immunologically mediated disease processes have been considered as primarily T_H1 or T_H2 mediated. However, inconsistencies between the T_H1/T_H2 model and clinical observations and animal data suggested that not all $CD4^+$-driven processes could be attributed to cytokines predicted to arise from T_H1 or T_H2 responses. In the past 2 years, strong evidence for additional T_H diversity has arisen.[12] T_H17 cells are induced by IL-6 and TGF-β and express the transcription factor RORγt (retinoic acid receptor related orphan receptor γt). T_H17 cells produce IL-17, a group of 5 homologous molecules designated IL-17A-F. T_H17 cells produce mainly IL-17A and IL-17F, and IL-17E is now called IL-25. IL-17A and IL-17F are potent proinflammatory cytokines capable of inducing IL-6 and TNF production, as well as driving granulocyte recruitment and tissue damage. T_H17 cells are thought to be important in autoimmunity; IL-17 is present in the inflamed tissues of patients with arthritis, multiple sclerosis, and systemic lupus erythematosus. In animal models genetic deletion or antibody inhibition of IL-17 blocks experimental autoimmune diseases, such as experimental autoimmune encephalomyelitis. T_H17 cells are also prominent in chronic allergic inflammatory processes, such as asthma.[13] Defects that impair T_H17 production in human

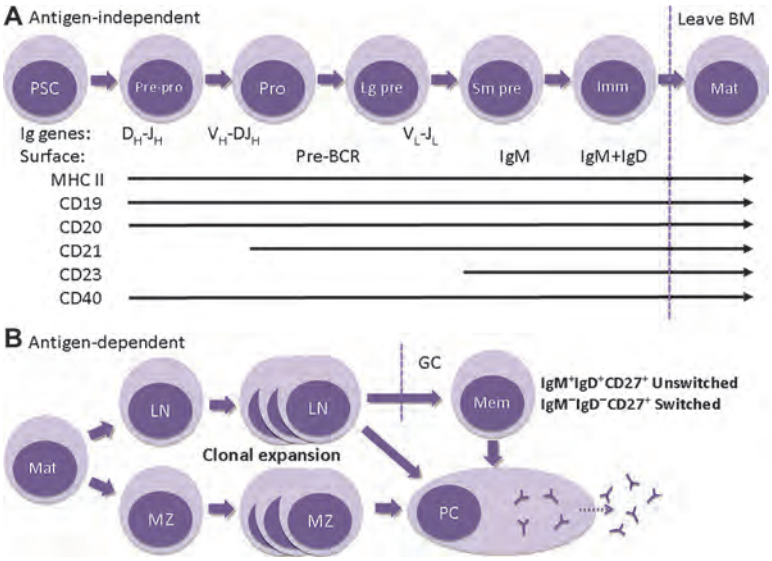

FIG 4. A, Antigen-independent B-cell development in the bone marrow. The earliest recognized committed stage is the pre-/pro-B cell, where immunoglobulin D_H and J_H genes rearrange. In the pro-B cell stage, a V_H segment is joined to the DJ_H unit. At this point, if heavy chain gene rearrangement on at least 1 chromosome has been successful, an IgM heavy chain might form and pair with the surrogate light chain heterodimer (lambda 5 and VpreB) to make the surface-expressed pre-B cell receptor *(BCR)* at the large pre-B cell *(Lg pre)* stage. Subsequent to signaling through the receptor, precursors undergo expansion through proliferation (not shown) and light chain genes rearrange (κ chains first and λ chains next, if κ rearrangement is unsuccessful) at the small pre-B cell *(Sm pre)* stage. If light chain assembly is successful, the cell might express a fully formed IgM receptor on its surface at the immature *(Imm)* stage and leave the bone marrow *(BM)*. Subsequently, the cell also expresses IgD on the surface in addition to IgM and becomes a mature *(Mat)* B cell. **B**, Antigen-dependent B-cell development in the periphery. Many B cells recirculate through the lymphatic system and lymph nodes *(LN)*, where they can encounter antigen. When activated, these cells proliferate and might become short-lived antibody-secreting plasma cells *(PC)*. Alternatively, they might enter follicles and establish germinal centers *(GC)*. Memory B cells are mainly formed in GCs. Memory cells can be subsequently activated and become long-lived plasma cells at some time in the future. Cells that have been activated acquire the CD27 surface marker. Cells that retain surface expression of IgM and IgD are called unswitched, whereas cells that have undergone immunoglobulin class-switching and have lost expression of IgM and IgD are called switched memory B cells. Cells can also enter the splenic marginal zone *(MZ)*, where they do not actively recirculate. If they are activated here in the absence of cognate T-cell help (see text), they also undergo clonal expansion and form plasma cells. However, little B-cell memory is generated in this pathway.

subjects, such as signal transducer and activator of transcription 3 mutations in the hyper-IgE syndrome, are associated with decreased inflammatory response and recurrent infections. It is likely that future investigations will uncover further diversity of T_H subsets. The existence of IL-9–producing T_H9 cells has recently been suggested by the observation that exposure of T_H2 cells to a combination of IL-4 and TGF-β reprograms them to produce IL-9, a potent mast cell growth factor and mediator of helminthic immunity.[14,15] A specialized subset of T_H cells, follicular T helper (T_{FH}) cells resides in lymph nodes and the spleen. T_{FH} cells are memory $CD4^+$ cells expressing the chemokine receptor CXCR5, which mediates their recruitment to follicles. These cells trigger B-cell activation, leading to germinal center formation.

The critical function of regulation of T-cell responses also resides within the $CD4^+$ αβ TCR subset of lymphocytes and is likely effected by several regulatory cell types. IL-10–producing regulatory T (T_R1) cells, as well as both naturally occurring and inducible $CD25^+CD4^+$ T cells expressing the transcription factor forkhead box protein 3, have been shown to quell T-cell responses. Absence of forkhead box protein 3, which is encoded on the X-chromosome, gives rise to a severe multisystem inflammatory disorder (immune dysregulation, polyendocrinopathy, X-linked syndrome). The complexity of the regulatory T-cell system has recently been well reviewed.[16]

$CD8^+$ T cells represent a major fraction of circulating T cells and act to remove both cells harboring intracellular pathogens, including viruses and transformed cells. Because CD8 serves as a coreceptor for MHC class I and $CD8^+$ thymocytes are selected on MHC class I, $CD8^+$ T cells primarily recognize antigenic peptides derived from cytosolic proteins. Cytolytic T lymphocytes (CTLs) kill target host cells in a contact-dependent mechanism. Recognition of foreign cytosolic peptides of the target cell in the context of host MHC class I by the CTL TCR leads to the formation of a conjugate with an immunologic synapse. Within minutes, the CTL activates apoptotic cell death in the target cell. This process is mediated by rapid mobilization of CTL granules to the synapse followed by fusion of granule membranes with the target cell plasma membrane and exocytosis of granule contents, including granzymes and perforin. The granzymes are serine proteases that target a number of proteins in the host cell, leading to activation of apoptosis. In a parallel proapoptotic pathway, TCR activation in the immune synapse drives expression of Fas ligand on the CTL. This in turn engages Fas (CD95) on the target cell membrane, again triggering apoptosis.

A small subset of T cells expresses a γδ TCR, and most are double negative (expressing neither CD4 nor CD8), with some variably $CD4^+$ or $CD8^+$. In human subjects these represent less than 5% of lymphocytes in most tissues but are found in higher

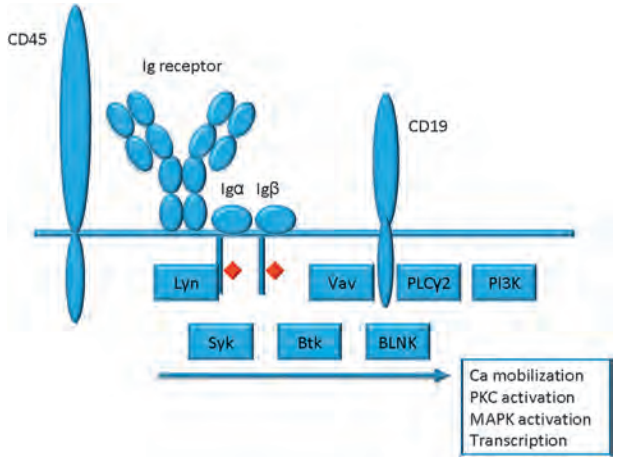

FIG 5. Signaling molecules in B-cell activation. The immunoglobulin receptor contains 2 signaling molecules called Ig-α and Ig-β (encoded by the *CD79A* and *CD79B* genes, respectively). The cytoplasmic domains of these molecules contain ITAMs *(red diamonds)*, which recruit signaling molecules to clusters of cross-linked or immobilized receptors on the cell surface. The tyrosine phosphatase CD45 is important for initiation of signaling, and the coreceptor molecule CD19 also assists in the recruitment of signaling molecules to the complex. Some of the proximal signaling molecules include the tyrosine kinases Lyn, Syk, and Btk; the adaptor protein B-cell linker protein *(BLNK)*; the guanine nucleotide exchange factor Vav; phospholipase Cγ2 *(PLCγ2)*; and phosphoinositide 3′ kinase *(PI3 K)*. Additional downstream signaling events include the release of intracellular calcium stores, the influx of extracellular calcium, activation of protein kinase C *(PKC)*, activation of mitogen-activated protein kinases *(MAPK)*, and activation of transcription regulated by a variety of factors, including OCA-B/OBF-1 (Oct binding factor 1, also called POU domain class 2 associating factor 1) and Pip/IRF-4 (interferon regulatory factor 4).

numbers in the gastrointestinal epithelium. Unlike αβ T cells, γδ cells recognize antigens not in the context of MHC class I or MHC class II molecules but rather as presented by nonclassical MHC molecules of the CD1 family. The γδ subset is expanded in the setting of mycobacterial infection, and it is thought that these T cells might respond to mycobacterial antigens. In addition to recognizing peptide antigens, the γδ TCR can bind to small molecules, including phospholipids and alkyl amines.

Natural killer T (NKT) cells represent another subset of T cells, which, like γδ T cells, recognize nonpeptide antigens presented by nonclassical MHC molecules of the CD1 family. NKT cells are defined by their simultaneous expression of T-cell (CD3, TCRαβ) and NK cell antigens (CD56). A large fraction of NKT cells is characterized by the expression of a single unique TCRα rearrangement, Vα24-Jα18 with Vβ11, and are referred to as invariant NKT cells. Activated NKT cells are capable of rapid and substantial production of cytokines, including IL-4, and have been implicated in allergic pathogenesis.[17] A currently very active area of research is the identification of endogenous and pathogen-derived ligands that might stimulate NKT expansion and activation.

B CELLS AND HUMORAL IMMUNITY

B-cell development

Adaptive humoral immunity is mediated by antibodies produced by plasma cells that develop from B cells under the direction of signals received from T cells and other cells, such as dendritic cells. B cells arise from hemopoietic stem cells in the bone marrow. Commitment to the B-cell lineage is under the control of several transcription factors, such as PU.1, IKAROS (IKAROS family zinc finger 1), E2A, EBF (early B cell factor 1), PAX5 (paired box gene 5) and IRF8 (interferon regulatory factor 8).[18-20] In the bone marrow B cells pass through several distinct developmental stages, during which they acquire their antigen specificity (Fig 4, *A*). Reaching the immature stage, B cells exit the marrow and complete development to the mature or naive stage. This is signaled by the appearance of IgD in addition to IgM on the cell surface. This entire developmental sequence occurs in the absence of any contact with exogenous antigen. Thus it is called antigen-independent B-cell development. Any genetic mutations affecting components of the pre-B cell receptor or the signaling pathways connected to it (Fig 5) lead to immunodeficiency with agammaglobulinemia and absence of B cells.[21]

The genes encoding immunoglobulins are assembled from segments in a manner entirely analogous to the process for TCR genes. Heavy chains are assembled from 4 segments (V_H, D, J_H and C_H); light chains are assembled from 3 segments (V_L, J_L, and C_L). There are 9 different heavy chain types (IgM, IgD, IgG1-4, IgA1 and IgA2, and IgE) and 2 light chain types (κ and λ). The heavy chain genes are on chromosome 14, and the κ and λ genes are on chromosomes 2 and 22, respectively. Immunoglobulin structure is considered in detail in the chapter "Structure and function of immunoglobulins."

TABLE I. B-cell subpopulations in peripheral blood

Surface phenotype	B-cell subset
$IgM^+IgD^-CD27^-$	Immature
$IgM^+IgD^+CD27^-$	Naive
$IgM^+IgD^+CD27^+$	Marginal zone (unswitched memory)
$IgM^-IgD^-CD27^+$	Germinal center (switched memory)*
$CD38^{low}CD21^{low}$	Uncharacterized†
$CD38^{high}IgM^{high}$	Transitional (activated)
$CD38^{high}IgM^-$	Plasmablast

*Reduction in this population is associated with several complications of common variable immunodeficiency.
†Expansion of this (thus far) otherwise uncharacterized population is seen in patients with autoimmune diseases, such as lupus, and in patients with common variable immunodeficiency with autoimmune complications.

B-cell subsets

In mice the presence of the surface marker CD5 distinguishes a population of B1 B cells with distinct characteristics: they develop early in ontogeny, they tend not to undergo somatic hypermutation (SHM; see below), and they secrete IgM antibody with polyspecificity, including binding to self-antigens.[22] The $CD5^-$ population is called B2 or conventional B cells. B cells expressing CD5 also exist in human subjects, and at least a subset of these cells might have characteristics similar to those of murine B1 cells. However, clear-cut phenotypically and functionally distinct B1- and B2-cell sublineages are not well described in human subjects. Furthermore, the marginal zone of the periarteriolar lymphoid sheath in the murine spleen contains B cells with a particular role in responding to so-called T-independent type 2 antigens (see below).[23] The histologic structure of the human spleen is distinct, and it is not yet clear whether an identical distinct population of marginal-zone B cells exists in human subjects.

Several subpopulations of B cells in peripheral blood can be distinguished based on surface-marker expression (Table I).

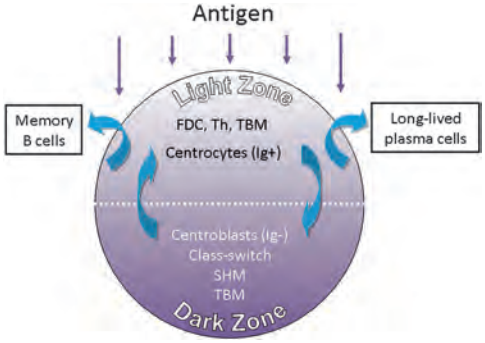

FIG 6. Diagram of a germinal center. Cells (and antigens) enter the light zone, which is positioned to facilitate exposure to sources of antigen (eg, intestinal lumen, splenic arterioles, and subcapsular sinus in lymph nodes). This area has a high concentration of follicular dendritic cells *(FDC)*, T_H cells, and tingible body macrophages *(TBM)*, which are engulfing apoptotic B cells. Light zone B cells (centrocytes) that interact effectively with FDCs and T_H cells lose expression of immunoglobulin and migrate to the dark zone and become centroblasts. Here they undergo immunoglobulin class-switching and SHM. If these processes destroy the ability to express immunoglobulin, the cells die by means of apoptosis and are engulfed by TBM. If they succeed in expressing immunoglobulin again, they migrate back to the light zone and interact with antigen on FDCs. If antigen specificity has been lost because of SHM, the cells die by means of apoptosis and are engulfed by TBM. The B cells with the highest affinity for antigen receive the most effective activating signals and are able to express more peptides for recognition by T_H cells. These cells might become memory cells or long-lived plasma cells (whereupon they leave the germinal center), or they might re-enter the dark zone and repeat the cycle.

These mainly represent different developmental stages and pathways, as described above (and below) and in Fig 4. Alterations of some of these populations have been associated with clinical phenotypes in immunodeficiency and autoimmune disease.[24]

B-cell activation

The second phase of B-cell development occurs after encounter with antigen and activation and is called the antigen-dependent phase (Fig 4, *B*). Depending on the various contacts and cytokine stimuli received by the activated cell (discussed below), it will become either a memory cell to be activated once again in the future or it will become a plasma cell producing large amounts of antibody.

T-independent antigens. Some antigens elicit antibody formation in the absence of T cells, and are called T-independent (TI) antigens. Although the phenomenon is most clearly seen in murine models, similar mechanisms of B-cell activation exist in human subjects. Certain molecules, such as some plant lectins (eg, pokeweed mitogen), are alone capable of inducing proliferation and antibody production from mature B cells. These are called TI type 1 antigens.[25]

Some macromolecules, such as polymerized proteins or polysaccharides, possess repeating molecular patterns that can interact with multiple immunoglobulin receptors on the cell surface and cross-link them. This might deliver a partially activating signal that can progress to memory or plasma cell development with only the additional signals provided by cytokines or other cell contacts provided by dendritic cells.[26] These are called TI type 2 antigens. In many cases the antigens themselves might also provide more than 1 activating signal because some might interact with other receptor systems, such as TLR.[27]

Another important signaling system in direct dendritic cell–B-cell interactions involves transmembrane activator and CamL interactor (TACI, also TNFRSF13B), which is expressed on activated B cells.[28] One TACI ligand, a proliferation inducing ligand (APRIL, also TNFSF13), is expressed on a broad range of leukocytes. Another TACI ligand, B cell–activating factor (BAFF, also TNFSF13B) is expressed on dendritic cells and myeloid cells. In combination with the signals described above, this system can promote immunoglobulin isotype switching (see below) independently of T cells. This process could underlie some rapid responses to polysaccharide antigens to provide adequate immunity before the recruitment of effective T-cell help.

T-dependent antigens. The vast majority of antibody responses to proteins and glycoproteins require participation of T cells, and these antigens are called T dependent. Mature B cells recirculate through secondary lymphoid organs, including lymph nodes, the spleen, and mucosal-associated lymphoid tissues. In the lymph nodes B cells are concentrated in the cortex in primary follicles in contact with follicular dendritic cells. T cells are in the paracortical areas. Low-molecular-weight antigens might diffuse directly into B-cell areas in secondary lymphoid tissues. Larger molecules require transport by means of cellular mechanisms that are still being elucidated.[29] Antigens complexed to varying to degrees with IgM, IgG, and complement might be carried on the surfaces of specialized macrophages, follicular dendritic cells, or even B cells themselves, all of which have receptors for IgG Fc and complement fragments. Antigen presented on these surfaces can stimulate B cells through immunoglobulin receptor cross-linking, expression of other interacting surface molecules, and cytokine secretion.

B cells require 2 principal types of signals to become activated. Signal 1 is delivered by cross-linking of the immunoglobulin receptor, as described above. This cross-linking leads to activation of intracellular signaling pathways (Fig 5) that render the cell capable of interacting with T cells and thereby receiving signal 2. B cells are active as APCs and express peptides along with MHC class II on their surface. These peptides can arise from processed antigen that was internalized after binding to the B-cell surface immunoglobulin receptor. When the B cell contacts a $CD4^+$ T cell specific for such a peptide with self-MHC class II and having been previously activated by an APC, the T cell is able to provide cognate (direct cellular contact) help and activate the B cell for further differentiation into memory cells or plasma cells.

The cognate interaction between T cells and B cells is analogous to the interaction between T cells and dendritic cells. B cells express many of the same costimulating molecules found on dendritic cells, such as CD40, B7-1 (CD80), and B7-2 (CD86). T cells and B cells form an analogous immunologic synapse, and the signaling pathways involved are similar. This initial interaction takes place at the margin between primary follicles and T-cell areas in secondary lymphoid tissues. The activated B cells enter one of 2 pathways. Either they immediately become short-lived plasma cells secreting low-affinity antibody without somatic mutation, or they enter a follicle to establish a germinal center (Fig 4).[30]

In the germinal center (Fig 6) B cells can change from the production of IgM and IgD to other isotypes, such as IgG, IgA, and IgE. This is called class-switching.[31] This process occurs through a mechanism of gene rearrangement somewhat analogous to the process of TCR and B-cell receptor gene segment rearrangement described above. In class-switching a DNA sequence between the VDJ unit and the genes encoding IgM and IgD is cut and ligated to a similar sequence in front of another immunoglobulin C-region gene encoding any of the subclasses of IgG, IgA, or IgE. The

result is the loss of the intervening DNA and the production of an antibody with the same specificity (same VDJ unit) with a new C-region isotype. The process of class-switching is partly under cytokine control. For example, IL-4 and IL-13 promote switching to IgE.[32] IFN-γ can antagonize this effect. IL-10 and TGF-β promote switching to IgA.[33]

At the same time that class-switching is occurring, a mechanism of nucleotide substitution is activated, leading to the accumulation of point mutations in the immunoglobulin heavy and light chain variable regions. This process is known as SHM.[34,35] The enzymes activation-induced cytidine deaminase and uracil nucleoside glycosylase, among others, are important for the DNA cutting and splicing events of class-switching, as well as for the nucleotide substitutions leading to SHM. Lack of either activation-induced cytidine deaminase or uracil nucleoside glycosylase enzymes leads to immunodeficiency (forms of hyper-IgM syndrome). As a result of the selection mechanisms operating in the germinal center, SHM leads to the production of antibodies with higher affinity for antigen. This is known as affinity maturation.

The immune response to the first exposure to an antigen is called the primary response. It is relatively slow (it takes a few weeks to develop fully) and leads to production of predominantly IgM antibody of relatively low affinity. Other isotypes, such as IgG, IgA, or IgE, appear relatively late (2 weeks or longer) and show higher affinity (affinity maturation). During the primary response, memory T cells and B cells are generated. In a subsequent exposure to the same antigens (pathogen), these cells are activated more quickly in comparison with a primary response, so that production of high-affinity IgG (or IgA or IgE) is established quickly (within 1 week). This is called a secondary response.

CONCLUSION

Phylogenetically ancient mechanisms of innate immunity are still critical for the protection of more highly evolved organisms from many pathogens. The evolution of pathogens that themselves had the capacity to alter their molecular patterns to evade innate immune mechanisms drove the counter-evolution of the mechanisms of adaptive immunity briefly reviewed above. The key feature of adaptive immunity is the vast repertoire of T- and B-lymphocyte receptor specificities generated through the somatic recombination of gene segments. Another important feature is the generation of immunologic memory or the ability of the system to learn or record its experiences of encounters with various pathogens in a manner leading to even more effective and rapid responses with subsequent challenges with the same or similar infections. The ability to generate such a wide repertoire of specificities vastly increases the opportunities for inappropriate attack against self-components. Thus a third principal feature of adaptive immunity is the requirement for complex and robust regulatory systems to prevent such attack.

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Structure and function of immunoglobulins

Harry W. Schroeder, Jr, MD, PhD,[a] and Lisa Cavacini, PhD[b] *Birmingham, Ala, and Boston, Mass*

Immunoglobulins are heterodimeric proteins composed of 2 heavy and 2 light chains. They can be separated functionally into variable domains that bind antigens and constant domains that specify effector functions, such as activation of complement or binding to Fc receptors. The variable domains are created by means of a complex series of gene rearrangement events and can then be subjected to somatic hypermutation after exposure to antigen to allow affinity maturation. Each variable domain can be split into 3 regions of sequence variability termed the complementarity-determining regions (CDRs) and 4 regions of relatively constant sequence termed the framework regions. The 3 CDRs of the heavy chain are paired with the 3 CDRs of the light chain to form the antigen-binding site, as classically defined. The constant domains of the heavy chain can be switched to allow altered effector function while maintaining antigen specificity. There are 5 main classes of heavy chain constant domains. Each class defines the IgM, IgG, IgA, IgD, and IgE isotypes. IgG can be split into 4 subclasses, IgG1, IgG2, IgG3, and IgG4, each with its own biologic properties, and IgA can similarly be split into IgA1 and IgA2. (J Allergy Clin Immunol 2010;125:S41-52.)

Key words: *Antibody structure, antibody function, immunoglobulin structure, immunoglobulin function, immunoglobulin gene rearrangement, class switching, somatic hypermutation*

Abbreviations used

ADCC:	Antibody-dependent cellular cytotoxicity
AID:	Activation-induced cytosine deaminase
C:	Constant
CDR:	Complementarity-determining region
CSR:	Class-switch recombination
FcR:	Fc receptor
FcRn:	Neonatal Fc receptor
FR:	Framework region
Fv:	Fab variable fragment
H:	Heavy
IgSF:	Immunoglobulin superfamily
J:	Joining
L:	Light
NHEJ:	Nonhomologous end-joining
pIgA:	Polymeric IgA
pIgR:	Polymeric immunoglobulin receptor
ΨLC:	Surrogate or pseudo-light chain
RAG:	Recombination-activating gene
RSS:	Recombination signal sequence
SC:	Secretory component
SHM:	Somatic hypermutation
sIgA:	Secretory IgA
V:	Variable

In 1890, von Behring and Kitasato reported the existence of an agent in the blood that could neutralize diphtheria toxin. The following year, reference was made to "Antikörper," or antibodies, in studies describing the ability of the agent to discriminate between 2 immune substances. Subsequently, the substance that induces the production of an antibody was referred to as the "Antisomatogen + Immunkörperbildner," or the agent that induces the antibody. The term "antigen" is a contraction of this term. Thus an antibody and its antigen represent a classic tautology.

In 1939, Tiselius and Kabat used electrophoresis to separate immunized serum into albumin, α-globulin, β-globulin, and γ-globulin fractions. Absorption of the serum against the antigen depleted the γ-globulin fraction, yielding the terms γ-globulin, immunoglobulin, and IgG. "Sizing" columns were then used to separate immunoglobulins into those that were "heavy" (IgM), "regular" (IgA, IgE, IgD, and IgG), and "light" (light chain dimers).

From [a]the Division of Clinical Immunology and Rheumatology, Departments of Medicine, Microbiology, and Genetics, University of Alabama at Birmingham, and [b]the Department of Medicine, Beth Israel Deaconess Medical Center, Boston.

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Reprint requests: Harry W. Schroeder, Jr, MD, PhD, Department of Medicine, University of Alabama at Birmingham, SHEL 176, 1530 3rd Ave S, Birmingham, AL 35294-2182. E-mail: hwsj@uab.edu.

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More than 100 years of investigation into the structure and function of immunoglobulin has only served to emphasize the complex nature of this protein. Typically, receptors bind to a limited and defined set of ligands. However, although individual immunoglobulin also bind a limited and defined set of ligands, immunoglobulins as a population can bind to a virtually unlimited array of antigens sharing little or no similarity. This property of adjustable binding depends on a complex array of mechanisms that alter the DNA of individual B cells. Immunoglobulins also serve 2 purposes: that of cell-surface receptors for antigen, which permit cell signaling and cell activation, and that of soluble effector molecules, which can individually bind and neutralize antigens at a distance. The molecular mechanisms that permit these many and varied functions are the focus of this chapter.

STRUCTURAL ELEMENTS

The immunoglobulin domain: The basic immunoglobulin superfamily building block

Immunoglobulins belong to the eponymous immunoglobulin superfamily (IgSF).[1-3] They consist of 2 heavy (H) and 2 light (L) chains (Fig 1), where the L chain can consist of either a κ or a λ chain. Each component chain contains one NH2-terminal variable (V) IgSF domain and 1 or more COOH-terminal constant (C) IgSF domains, each of which consists of 2 sandwiched β-pleated sheets pinned together by a disulfide bridge between 2 conserved cysteine residues.[1] Each V or C domain consists of approximately 110 to 130 amino acids, averaging 12,000 to 13,000 kd. Both immunoglobulin L chains contain only 1 C domain, whereas immunoglobulin H chains contain either 3 or 4 such domains. H chains

with 3 C domains tend to include a spacer hinge region between the first (C_H1) and second (C_H2) domains. A typical L chain will thus mass approximately 25 kd, and a 3 C domain Cγ H chain with its hinge will mass approximately 55 kd. Considerable variability is allowed to the amino acids that populate the external surface of the IgSF domain and to the loops that link the β strands. These solvent-exposed surfaces offer multiple targets for docking with other molecules.

Antigen recognition and the Fab

Early studies of immunoglobulin structure were facilitated by the use of enzymes to fragment IgG molecules. Papain digests IgG into 2 Fab fragments, each of which can bind antigen, and a single Fc fragment. Pepsin splits IgG into an Fc fragment and a single dimeric $F(ab)_2$ that can cross-link, as well as bind, antigens. The Fab contains 1 complete L chain in its entirety and the V and C_H1 portion of 1 H chain (Fig 1). The Fab can be further divided into a variable fragment (Fv) composed of the V_H and V_L domains, and a constant fragment composed of the C_L and C_H1 domains. Single Fv fragments can be genetically engineered to recapitulate the monovalent antigen-binding characteristics of the original parent antibody.[4]

Intriguingly, a subset of antibodies in a minority of species (camelids[5] and nurse shark[6]) lack light chains entirely and use only the heavy chain for antigen binding. Although these unusual variants are not found in human subjects, there are a number of ongoing attempts to humanize these types of antibodies for therapeutic and diagnostic purposes.[7]

Paratopes, epitopes, idiotypes, and isotypes

Immunoglobulin-antigen interactions typically take place between the paratope, the site on the immunoglobulin at which the antigen binds, and the epitope, which is the site on the antigen that is bound. *In vivo* immunoglobulins tend to be produced against intact antigens in soluble form and thus preferentially identify surface epitopes that can represent conformational structures that are noncontiguous in the antigen's primary sequence. This ability to identify component parts of the antigen independently of the rest makes it possible for the B cell to discriminate between 2 closely related antigens, each of which can be viewed as a collection of epitopes. It also permits the same antibody to bind divergent antigens that share equivalent or similar epitopes, a phenomenon referred to as cross-reactivity.

Immunization of heterologous species with mAbs (or a restricted set of immunoglobulins) allowed the identification of both common and individual immunoglobulin antigenic determinants. Individual determinants, termed idiotypes, are contained within V domains. Common determinants, termed isotypes, are specific for the constant portion of the antibody and allow grouping of immunoglobulins into recognized classes, with each class defining an individual type of C domain. Determinants common to subsets of individuals within a species yet differing between other members of that species are termed allotypes and define inherited polymorphisms that result from gene alleles.[8]

IMMUNOGLOBULIN GENE ORGANIZATION AND REARRANGEMENT

Immunoglobulin heavy and light chains are each encoded by a separate multigene family,[9,10] and the individual V and C domains are each encoded by independent elements: *V(D)J* gene segments for the V domain and individual exons for the C domains. The primary sequence of the V domain is functionally divided into 3 hypervariable intervals termed complementarity-determining regions (CDRs) that are situated between 4 regions of stable sequence termed framework regions (FRs; Fig 1).

Immunoglobulin rearrangement

Each *V* gene segment typically contains its own promoter, a leader exon, an intervening intron, an exon that encodes the first 3 framework regions (FRs 1, 2, and 3), CDRs 1 and 2 in their entirety, the amino-terminal portion of CDR3, and a recombination signal sequence (RSS). Each joining (J) gene segment begins with its own recombination signal, the carboxy terminal portion of CDR3, and the complete FR4 (Figs 1 and 2).

The creation of a V domain is directed by the RSSs that flank the rearranging gene segments. Each RSS contains a strongly conserved 7-bp (or heptamer) sequence (eg, *CACAGTG*) that is separated from a less well-conserved 9-bp (or nonamer) sequence (eg, *ACAAAACCC*) by either a 12- or 23-bp spacer. These spacers place the heptamer and nonamer sequences on the same side of the DNA molecule separated by either 1 or 2 turns of the DNA helix. A 1-turn *RSS* (12-bp spacer) will preferentially recognize a 2-turn signal sequence (23-bp spacer), thereby avoiding wasteful *V-V* or *J-J* rearrangements.

Initiation of the *V(D)J* recombination reaction requires recombination-activating genes (RAGs) 1 and 2, which are almost exclusively expressed in developing lymphocytes.[11] RAG1 and RAG2 introduce a DNA double-strand break between the terminus of the rearranging gene segment and its adjacent RSS. These breaks are then repaired by ubiquitously expressed components of a DNA repair process, which is known as nonhomologous end-joining (NHEJ), that are common to all cells of the body. Thus although mutations of RAG affect only lymphocytes, loss or alteration-of-function mutations in NHEJ proteins yield susceptibility to DNA damage in all cells of the body. The NHEJ process creates precise joins between the *RSS* ends and imprecise joins of the coding ends. Terminal deoxynucleotidyl transferase (TdT), which is expressed only in lymphocytes, can variably add non–germline-encoded nucleotides (N nucleotides) to the coding ends of the recombination product.

Typically, the initial event in recombination will be recognition of 12-bp spacer *RSS* by RAG1. RAG2 then associates with RAG1 and the heptamer to form a synaptic complex. Binding of a second RAG1 and RAG2 complex to the 23-bp, 2-turn *RSS* permits the interaction of the 2 synaptic complexes to form what is known as a paired complex, a process that is facilitated by the actions of the DNA-bending proteins HMG1 and HMG2.

After paired complex assembly, the RAG proteins single-strand cut the DNA at the heptamer sequence. The 3′ OH of the coding sequence ligates to 5′ phosphate and creates a hairpin loop. The clean-cut ends of the signal sequences enable formation of precise signal joints. However, the hairpin junction created at the coding ends must be resolved by renicking the DNA, usually within 4 to 5 nucleotides from the end of the hairpin. This forms a 3′ overhang that is amenable to further modification. It can be filled in through DNA polymerases, be nibbled, or serve as a substrate for TdT-catalyzed N addition. DNA polymerase μ, which shares homology with TdT, appears to play a role in maintaining the integrity of the terminus of the coding sequence.

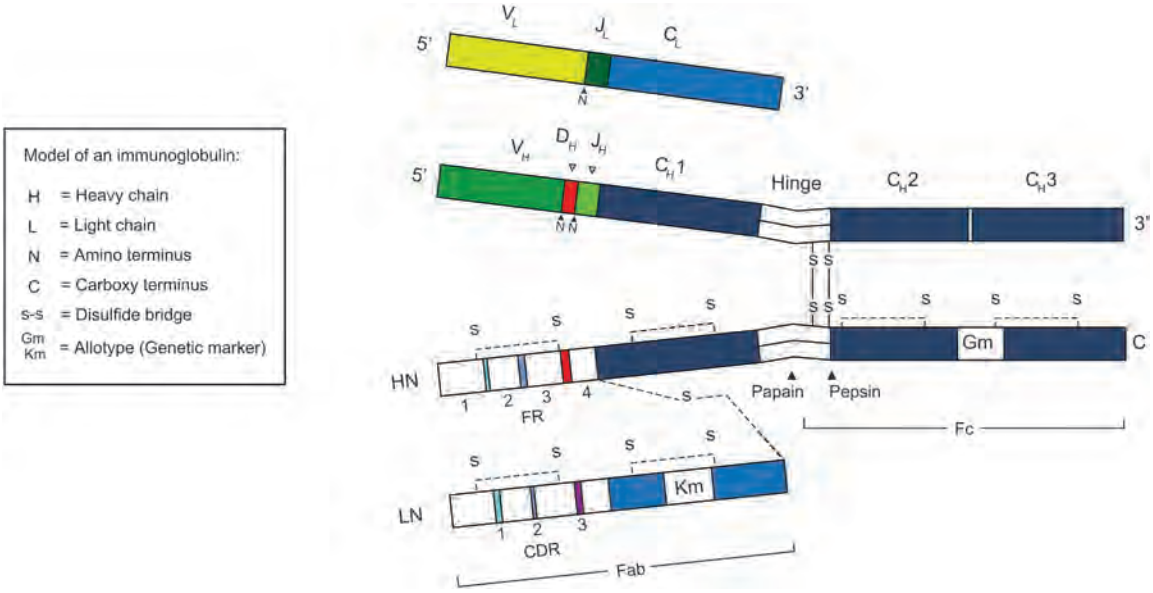

FIG 1. Two-dimensional model of an IgG molecule. The H and L chains at the top deconstruct the antibody at a nucleotide level. The chains at the bottom deconstruct the protein sequence. See the text for further details.

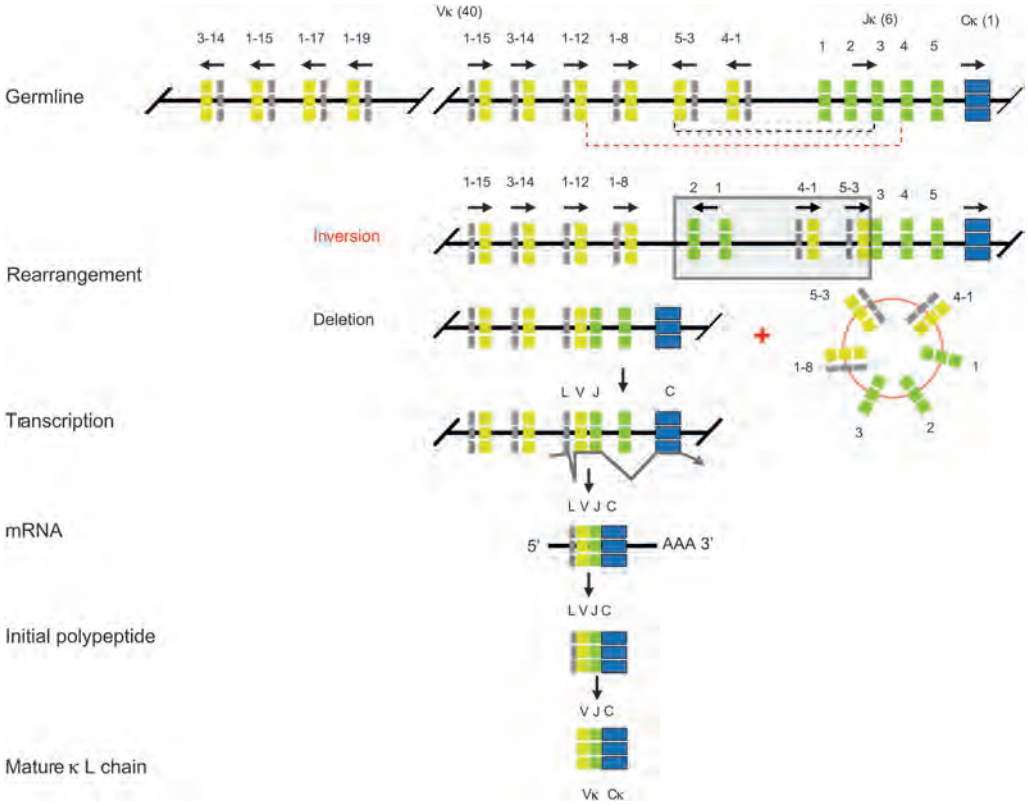

FIG 2. Rearrangement events in the human κ locus. See the text for further details.

The cut ends of the coding sequence are then repaired by the NHEJ proteins. NHEJ proteins involved in *V(D)J* recombination include Ku70, Ku80, DNA-PKcs, Artemis, XRCC4, and ligase.[4] Ku70 and Ku80 form a heterodimer (Ku) that directly associates with DNA double-strand breaks to protect the DNA ends from degradation, permit juxtaposition of the ends to facilitate coding end ligation, and help recruit other members of the repair complex. DNA-PKcs phosphorylates Artemis, inducing an endonuclease activity that plays a role in the opening of the coding joint hairpin. Finally, XRCC4 and ligase 4 help rejoin the ends of the broken DNA. Deficiency of any of these proteins creates sensitivity to DNA breakage and can lead to a severe combined immunodeficiency phenotype.

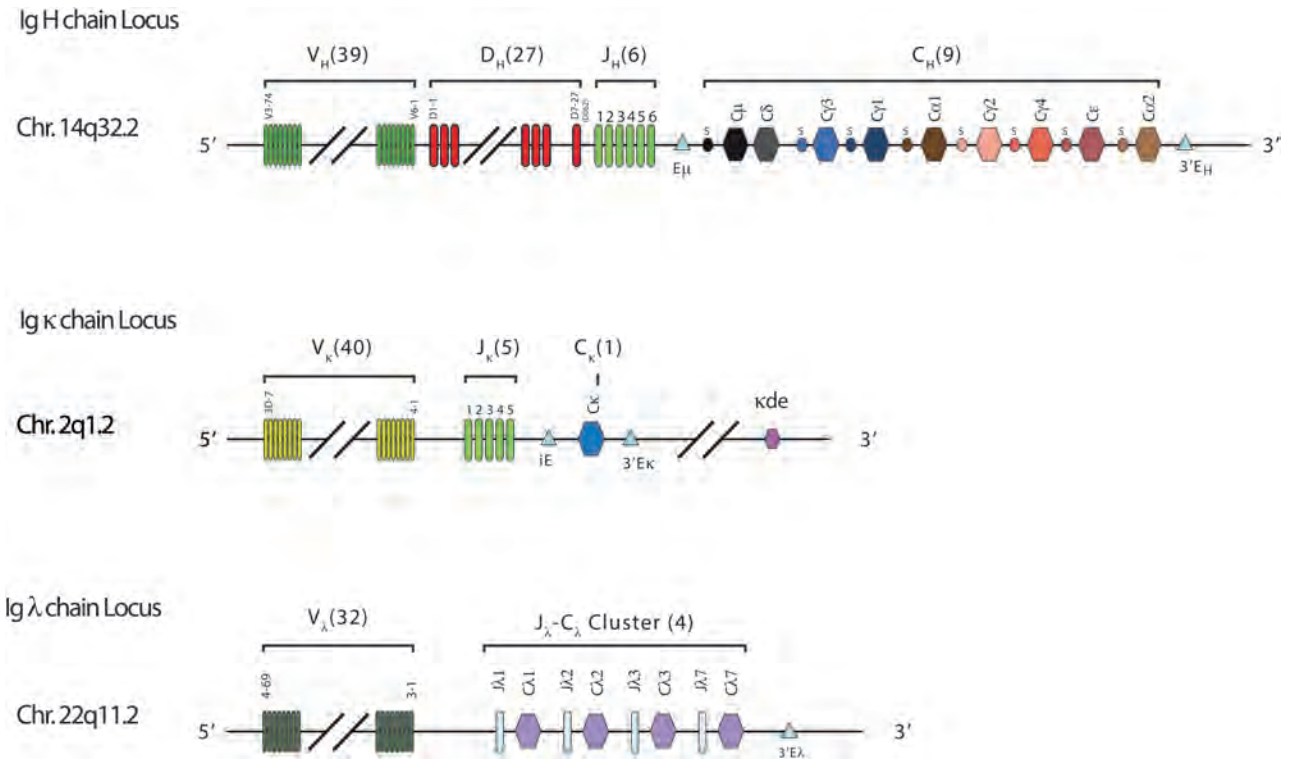

FIG 3. Representation of the chromosomal organization of the immunoglobulin H, κ, and λ gene clusters. The typical numbers of functional gene segments are shown. The κ gene cluster includes a κ-deleting element that can rearrange to sequences upstream of Cκ in cells that express λ chains, reducing the likelihood of dual κ and λ light chain expression.

The κ locus

The κ locus is located on chromosome 2p11.2.[12] κ V domains represent the joined product of *V*κ and *J*κ gene segments (Fig 2), whereas the κ C domains are encoded by a single *C*κ exon. The locus contains 5 *J*κ and 75 *V*κ gene segments upstream of *C*κ (Fig 3). One third of the *V*κ gene segments contain frameshift mutations or stop codons that preclude them from forming functional protein, and of the remaining sequences, less than 30 of the *V*κ gene segments have actually been found in functional immunoglobulins. *V* gene segments can be grouped into families on the basis of sequence and structural similarity.[13,14] There are 6 such families for *V*κ.

Each active *V*κ gene segment has the potential to rearrange to any of the 5 *J*κ elements, generating a potential "combinatorial" repertoire of more than 140 distinct *VJ* combinations. The *V*κ gene segment contains FR1, FR2, and FR3; CDR1 and CDR2; and the amino-terminal portion of CDR3. The *J*κ element contains the carboxy terminus of CDR3 and FR4 in its entirety. The terminus of each rearranging gene segment can undergo a loss of 1 to 5 nucleotides during the recombination process, yielding additional junctional diversity. In human subjects TdT can introduce random N nucleotides to either replace some or all of the lost *V*κ or *J*κ nucleotides or to add to the original germline sequence.[15] Each codon created by N addition increases the potential diversity of the repertoire 20-fold. Thus the initial diversification of the κ repertoire is focused at the *VJ* junction that defines the light chain CDR3, or CDR-L3.

The λ locus

The λ locus, which is located on chromosome 22q11.2, contains 4 functional *C*λ exons, each of which is associated with its own *J*λ (Fig 3). *V*λ genes are arranged in 3 distinct clusters, each containing members of different *V*λ families.[16] Depending on the individual haplotype, there are approximately 30 to 36 potentially functional *V*λ gene segments and an equal number of pseudogenes.

During early B-cell development, H chains form a complex with unconventional λ light chains, known as surrogate or pseudo-light chains (ΨLC), to form a pre–B-cell receptor. The genes encoding the ΨLC proteins λ14.1 (λ5) and V_{preB} are located within the λ light chain locus on chromosome 22. Together, these 2 genes create a product with considerable homology to conventional λ light chains. A critical difference between these unconventional *ΨLC* genes and other L chains is that *λ14.1* and V_{preB} gene rearrangement is not required for *ΨLC* expression. The region of the *ΨLC* gene that corresponds to CDR-L3 covers CDR-H3 in the pre–B-cell receptor, allowing the pre–B cell to avoid antigen-specific selection.[17]

The H chain locus

The H chain locus, which is located on chromosome 14q32.33, is considerably more complex than the light chain clusters. The approximately 80 V_H gene segments near the telomere of the long arm of chromosome 14 can be grouped into 7 different families of related gene segments.[18] Of these, approximately 39 are

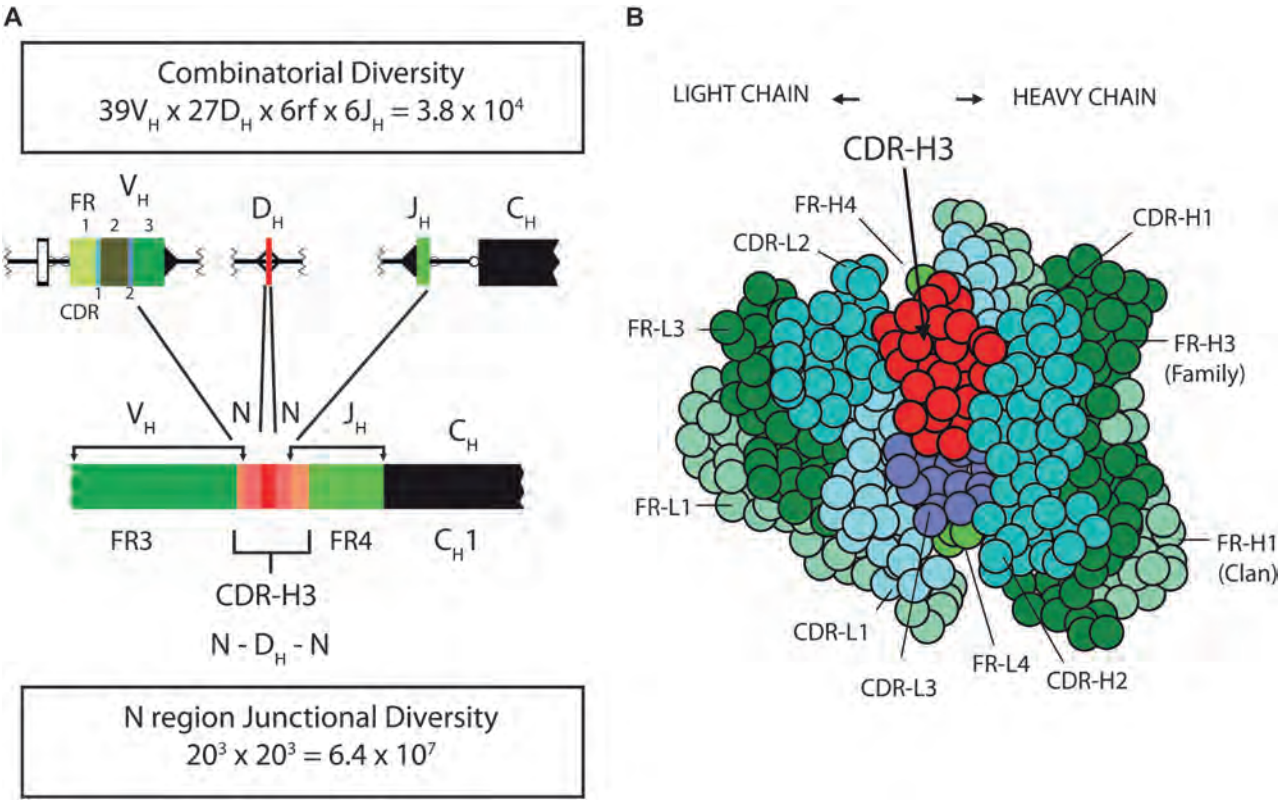

FIG 4. The antigen-binding site is the product of a nested gradient of diversity. **A**, H chain rearrangement can yield as many as 38,000 different VDJ combinations. The addition of 9 N nucleotides on either side of the D gene segment can yield up to 64,000,000 different CDR-H3 junctional sequences. **B**, The view is looking into the binding site as an antigen would see the antigen-binding site. This site is created by the juxtaposition of the 3 CDRs of the H chain and the 3 CDRs of the light chain. The V_H domain is on the right side. The central location of CDR-H3, which because of N addition is the focus for repertoire diversity, is readily apparent.

functional. Adjacent to the most centromeric V_H, *V6-1*, are 27 D_H (D for diversity) gene segments (Fig 3)[19] and 6 J_H gene segments. Each V_H and J_H gene segment is associated with a 2-turn *RSS*, which prevents direct $V \rightarrow J$ joining. A pair of 1-turn *RSS*s flanks each D_H segment. Recombination begins with the joining of a D_H to a J_H gene segment, followed by the joining of a V_H element to the amino-terminal end of the *DJ* intermediate. The V_H gene segment contains FR1, FR2, and FR3; CDR1 and CDR2; and the amino-terminal portion of CDR3. The D_H gene segment forms the middle of CDR3, and the J_H element contains the carboxy terminus of CDR3 and FR4 in its entirety (Fig 1). Random assortment of one of approximately 39 active V_H and one of 27 D_H gene segments with one of the 6 J_H gene segments can generate more than 10^4 different *VDJ* combinations (Fig 4).

Although combinatorial joining of individual *V*, *D*, and *J* gene segments maximizes germline-encoded diversity, the junctional diversity created by *VDJ* joining is the major source of variation in the preimmune repertoire (Fig 4). First, D_H gene segments can rearrange by either inversion or deletion, and each D_H gene segment can be spliced and translated in each of the 3 potential reading frames. This gives each D_H gene segment the potential to encode 6 different peptide fragments.

Second, the rearrangement process proceeds through a step that creates a hairpin ligation between the 5′ and 3′ termini of the rearranging gene segment. Nicking to resolve the hairpin structure leaves a 3′ overhang that creates a palindromic extension, termed a P junction, that can add germline-encoded nucleotides.

Third, the terminus of each rearranging gene segment can undergo a loss of 1 to several nucleotides during the recombination process.

Fourth, TdT can add numerous N nucleotides at random to replace or add to the original germline sequence. N nucleotides can be inserted between the *V* and *D* segments, as well as between the *D* and *J* segments. The imprecision of the joining process and variation in the extent of N addition permits generation of CDR-H3s of varying length and structure. As a result, more than 10^7 different H chain *VDJ* junctions, or CDR-H3s, can be generated at the time of gene segment rearrangement. Taken as a whole, somatic variation in CDR3, combinatorial rearrangement of individual gene segments, and combinatorial association between different L and H chains can yield a potential preimmune antibody repertoire of greater than 10^{16} different immunoglobulins.

Class-switch recombination

Located downstream of the VDJ loci are 9 functional C_H genes (Fig 3).[20] These constant genes consist of a series of exons, each encoding a separate domain, hinge, or terminus. All C_H genes can undergo alternative splicing to generate 2 different types of carboxy termini: either a membrane terminus that anchors immunoglobulin on the B-lymphocyte surface or a secreted terminus that occurs in the soluble form of the immunoglobulin. With the exception of $C_H1\delta$, each C_HI constant region is preceded by both an exon that cannot be translated (an *I* exon) and a region of repetitive DNA

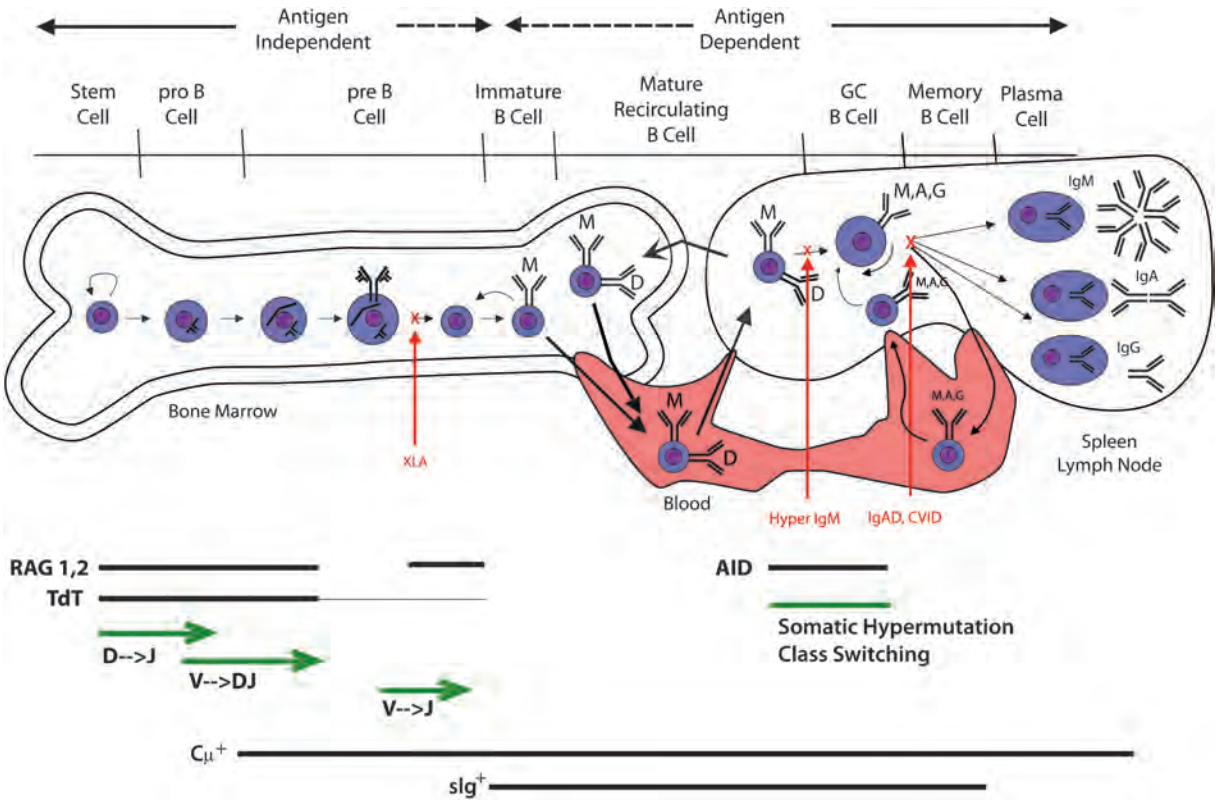

FIG 5. Immunoglobulin diversification and B-cell development. B-cell development as a function of immunoglobulin rearrangement and modification is shown. After birth, B-cell development begins in the bone marrow and is independent of antigen stimulation. The pre–B cell is defined by the presence of cytoplasmic μ protein ($C\mu^+$). With development, the fate of the B cell becomes increasingly dependent on its response to antigen. Immature B cells leave the bone marrow and begin to express IgD. They recirculate through the blood, the secondary lymphoid organs, and the bone marrow. Encounter with cognate antigen can cause the cell to become a memory B cell or a plasma cell. Patients with X-linked agammaglobulinemia *(XLA)* lack Bruton tyrosine kinase function and have difficulty making immature B cells and IgM. Patients with hyper-IgM syndrome *(Hyper IgM)* are unable to class-switch. Patients with selective IgA deficiency *(IgAD)* or common variable immune deficiency *(CVID)* can class-switch but have difficulty becoming plasma cells or memory B cells.

termed the switch. Cocktails of cytokine signals transmitted by T cells or other extracellular influences variably activate the I exon, initiating transcription and thus activating the gene. Through recombination between the $C\mu$ switch region and one of the switch regions of the 7 other H chain constant regions (a process termed class-switching or class-switch recombination [CSR]), the same VDJ heavy chain variable domain can be juxtaposed to any of the H chain classes.[20] This enables the B cell to tailor both the receptor and the effector ends of the antibody molecule to meet a specific need.

Somatic hypermutation

A final mechanism of immunoglobulin diversity is engaged only after exposure to antigen. With T-cell help, the variable domain genes of germinal center lymphocytes undergo somatic hypermutation (SHM) at a rate of up to 10^{-3} changes per base pair per cell cycle. SHM is correlated with transcription of the locus, and in human subjects 2 separate mechanisms are involved: the first mechanism targets mutation hot spots with the *RGYW* (purine/G/pyrimidine/A) motif,[21] and the second mechanism incorporates an error-prone DNA synthesis that can lead to a nucleotide mismatch between the original template and the mutated DNA strand.[22] Other species use gene conversion between functional and nonfunctional V sequences to introduce additional somatic diversity. SHM allows affinity maturation of the antibody repertoire in response to repeated immunization or exposure to antigen.

Activation-induced cytidine deaminase

Activation-induced cytidine deaminase (AID) plays a key role in both CSR and SHM.[11,23] AID is a single-strand DNA cytidine deaminase that can be expressed in activated germinal center B cells.[24] Transcription of an immunoglobulin *V* domain or of the switch region upstream of the C_H1 domain opens the DNA helix to generate single-strand DNA that can then be deaminated by AID to form mismatched dU/dG DNA base pairs. The base excision repair protein uracil DNA glycosylase removes the mismatched dU base, creating an abasic site. Differential repair of the lesion leads to either SHM or CSR. The mismatch repair proteins MSH2 and MSH6 can also bind and process the dU:dG mismatch. Deficiencies of AID and uracil DNA glycosylase underlie some forms of the hyper-IgM syndrome.

Generation of immunoglobulin diversity occurs at defined stages of B-cell development

Creation of immunoglobulin diversity is hierarchical. In pro–B cells $D_H \rightarrow J_H$ joining precedes $V_H \rightarrow DJ_H$ rearrangement, and $V_L \rightarrow J_L$ joining takes place at the late pre–B-cell stage. Production of a properly functioning B-cell receptor is essential for development beyond the pre–B-cell stage. For example, function-loss mutations in RAG1/2 and DNA-dependent protein kinase (DNA-PKcs and Ku 70/80) preclude B-cell development, as well as T-cell development, leading to severe combined immune deficiency. In frame, functional VDJ_H rearrangement allows the pro–B cell to produce μ H chains, most of which are retained in the endoplasmic reticulum. The appearance of cytoplasmic μ H chains defines the pre–B cell.

Pre–B cells whose μ H chains can associate V_{preB} and λ14.1 (λ5), which together form the surrogate light chain (ΨLC), begin to express a pre–B-cell receptor. Its appearance turns off RAG1 and RAG2, preventing further H chain rearrangement (allelic exclusion). This is followed by 4 to 6 cycles of cell division.[25] Late pre–B daughter cells reactivate RAG1 and RAG2 and begin to undergo $V_L \rightarrow J_L$ rearrangement. Successful production of a complete κ or λ light chain permits expression of conventional IgM on the cell surface (sIgM), which identifies the immature B cell. Immature B cells that have successfully produced an acceptable IgM B-cell receptor extend transcription of the H chain locus to include the $C\delta$ exons downstream of $C\mu$. Alternative splicing permits co-production of IgM and IgD. These now newly mature IgM^+IgD^+ B cells enter the blood and migrate to the periphery, where they form the majority of the B-cell pool in the spleen and the other secondary lymphoid organs. The IgM and IgD on each of these cells share the same variable domains.

The lifespan of mature B cells expressing surface IgM and IgD appears entirely dependent on antigen selection. After leaving the bone marrow, unstimulated cells live only days or a few weeks. As originally postulated by Burnet's "clonal selection" theory, B cells are rescued from apoptosis by their response to a cognate antigen. The reaction to antigen leads to activation, which might then be followed by diversification. The nature of the activation process is critical. T cell–independent stimulation of B cells induces differentiation into short-lived plasma cells with limited class switching. T-dependent stimulation adds additional layers of diversification, including SHM of the variable domains, which permits affinity maturation, class-switching to the entire array of classes available, and differentiation into the long-lived memory B-cell pool or into the long-lived plasma cell population.

H CHAIN C DOMAIN STRUCTURE AND FUNCTION

In general, the C domain of the H chain defines effector function, whereas the paired V domains of the antibody confer antigenic specificity. The H chain constant domain is generally defined as C_H1-C_H2-C_H3 (IgG, IgA, and IgD), with an additional domain (C_H4) for IgM and IgE. As described above, the C_H1 domain is located within the F(ab) region, whereas the remaining C_H domains (C_H2-C_H3 or C_H2-C_H4) comprise the Fc fragment. This Fc fragment defines the isotype and subclass of the immunoglobulin. Despite amino acid differences between the isotypes and subclasses, each C_H region folds into a fairly constant structure consisting of a 3-strand/4-strand β sheet pinned together by an intrachain disulfide bond. The Fc fragment mediates effector function by binding to the Fc receptor (FcR) on effector cells or activating other immune mediators, such as complement.[26] For this reason, changes in the Fc region can significantly affect the end result of an antibody-antigen interaction. The Fc region can also affect the affinity or kinetics of binding of the antibody by the Fv region and thus influence antigen recognition or binding.[27]

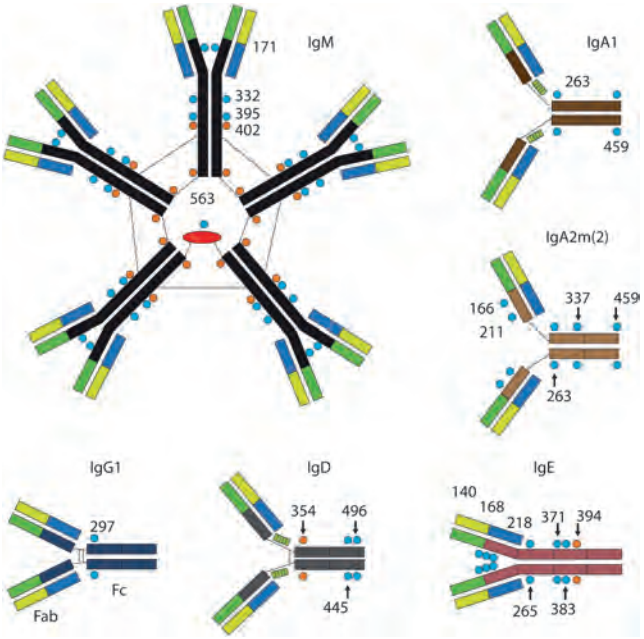

FIG 6. Structural and glycosylation properties of immunoglobulins. Depiction of the structure and glycosylation sites (indicated by amino acid location) for human IgM, IgG, IgD, IgE, IgA2, and IgA2. Adapted from Arnold et al.[28]

Role of glycosylation

Immunoglobulins are glycoproteins, and the glycans associated especially with the Fc domain of immunoglobulins have been shown to affect antibody function. The extent of glycosylation varies by isotype (Fig 6).[28] For IgG molecules, there is an N-linked glycosylation site located at Asn297 on each of the 3 C_H2 domains. The core of this complex biantennary type of sugar is a heptasaccharide consisting of N-acetylglucosamine and mannose. Variation in glycosylation is seen between IgG molecules, as well as within the 3 sites on the same molecule because of differences in terminal sialic acid, galactose, N-acetylglucosamine, and fucosylation of the core. These differences can lead to as many as 32 possible glycosylation patterns. The glycans at this site interact with a hydrophobic pocket on the Fc domain that stabilizes the immunoglobulin structure.[29,30] At a similar site in the C_H2 domain of IgD, Asn354, mutations that prevent glycosylation are associated with the loss of IgD production, suggesting that glycans in the C_H2 domain can be essential for immunoglobulin stability.

Glycans on immunoglobulins profoundly influence binding to FcRs on effector cells, as well as immune mediators. When IgG sequences are mutated such that glycosylation is eliminated, there is reduced or no binding of the aglycosylated IgG to FcγR. This led to the suggestion that the N-glycan at Asn297 was critical for the engagement of IgG with FcγR. This is in contrast to engagement between IgA and IgE and FcαR and FcεR,

TABLE I. Properties of immunoglobulin isotypes/subclasses

	Serum (%)	Structure	Complement fixation	Opsonizing	Cross-placenta	Other functions	FcR
IgG	75	Monomer	+	+++	+	For all IgG subclasses:	FcγR
IgG1	67% IgG	Monomer	Yes	Yes	+	Secondary response	I, II, III
IgG2	22% IgG	Monomer	Yes	Yes	+	Neutralize toxins and	II
IgG3	7% IgG	Monomer	Yes	Yes	+	virus	I, II, III
IgG4	4% IgG	Monomer	No	No	+		I, II
IgM	10	Pentamer	+++	+	−	Primary response	
IgA	15	Monomer, dimer	−	−	−	Mucosal response	FcαR (CD89)
IgA1		Monomer, dimer	−	−	−		
IgA2		Monomer, dimer	−	−	−		
IgD	<0.5	Monomer	−	−	−	Homeostasis	FcδR
IgE	<0.01	Monomer	−	−	−	Allergy	FcεR I, II

respectively, where it had been shown that glycans were not required for interaction. Subsequently, it was shown that point mutations could be introduced to the C_H domains that would permit binding to FcγR in the absence of glycosylation.[31] However, the observation that the Asn297 site is conserved across evolution and that IgG is subjected to posttranslational modifications at this site suggests that glycosylation at this position makes a significant contribution to antibody function *in vivo*.

A key effector function for IgG antibodies is antibody-dependent cellular cytotoxicity (ADCC) in which antibody-coated antigens activate effector cells, such as natural killer cells or monocytes, to destroy the antibody-coated target by binding of the complex to the FcγR. The ADCC activity was shown to be significantly dependent on the glycan composition of the IgG and, furthermore, the net result of binding to activating and inhibitory FcγR. A number of engineered cell lines, as well as glycosidase inhibitors, are available to direct the sugar composition of glycans on an immunoglobulin. Through these studies, it was demonstrated that ADCC activity increases after a reduction in the fucose content of an antibody.[32,33]

Complement-dependent cytotoxicity is another effector function of IgG that is dependent on the binding of C1q to the Fc domain. Glycosylation also plays a role in complement-dependent cytotoxicity, requiring the presence of a complex structure containing at least 2 N-acetylglucosamines with multiple galactoses and sialic acids.

There are also experiments of nature in which aberrantly glycosylated immunoglobulins are associated with detrimental effects. For example, higher than normal levels of IgG lacking sialic acid or galactose are found in patients with a number of autoimmune diseases, rheumatoid arthritis in particular. N-glycans terminate with N-acetylglucosamine, which can activate the complement cascade through mannose-binding lectin and create an inflammatory state.[34] Removal of the majority of the secondary glycan structure with Endo-S (1 N-acetylglucosamine, a terminal fucose, or both remains with the core sugar) has been shown to reduce the pathogenesis and proinflammatory properties of autoantibodies in murine models.[35] This is not limited to IgG because a reduction in terminal galactose on IgA has been associated with decreased clearance of IgA from the circulation, with the subsequent development of nephropathy.

Intravenous immunoglobulin can be used in selected circumstances to ameliorate inflammatory diseases. The anti-inflammatory activity of intravenous immunoglobulin has shown to be associated with sialic acid in a 2,6 linkage to a terminal galactose on IgG. Recently, a receptor specific to this sialyated Fc has been identified on myeloid cells.[36] Engagement of this receptor with sialyated IgG might upregulate inhibitory FcγR to reduce inflammation by means of IgG/activating FcγR engagement.

Immunoglobulin glycosylation can also alter other antibody functions. For example, it has been shown that an anti-HIV antibody that fails to neutralize acquires neutralization activity when expressed in a cell line that results in posttranslational modification of an antibody with a marked increase in sialic acid, fucose, and N-acetylglucosamine levels.[37] It is hypothesized that the glycan interactions between the antibody and virus interfere with the normal infection process.

O-linked glycans also play a pivotal role in the immune response. There are several potential O-linked sites in the hinge region of IgD and IgA antibodies, which serve to protect the hinge from proteases, bind bacteria, or both. Understanding the effect of differential glycosylation on immunoglobulin function is contributing to the design of more effective immunotherapies through either engineered passive immunotherapy[4] or *in vivo* treatment with glycan modifiers.[38]

Heavy chain isotypes

Early in B-cell development, productively rearranged variable domains (V_H and V_L) are expressed in association with the μ heavy chain to produce IgM and then IgD by means of alternative splicing. Later during development and in response to antigenic stimulation and cytokine regulation, these variable domains can associate with the other isotypes (IgG, IgA, and IgE) in a controlled process; that is, isotype switching does not occur merely by chance. The C_H genes for each isotype are aligned in the same transcriptional orientation on human chromosome 14. Isotypes differ in a number of properties, including size, complement fixation, FcR binding, and isotype response to antigen. The choice of isotype is dependent on the antigen itself and the signaling pathways that are activated, as well as the local microenvironment, as summarized in Table I.

IgM. IgM is the first immunoglobulin expressed during B-cell development. Naive B cells express monomeric IgM on their surface and associate with CD79a and CD79b, polypeptide chains that participate in IgM cell signaling. On maturation and antigenic stimulation, multimeric (usually pentameric and rarely hexameric) IgM, in which single IgM units link to each other by disulfide bonds in the C_H4 region, is secreted (Figure 5). The pentamer also contains a polypeptide chain, the J-chain, which is bound to 2 of the monomers by means of a disulfide bond. The

J-chain facilitates secretion at mucosal surfaces (see below). Generally, although monomeric IgM molecules have low affinity because of their immaturity, high avidity can be attained by means of multimeric interactions between the pentameric secreted antibody and the antigen, especially if that antigen contains multiple repeating epitopes itself. IgM functions by opsonizing (coating) antigen for destruction and fixing complement. The pentameric nature of the antibody renders it very efficient in this process.

IgM antibodies are associated with a primary immune response and are frequently used to diagnose acute exposure to an immunogen or pathogen. Given that IgM is expressed early in B-cell development, the μ heavy chain associates with V_H and V_L regions that have not undergone much somatic mutation in response to antigen. As a result, IgM antibodies tend to be more polyreactive than other isotypes, which allows IgM-bearing B cells to respond quickly to a variety of antigens. These relatively low-affinity IgM antibodies are also called natural antibodies. Some of these natural antibodies not only participate as a first line of defense but also play a role in immunoregulation.[39] Natural antibodies might react with autoantigens but are rarely responsible for autoimmune disease or pathogenesis. Pathogenic autoantibodies tend to be drawn from the somatically mutated, high-affinity IgG population.

IgD. Circulating IgD is found at very low levels in the serum, with a short serum half-life, which can be attributed to the sensitivity of the molecule, with the hinge region in particular, to proteolysis. The function of circulating IgD is unclear because it is not known to participate in the major antibody effector mechanisms. Circulating IgD can react with specific bacterial proteins, such as the IgD-binding protein of *Moraxella catarrhalis*, independently of the variable regions of the antibody.[40] The binding of these bacterial proteins to the constant region of IgD results in B-cell stimulation and activation.

Although the membrane-bound form of IgD has been more extensively studied, even here its function remains poorly understood. Similar to IgM, membrane-bound IgD is associated with CD79a and CD79b for signaling. IgD is expressed on the membranes of B cells when they leave the bone marrow and populate secondary lymphoid organs. Most IgD^+ B cells also coexpress IgM, and both participate in B-cell receptor signaling through CD79a and CD79b. IgD can replace IgM and *vice versa* on IgD^+IgM^+ B cells. It has been proposed that membrane-bound IgD regulates B-cell fate at specific developmental stages through changes in activation status.[41]

IgG. IgG is the predominant isotype found in the body. It has the longest serum half-life of all immunoglobulin isotypes. It is also the most extensively studied class of immunoglobulins. Based on structural, antigenic, and functional differences in the constant region of the heavy chain, C_H1 and C_H3 in particular, 4 IgG subclasses (IgG1, IgG2, IgG3, and IgG4) were identified. These IgG subclasses were numbered in reference to the rank order (IgG1 > IgG2 > IgG3 > IgG4) of the serum levels of these antibodies in the blood of healthy subjects living in an affluent western European environment. The differences in the C_H domains affect antibody flexibility and functional affinity, some of which facilitate cooperative interactions with multivalent antigens. The mobility or flexibility of the F(ab) and Fv portions of the antibody are primarily controlled by the C_H1 domain and hinge region. The IgG subclasses exhibit different functional activities. Activation of the complement cascade is an important means of clearance of opsonized pathogens. Although IgG4 is the only subclass that fails to fix complement, affinity for C1q, which is the first component of the complement pathway and binds to the C_H2 domain of IgG, differs between members of the other 3 IgG subclasses (IgG3 > IgG1 > IgG2). There are also defined differences in the affinity to the 3 classes of FcγR (I, II, and III). IgG1 and IgG3 bind to all 3 FcγR classes. IgG4 binds only FcγRII and FcγRIII, although this binding is significantly weaker than that of IgG1. IgG2 binds only to FcγRII.

There are also similarities within the subclasses, such as transplacental transport and participation in the secondary immune response. Within the secondary antibody response, there is skewing in the predominant subclass that is induced. For example, IgG1 and IgG3 antibodies are generally induced in response to protein antigens, whereas IgG2 and IgG4 antibodies are associated with polysaccharide antigens. The response to a given antigen can also result in a skewed IgG subclass response, and this is frequently a source of investigation as to correlates of protection or for the design of vaccines.

Specific subclasses can be associated with individual disease processes. For example, in patients with pemphigus vulgaris, a mucocutaneous blistering disease, IgG4 antibodies to desmoglein 3 are pathogenic,[42,43] whereas first-degree relatives with IgG1 autoantibodies to the same protein show no evidence of the disease.

IgG antibodies also contribute directly to an immune response, including neutralization of toxins and viruses. Here again, IgG subclass affects the outcome of this interaction. In patients with HIV, it has been shown that IgG3 antibodies can be more effective at neutralizing virus than IgG1 antibodies, presumably through an increase in antibody flexibility, improving antibody access or inducing changes in the oligomer structure of the virus.[44,45]

IgA. IgA serum levels tend to be higher than IgM levels but considerably lower than IgG levels. Conversely, IgA levels are much higher than IgG levels at mucosal surfaces and in secretions, including saliva and breast milk.[46] In particular, IgA can contribute up to 50% of the protein in colostrum, the "first milk" given to the neonate by the mother. Although generally a monomer in the serum, IgA at the mucosa, termed secretory IgA (sIgA), is a dimer (sometimes trimer and tetramer) associated with a J-chain and another polypeptide chain, the secretory component (SC; discussed below). Similar to IgM, the C_H3 domains of IgA have short tailpieces to which the J-chain binds through disulfide bonds, whereas the SC is disulfide bonded to one of the C_H2 domains of the dimer. There are 2 subclasses of IgA, IgA1 and IgA2, with structures that differ mainly in their hinge regions. IgA1 has a longer hinge region with a duplicated stretch of amino acids that is lacking in IgA2. This elongated hinge region increases the sensitivity of IgA1 to bacterial proteases in spite of partial protection by glycans. Such increased protection against protease digestion might explain why IgA2 predominates in many mucosal secretions, such as the genital tract, whereas more than 90% of serum IgA is in the form of IgA1.

IgA is critical at protecting mucosal surfaces from toxins, viruses, and bacteria by means of direct neutralization or prevention of binding to the mucosal surface. Intracellular IgA might also be important in preventing bacterial or viral infection, pathogenesis, or both. The polymeric nature of sIgA might be particularly important. For example, polymeric IgA (pIgA) is more effective than monomeric IgA at preventing *Clostridium difficile* toxin A–induced damage to epithelial cells.[47] Although complement fixation by IgA does not appear to be a major effector mechanism at the mucosal surface, the IgA receptor is expressed

on neutrophils, which might be activated to mediate ADCC locally. As described above, specific bacteria can be trapped by the glycans on IgA. Finally, it has been proposed that sIgA might also act as a potentiator of the immune response in intestinal tissue by means of uptake of antigen to dendritic cells.[48]

IgE. Although it is present at the lowest serum concentration and has the shortest half-life, IgE is a very potent immunoglobulin. It is associated with hypersensitivity and allergic reactions, as well as the response to parasitic worm infections. IgE binds with extremely high affinity to FcεRI, which is expressed on mast cells, basophils, Langerhans cells, and eosinophils. Circulating IgE upregulates FcεR expression on these cells. The combination of strong binding and upregulation of FcεR expression contributes to the remarkable potency of this immunoglobulin.

Recently, there has been the development of anti-IgE antibodies as therapy for allergy and asthma.[49] Antibodies are designed to target free IgE, as well as B cells with membrane-bound IgE, but not IgE bound to FcεR because the latter would stimulate degranulation and the release of inflammatory mediators. IgE has a much lower affinity for FcεRII, or CD23, which is expressed both on the same cells as FcεRI and on B cells, natural killer cells, and platelets.

Higher-order structures

The J-chain. The J-chain is a relatively conserved, 15- to 16-kd polypeptide (137 amino acids) incorporated into pIgA or polymeric IgM in the antibody-producing cell during the secretory pathway. There are 6 cysteine residues for intrachain disulfide bonds plus the 2 cysteines for attachment to the IgA or IgM tailpiece. There is a single N-linked glycan that contributes approximately 8% of the mass to the molecule. This glycan is critical to association with monomeric IgA. Although the J-chain is produced by B cells, it is not necessarily produced by all B cells. It appears that J-chain expression might be restricted to those areas, such as the lamina propria, in which mucosal antibody is important, as opposed to B cells in the distal bone marrow. Free J-chain is not found outside the cell and is only found as part of the polymeric immunoglobulin complex. It has been shown that the J-chain is essential for polymerization and secretion of IgA. In contrast, pentameric IgM requires the J-chain for secretion (but not formation), and hexameric IgM does not require the J-chain at all.

Dimers, pentamers, and hexamers. Polymeric immunoglobulin is generally more effective than monomeric immunoglobulin in terms of binding to FcR on the cell surface. As described above, IgA and IgM molecules have the capacity to be naturally expressed as multimeric antibodies. Both immunoglobulins have a short tailpiece (18 amino acids) in the C_H3 domain, with a penultimate cysteine residue to which the J-chain forms a disulfide bond with one of the monomers, with the other forming a tailpiece-to-tailpiece disulfide bond. Typically dimeric structures are formed for IgA, and pentameric structures are formed for IgM.

FCRS

FcγR

FcRs for immunoglobulin link the humoral immune compartment to the cellular immune compartment. The net result of binding of immunoglobulin to receptor is a function of the receptor, the cell on which it is expressed, and any ancillary signals. Tight regulation of binding to the FcR is necessary to maintain a healthy immune system.

The most extensively studied FcRs are the IgG-binding receptors, termed FcγR. In human subjects 3 classes of FcγR have been identified: I, II, and III. FcγRII and FcγRIII each have 2 isoforms, A and B. These FcγRs are expressed, to varying degrees, on many hematopoietic cells, as well as other cells, such as endothelial cells. T cells have proved to be a stark exception. The FcγRs differ in their binding affinity to IgG, with FcγRI showing the highest affinity, whereas FcγRII and FcγRIII bind with lower affinity. For that reason, only FcγRI binds monomeric IgG, whereas the other 2 receptors bind aggregated IgG or immune complexes. Of note, FcγRI has 3 extracellular domains, whereas FcγRII and FcγRIII have only 2 extracellular domains.

As described above, there are differences in binding of IgG subclasses to FcγR. There are also differences in the signaling pathway that is associated with each FcγR. FcγRI, FcγRIIA, and FcγRIIIA all transduce an activating signal when IgG binds. However, FcγRIIB transmits an inhibitory signal, and no signal is associated with binding to FcγRIIIB. Although the other FcγRs are typical transmembrane proteins, FcγRIIIB lacks this feature and instead is attached by glycophosphatidylinositol tail. The end result of the interaction of antibody and antigen with FcγR tends to be a balancing act between inhibitory and stimulatory activities and a complex function of the IgG subclass, the particular FcγR bound, and the cells expressing the FcγR.[50]

The neonatal FcR

There is another FcγR, the neonatal Fc receptor (FcRn), which was originally shown to mediate the transcytosis of maternal IgG to the neonate. Subsequently, it was determined that the FcRn is also responsible for the regulation of serum IgG levels. IgG binds to FcRn in the acidic environment of the endosomes, which protects it from destruction by lysosomes. The IgG is recycled to the surface and released into circulation by the pH change. The FcRn is saturable, and once IgG levels exceed a threshold, it is degraded by the lysosomes. Whereas the C_H3 domain of IgG Fc binds to FcγR, it is the C_H2-C_H3 region that binds to FcRn. Binding is thus independent of the sugar moiety, which is attached to the C_H2 domain. It should also be noted that binding to FcRn is strictly pH dependent, whereas this is not the case with FcγR. Mutagenesis studies have demonstrated that mutations in the Fc region can increase or decrease interactions with FcRn. For example, mutations at positions 250 and 428 of IgG1 resulted in an increase in serum half-life for the single mutant M428L and the double mutant T250Q/M428L.[51] Others have shown that a single mutation of human IgG1, N434A, and a triple mutant, T307A/E380A/N434A, also show an enhanced half-life when tested in human FcRn transgenic mice.[52] That affinity for FcRn can be increased, resulting in increased immunoglobulin half-life, suggests that improved therapeutics might be designed to decrease dosing.

FcεR

The FcRs for IgE are also relatively well studied, especially in terms of the development of therapeutic anti-IgE antibodies for the treatment of allergy and asthma, as described above. It is the C_H3 domain of IgE that binds to FcεRI and CD23; however, there

are distinct differences in binding. FcεRI captures both C_H3 domains of IgE because of the unique shape of the IgE molecule. On the other hand, CD23 consists of a trimer on the cell surface, and 2 heads of this trimer must separately contact a C_H3 domain of IgE for strong binding.

FcαR

The FcR for IgA, CD89, is expressed on myeloid cells, including PMNs, monocytes, and a population of dendritic cells. There are 5 exons, including 2 extracellular domains, EC1 and EC2, each of which encodes a single immunoglobulin-like domain. IgA binds to membrane-distal EC1 in contrast to the usual binding of IgG to the membrane-proximal extracellular domain of FcγR. Multiple splice variants have been demonstrated, and whereas full-length CD89 binds pIgA with higher affinity than serum IgA, there is no difference in binding to truncated CD89. Signaling through the FcαR is accomplished through the FcR γ-chain which contains an immunoreceptor tyrosine-based activation motif signaling motif. Not all FcαRs associate with γ-chain, resulting in "γ-less" FcRs that endocytose bound IgA to early endosomes and then recycle IgA back to the cell surface. Cross-linking of FcαR with an associated γ-chain results in the activation of a number of signaling molecules in the lipid rafts, calcium release, and induction of nicotinamide adenine dinucleotide phosphate oxidase activity. Outside of endocytosis, the biologic and cellular functions of PMNs after FcαR stimulation are dependent on tyrosine kinase activity of the associated γ-chain. Cross-linking of FcαR has also been shown to induce effector functions, such as phagocytosis and ADCC.

FcδR

The FcR for IgD is less well understood. A receptor for IgD has been reported to be present on human CD4 and CD8 T cells. Its expression is upregulated by mitogenic stimulation of the T cells. Binding of IgD to this putative FcδR is mediated by glycans on the IgD surface and might not necessarily be a function of a defined FcδR. Binding of IgD to receptors with putative FcδR activity on T cells has been proposed to serve as a bridge for stimulation of IgD-expressing B cells or as antigen presentation by the B cells to the T cells, but this remains controversial.

IMMUNOGLOBULIN TRANSPORT

The transport of polymeric immunoglobulin into mucosal secretions is a function of the polymeric immunoglobulin receptor (pIgR). This receptor is found on the basolateral surface of epithelial cells lining the mucosal surface. Membrane-bound pIgR consists of 5 immunoglobulin-like domains (extracellular portion) with a transmembrane and cytoplasmic domain. pIgA (with the J-chain) binds to the pIgR on the epithelial cell. It is then internalized and transcytosed to the apical cell membrane. The extracellular portion of the pIgR is cleaved to form the SC and covalently associates with the pIgA. The complex of pIgA with SC forms sIgA. The SC forms a disulfide link with Cys311 in Cα2 of one of the monomers of the pIgA. Although the SC is not physically associated with the J-chain of the pIgA, the J-chain is required for SC to associate with pIgA. SC is not covalently linked to pentameric IgM but rather associates noncovalently with pentameric IgM because of excess free SC.

Extensive analysis of the glycosylation patterns of the components of sIgA has predicted a model in which most of the molecule is covered in glycans, with the exception of the F(ab) or antigen-binding sites. In this manner sIgA participates in both the adaptive (antigen binding) and innate (adhesion caused by glycans) arms of the immune system. Although the SC does not have a direct role in the biologic activity of sIgA, it does confer some protection from proteolytic cleavage after secretion and anchors the sIgA to mucus lining the epithelium. Moreover, as a result of covalent binding of SC to pIgA, sIgA is the most stable immunoglobulin in secretions.

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Immunologic messenger molecules: Cytokines, interferons, and chemokines

Scott P. Commins, MD, PhD, Larry Borish, MD, and John W. Steinke, PhD *Charlottesville, Va*

Cytokines and chemokines are secreted proteins involved in numerous aspects of cell growth, differentiation, and activation. A prominent feature of these molecules is their effect on the immune system with regard to cell trafficking and development of immune tissue and organs. The nature of an immune response determines which cytokines are produced and ultimately whether the response is cytotoxic, humoral, cell mediated, or allergic. For this chapter, cytokines are grouped according to those that are predominantly antigen-presenting cell or T lymphocyte derived; that mediate cytotoxic, humoral, cell mediated, and allergic immunity; or that are immunosuppressive. A discussion of chemokine function and their role in cell trafficking and disease follows. (J Allergy Clin Immunol 2010;125:S53-72.)

Key words: *Cytokine, chemokine, interferon, antigen-presenting cell, T lymphocyte*

Cytokines are secreted proteins with growth, differentiation, and activation functions that regulate and determine the nature of immune responses. For this review, cytokines are grouped according to those that are predominantly antigen-presenting cell (APC) or T lymphocyte derived; that predominantly mediate cytotoxic (antiviral and anticancer), humoral, cell-mediated (T_H1 and T_H17), or allergic immunity (T_H2); or that are immunosuppressive (regulatory T [Treg]). This is followed by a discussion of the complementary family of secreted immune proteins, the chemokines. Cytokine families are summarized in Table I.

Abbreviations used

ABPA:	Allergic bronchopulmonary aspergillosis
AHR:	Airway hyperreactivity
APC:	Antigen-presenting cell
APRIL:	A proliferation-inducing signal
BAFF:	B-cell activation factor from the TNF family
DC:	Dendritic cell
Foxp3:	Forkhead box protein 3
gp130:	Glycoprotein 130
ICAM:	Intercellular adhesion molecule
IFNGR:	IFN-γ receptor
IL-1ra:	IL-1 receptor antagonist
IL-2R:	IL-2 receptor
IL-4R:	IL-4 receptor
IL-5R:	IL-5 receptor
IL-6R:	IL-6 receptor
IL-10R:	IL-10 receptor
IL-12R:	IL-12 receptor
IL-13R:	IL-13 receptor
IL-17R:	IL-17 receptor
IL-20R:	IL-20 receptor
IL-22R:	IL-22 receptor
IRS:	Insulin response element
iTreg:	Induced regulatory T
JAK:	Janus kinase
MAPK:	Mitogen-activated protein kinase
MCP:	Monocyte chemoattractant protein
M-CSF:	Macrophage colony-stimulating factor
MIP:	Macrophage inflammatory protein
NK:	Natural killer
nTreg:	Natural regulatory T
ROR:	Retinoic acid receptor–related orphan receptor
SCF:	Stem cell factor
STAT:	Signal transducer and activator of transcription
TACI:	Transmembrane activator and calcium modulator and cyclophilin ligand interactor
T-bet:	T-box expressed in T cells
Treg:	Regulatory T
TSLP:	Thymic stromal lymphopoietin
VCAM:	Vascular cell adhesion molecule

CYTOKINE PRODUCTION BY ANTIGEN-PRESENTING CELLS

Cytokines primarily derived from dendritic cells (DCs), mononuclear phagocytes, and other APCs are particularly effective in subserving the dual functions of generating a potent innate immune response and providing signals contributing to initiation and guidance of the nature of the adaptive immune response. The processing of antigens as they are taken up by APCs, metabolized, and presented to T_H lymphocytes provides one pathway for this class of cytokine production. Alternatively, APCs are potently triggered to produce cytokines through their pattern recognition receptors. The cytokines predominantly produced by APCs include TNF, IL-1, IL-6 (and other glycoprotein 130 [gp130]–utilizing factors), CXCL8 (IL-8), and other members of the chemokine family (discussed later), as well as IL-12, IL-15, IL-18, IL-23, IL-27, and IL-32.

TNF

TNF represents 2 homologous proteins primarily derived from mononuclear phagocytes (TNF-α) and lymphocytes (TNF-β).[1] TNF-α is also produced by neutrophils, lymphocytes, natural killer (NK) cells, endothelium, and mast cells. TNF-α is

From the University of Virginia Health System, Carter Center for Immunology Research, Asthma and Allergic Disease Center.

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Reprint requests: Larry Borish, MD, University of Virginia Health System, Asthma and Allergic Disease Center, Box 801355, Charlottesville, VA 22908-1355. E-mail: lb4m@virginia.edu.

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TABLE I. Cytokine families

Family	Members
Hematopoietic	
Common γ chain	IL-2, IL-4, IL-7, IL-9, IL-15, IL-21
Shared β chain (CD131)	IL-3, IL-5, GM-CSF
Shared IL-2β chain (CD122)	IL-2, IL-15
Other hematopoietic	IFN-γ, IL-7, IL-13, IL-21, IL-31, TSLP
IL-1 family	IL-1α, IL-1β, IL-1ra, IL-18, IL-33
gp130-utilizing	IL-6, IL-11, IL-27, IL-31, ciliary neurotrophic factor (CNTF), cardiotrophin 1 (CT-1), leukemia inhibitory factor (LIF), oncostatin M (OSM), osteopontin
IL-12	IL-12, IL-23, IL-35
IL-10 superfamily	IL-10, IL-19, IL-20, IL-22, IL-24, IL-26, IL-28, IL-29
IL-17	IL-17A-F, IL-25 (IL-17E)
Interferons	
Type I interferons	IFN-α, IFN-β, IFN-ω
Type II interferon	IFN-γ (also a hematopoietic cytokine)
Type III interferons	IFN-λ1 (IL-29), IFN-λ2 (IL-28A), IFN-λ3 (IL-28B)
TNF superfamily	TNF-α, TNF-β, BAFF, APRIL

processed as a membrane-bound protein from which the soluble active factor is cleaved by using the enzyme TNF-α converting enzyme.[2] TNF-β (also known as lymphotoxin α) can be synthesized and processed as a typical secreted protein but is usually linked to the cell surface by forming heterotrimers with a third membrane-associated member of this family, lymphotoxin β. TNF-α and TNF-β bind to the same 2 distinct cell-surface receptors, TNF receptor I (p75) and TNF receptor II (p55), with similar affinities and produce similar, although not identical, effects.[3] Notably, the active form of both cytokines is a homotrimer. TNFs induce antitumor immunity through direct cytotoxic effects on cancerous cells and by stimulating antitumor immune responses. TNFs interact with endothelial cells to induce intercellular adhesion molecule (ICAM) 1, vascular cell adhesion molecule (VCAM) 1, and E-selectin, permitting the egress of granulocytes into inflammatory loci. TNFs are a potent activator of neutrophils, mediating adherence, chemotaxis, degranulation, and the respiratory burst. TNFs are responsible for the severe cachexia that occurs in chronic infections and cancer.[1] Furthermore, TNFs induce vascular leakage and have negative inotropic effects, and because the most potent inducer of TNF is endotoxin, it is the primary mediator of septic shock.[4]

IL-1

The IL-1 family represents 5 peptides (IL-1α, IL-1β, the IL-1 receptor antagonist [IL-1ra], IL-18, and IL-33).[5] IL-1α and IL-1β have similar biologic activities and, along with IL-1ra, have similar affinities for the 2 IL-1 receptors. Type I receptors transduce the biologic effects attributed to IL-1.[6] Type II receptors have a minimal intracellular domain, and the capture and sequestration of IL-1 by these inactive receptors serves an anti-inflammatory function. The capacity of IL-1ra to bind IL-1 receptor without transducing activities is the basis for its antagonist function.[7] IL-1ra is secreted in inflammatory processes in response to many cytokines, including IL-4, IL-6, IL-13, and TGF-β. Production of IL-1ra moderates the potentially deleterious effects of IL-1 in the natural course of inflammation.

IL-1 is primarily produced by cells of the mononuclear phagocytic lineage but is also produced by endothelial cells, keratinocytes, synovial cells, osteoblasts, neutrophils, glial cells, and numerous other cells. IL-1 production is stimulated by a variety of agents, including endotoxin, that stimulate molecular pattern receptors. Both IL-1α and IL-1β, as well as the related proteins IL-18 and IL-33 (discussed later), are synthesized as inactive precursors without a secretory sequence. The mechanism for their secretion depends on cleavage by a specific converting enzyme, termed IL-1 converting enzyme or caspase-1, contained within a specialized intracellular complex termed the inflammasome, which cleaves the procytokines into their active secreted forms.[8]

One of the most important biologic activities of IL-1 is its ability to activate T lymphocytes by enhancing the production of IL-2 and the expression of IL-2 receptors. In the absence of IL-1, a diminished immune response or tolerance develops. The production of IL-1 (and other APC-derived cytokines) during the immune response produces a spectrum of changes associated with being ill. IL-1 interacts with the central nervous system to produce fever, lethargy, sleep, and anorexia. An IL-1–hepatocyte interaction inhibits production of housekeeping proteins (eg, albumin) and stimulates the synthesis of acute-phase response peptides (eg, amyloid peptide, C-reactive peptide, and complement). IL-1 stimulates endothelial cell adherence of leukocytes through the upregulation of ICAM-1, VCAM-1, and E-selectin. IL-1 contributes to the hypotension of septic shock. TNF and IL-1 share numerous biologic activities, the major distinction being that TNF has no direct effect on lymphocyte proliferation.

IL-6

Mononuclear phagocytic cells are the most important source of IL-6[9]; however, IL-6 is also produced by T and B lymphocytes, fibroblasts, endothelial cells, keratinocytes, hepatocytes, and bone marrow cells. IL-6 signals through a ligand-binding IL-6 receptor (IL-6R) α chain (CD126) and the signal-transducing component gp130 (CD130). CD130 is the common signal transducer for several cytokines in the IL-6 family and is ubiquitously expressed. In contrast, the expression of IL-6Rα is restricted. In addition to the membrane-bound receptor, a soluble form of IL-6R can capture circulating IL-6 and make it available to bind and activate gp130.[10] In contrast, soluble gp130 functions as an anti-inflammatory decoy receptor. Other cytokines that signal through gp130-containing receptors are IL-11, IL-27, IL-31, ciliary neurotrophic factor, leukemia inhibitory factor, oncostatin M, and osteopontin. These cytokines are referred to as the IL-6–like or gp130-utilizing cytokines (Table II).[11]

Under the influence of IL-6, B lymphocytes differentiate into mature plasma cells and secrete immunoglobulins. IL-6 mediates T-cell activation, growth, and differentiation. In addition to lymphocyte activation, IL-6 shares several activities with IL-1, including the induction of pyrexia and the production of acute-phase proteins. IL-6 is the most important inducer of acute-phase proteins. As discussed below, IL-6 has a primary role in T_H17 immune deviation.

TABLE II. IL-6–like (gp130-utilizing) cytokines

IL-6–like cytokine	Characteristics
IL-31	Primarily expressed by T_H lymphocytes under T_H2 conditions. Induces chemokines that recruit neutrophils, monocytes, T cells. Overexpression in mice leads to atopic dermatitis model. Increased IL-31 receptor levels in murine model of AHR.
IL-11	Increases production of acute-phase proteins. Induces lymphoid cell differentiation. Stimulatory factor for fibroblasts. Expression in severe asthma with remodeling.
Osteopontin	Induced by IFN-γ, IL-1β, and TNF-α. Expression inhibited by IL-4 and IL-13. Upregulated in patients with chronic sinusitis, nasal polyps, and asthma.
Oncostatin	Synthesized by T cells and monocytes. Proinflammatory or anti-inflammatory functions. Roles in liver development, hematopoiesis, inflammation, and possibly CNS development. Signals through a shared type I receptor of gp130/LIFR-β and a specific type II receptor of gp130/OSMRβ.
LIF	Induces terminal differentiation of myeloid leukemia cells. Influences bone metabolism, cachexia, neural development, embryogenesis, and inflammation. Binds to the specific LIF receptor (gp130/LIFR-α).

IL-12, IL-18, and IL-23

IL-12 and IL-23 are heterodimers that share a larger (IL-12p40) subunit. Both are primarily derived from DCs.[12,13] Their receptors are also heterodimers having distinct α chains and shared use of the IL-12 receptor (IL-12R) β1 chain. These cytokines are involved in T-cell activation and immune deviation of T_H1 and T_H17 cells, respectively (discussed later).

IL-12 is derived most importantly from DCs but also from Langerhans cells, mononuclear phagocytic cells, B cells, PMNs, and mast cells. The biologically active form is a heterodimer. The larger subunit (IL-12p40) is homologous to the soluble receptor for IL-6, whereas the smaller subunit (IL-12p35) is homologous to IL-6. Homodimers of IL-12p40 are also functional (IL-12p80). IL-12 stimulates IFN-γ production and activates and induces proliferation, cytotoxicity, and cytokine production of NK cells. Other activities attributed to IL-12 include proliferation of T_H and cytotoxic lymphocytes.

IL-18, along with IL-12 and IL-23, is an inducer of IFN-γ.[14] Similar to IL-1, IL-18 requires a specific converting enzyme (caspase-1) to permit secretion and activation. In contrast to most cytokines, IL-18 is constitutively expressed, and release of its active form is regulated through activation of this converting enzyme. IL-18 has an important role in cellular adhesion, being the final common pathway used by IL-1 and TNF that leads to ICAM-1 expression. IL-18 binds to a unique heterodimer receptor, the expression of which is upregulated by IL-12, and hence these 2 cytokines synergize to stimulate IFN-γ release.

Finally, as noted, IL-23 is a heterodimer consisting of a larger subunit shared with IL-12 (IL-12p40) and a unique subunit (IL-23p19). Its inflammatory response includes induction of remodeling through activation of matrix metalloproteinases, increased angiogenesis, and reduced CD8 T-cell infiltration. Its important synergistic role in T_H17 differentiation is discussed below.

IL-15

Mononuclear phagocytic cells are the main source of IL-15, whereas epithelium, fibroblasts, and placenta are additional sources. IL-15 is distinguished from IL-2 through its use of a unique α chain as part of its receptor signaling complex.[15] Both receptors share the use of the IL-2 receptor (IL-2R) β and common γ chain (Table I). IL-15, similar to IL-2, is a T-cell growth factor and is chemotactic for T lymphocytes. The most important activity of IL-15 might be its activation of NK cells. IL-2 and IL-15 are contrasted in their roles in adaptive immune responses in which IL-2, but not IL-15, is involved in the generation and maintenance of Treg cells, whereas IL-15 is necessary for maintaining the survival of CD8 memory T cells. IL-15 is also active as an accessory mast cell growth factor.

IL-27

The cells responsible for most of the production of IL-27 are macrophages and DCs. IL-27 is a heterodimer composed of IL-27B (EBV-induced gene B) and IL-27p28 (also known as IL-30).[16] IL-27 subserves important functions in T_H1 immunity, reflecting its ability to synergize with IL-12 to induce IFN-γ production from NK and T_H cells (T_H1 immune deviation). The effects of IL-27 are mediated through interaction with a receptor complex consisting of IL-27 receptor α and gp130.[17]

IL-32

IL-32 was discovered in a search for IL-18–inducible genes.[18] Its biologic activities include induction of proinflammatory cytokines (eg, TNF-α) and chemokines from differentiated macrophages. The highest levels of expression are observed in NK and T cells; however, expression can also be observed in epithelial cells in response to IFN-γ and IL-1β. IL-32 synergizes with nucleotide-binding oligomerization domain 1 and 2 ligands to stimulate IL-6 and IL-1β release in a caspase-1–dependent manner.[19]

CYTOTOXIC IMMUNITY

Immune responses directed against virus-infected and neoplastic cells are primarily mediated by $CD8^+$ cytotoxic lymphocytes and NK cells. As discussed elsewhere, numerous cytokines contribute to cytotoxic immunity, as well as IL-11 and the interferons.

IL-11

In addition to its functions in promoting cytotoxic antiviral immune responses, IL-11 was originally described as a stimulatory factor for hematopoietic cells, synergizing with other growth factors to produce erythrocytes and platelets. IL-11 increases the production of acute-phase proteins and induces lymphoid cell differentiation. It is an important stimulatory factor for connective tissue cells, such as fibroblasts, that stimulate proliferation and collagen deposition. A role for IL-11 in asthma remodeling is suggested by studies demonstrating expression of IL-11 in patients with severe asthma.[20,21]

Interferons

Interferons derive their name from their ability to interfere with viral growth. There are 3 major classes of interferons. Type I

TABLE III. IL-10 superfamily

Interleukin	1° Cell source	Receptor	Activated signal transducer	Biologic effect	Clinical association
IL-10	Monocytes, B cells, Treg cells	IL-10R1/IL-10R2	JAK1, TYK2, STAT1, STAT3	Immune suppression, anti-inflammatory	Burkitt lymphoma, malignant B-cell lymphomas
IL-19	Monocytes	IL-20R1/IL-20R2	STAT1, STAT3	Skin development, immunoregulatory	Psoriasis, asthma
IL-20	Monocytes, skin keratinocytes	IL-20R1/IL-20R2, IL-22R1/IL-10R2	JAK/STAT	Skin development, innate immunity, hematopoiesis	Psoriasis, atherosclerosis, angiogenesis
IL-22	Activated T cells, activated NK cells, T_H17 cells	IL-22R1/IL-10R2	STAT3	Acute-phase response, innate immunity	Crohn disease, interstitial lung disease, rheumatoid arthritis, psoriasis
IL-24	Melanocytes, monocytes, T_H2 cells	IL-20R1/IL-20R2, IL-22R1/IL-20R2 (skin only)	STAT3	Proapoptosis, epidermal functions, inflammatory cascade	Melanoma, psoriasis, inflammation
IL-26	Monocytes, memory T cells	IL-20R1/IL-10R2	STAT1, STAT3	Mucosal and cutaneous immunity	T-cell transformation
IL-28, IL-29	DCs	IFNLR1/IL-10R2	JAK1, STAT1, STAT2, STAT3, and STAT5	Antiviral immunity	Hepatitis B/C infections

interferons (IFN-α/β/ω) are primarily derived from monocytes, macrophages, B lymphocytes, and NK cells. An important source of IFN-α is plasmacytoid DCs, reflecting their activation by viral RNA acting through Toll-like receptors 3 and 7. The antiviral activity of type I interferons is mediated through their ability to inhibit viral replication within virus-infected cells, protect uninfected cells from infection, and stimulate antiviral immunity by cytotoxic ($CD8^+$) lymphocytes and NK cells. IFN-α has other important biologic actions, including upregulation of class I MHC antigens and mediation of antitumor activity. IFN-ω[22] displays a high degree of homology with various IFN-α species, including positions of the cysteine residues involved in disulfide bonds[23]; however, sequence divergence allows classification as a unique protein family. IFN-ω binds to the same receptors as IFN-α and IFN-β.[24]

A sole member makes up the class of type II interferons: IFN-γ. IFN-γ is a homodimer primarily made by T cells and NK cells and to a lesser degree by macrophages. The biologic activities of IFN-γ include only modest antiviral activity, and its derivation primarily from T lymphocytes suggests that it is more of an interleukin than an interferon. IFN-γ and its role in cellular immunity are discussed below.

The type III interferons consist of IFN-λ1, IFN-λ2, and IFN-λ3, also called IL-29, IL-28A, and IL-28B, respectively. Type III interferons share with type I interferons the same Janus kinase (JAK) and signal transducer and activator of transcription (STAT) signaling pathways. IFN-λs exhibit several other common features with type I interferons, including antiviral, antiproliferative, and antitumor activities. Despite amino acid homology with type I interferons, the intron-exon structure of the IFN-λ family more closely resembles that of IL-10.[25] Moreover, IFN-λs act through a cell-surface heterodimer receptor, one chain being IFN-γ–specific (IFNLR1) and the second, IL-10 receptor (IL-10R) 2, being shared by IL-10, IL-22, and IL-26 (Table III). In addition to the full-length IFNLR1, 2 inhibitory splice variants have been identified, one variant deletes 29 amino acids in its intracytoplasmic portion, likely disabling its signaling capacity, and the second encodes a secreted (decoy) receptor.[25] Although IL-10R2 is ubiquitously expressed, IFNLR1 is more tightly regulated. IFN-λ subtypes are induced on infection by multiple viruses, which is consistent with their antiviral activities,[25,26] and pretreating hepatocellular cells prevents viral infection.[25] One notable difference between IFN-λ and type I interferons is that IFN-λ shifts immature DCs toward a program characterized by the ability to produce forkhead box protein 3 (Foxp3)–expressing $CD4^+CD25^+$ Treg cells.[27]

HUMORAL IMMUNITY

At least 2 cytokines contribute to B-lymphocyte maturation in the bone marrow: the lymphoid stem cell growth factors IL-7 and IL-11. IL-7 is critically important to the development of both B and T lymphocytes through its production by stromal tissue of the bone marrow and thymus, from which it interacts with lymphoid precursors. In addition, IL-7 stimulates the proliferation and differentiation of cytotoxic T and NK cells and stimulates the tumoricidal activity of monocytes and macrophages. The central importance of IL-7 to lymphoid maturation is reflected in severe combined immune deficiency resulting from the absence of either IL-7 or functional IL-7 receptors (IL-7 receptor α [CD127] or common γ chain).

IL-21

IL-21 is increasingly recognized as being central to B-cell proliferation, survival, and differentiation into immunoglobulin-producing plasma cells.[28] Its induction of activation-induced cytidine deaminase contributes to class-switch recombination. IL-21 receptors are expressed on activated B, T, and NK cells. It shares numerous biologic activities with IL-2 and IL-15, with which it is homologous, including the capacity to activate NK cells and promote the proliferation of B and T lymphocytes.[29] Its receptor shares the common γ chain with IL-2, IL-4, IL-7, IL-9, and IL-15. Among T cells, it is preferentially expressed by T_H17 cells and is involved in T_H17 differentiation (discussed below).

B-cell activation factor from the TNF family and a proliferation-inducing ligand

Two other TNF family cytokines, B-cell activation factor from the TNF family (BAFF) and a proliferation-inducing ligand (APRIL), enhance the maturation and survival of transitional and mature B cells. BAFF and APRIL are expressed in bone marrow nonlymphoid cells, with low levels also in developing B cells. BAFF overexpression leads to an expanded B-cell compartment, and increased amounts of BAFF have been found in autoimmune patients. BAFF knockout mice have a severe block in B-cell development in the spleen, although not in bone marrow. Three receptors from the TNF receptor family bind to BAFF and APRIL: transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI), B-cell maturation antigen, and BAFF-R.[30,31] BAFF-R binds specifically to BAFF, whereas TACI and BMCA bind primarily to APRIL. Similar to BAFF-deficient mice, BAFF-R–null mice show defective splenic B-cell maturation. Mutations in TACI have been identified as an important factor in common variable immunodeficiency.[32,33]

After B cells egress from the bone marrow, isotype switching, the activation of mature B cells into immunoglobulin-secreting B cells, and their final differentiation into plasma cells are processes that are under T-cell control.[34] Cytokines that trigger isotype switching include IL-4 and IL-13, which induces the IgE isotype TGF-β, which triggers IgA, and IL-10, which contributes to the generation of IgG4.

CELLULAR IMMUNITY

IL-2

Stimulation of T cells by antigen (signal 1) in the presence of accessory signals provided by the cognate interaction of the B7 molecules (CD80 or CD86) with CD28 (signal 2) and the cytokines IL-1 and IL-6 (signal 3) induces the simultaneous secretion of IL-2 and the expression of high-affinity IL-2R by effector T cells. Subsequently, the binding of secreted IL-2 to these IL-2R–expressing T cells induces clonal T-cell proliferation. The requirement for both IL-2 production and IL-2R expression for T-cell proliferation ensures that only effector T cells specific for the antigen inciting the immune response become activated. This is in contrast to Treg cells, which constitutively express IL-2R and can thereby be spontaneously activated in the presence of IL-2. IL-2 is also necessary during Treg cell development in the thymus.[35] IL-2 signals through a receptor complex consisting of IL-2–specific IL-2Rα (CD25), IL-2Rβ (CD122), and the common γ chain. In addition to its role as an effector and Treg cell growth factor, IL-2 is also involved in activation of NK cells, B cells, cytotoxic T cells, and macrophages. Many of the immunosuppressive drugs used in the treatment of autoimmune diseases, such as corticosteroids, cyclosporine, and tacrolimus, work, in part, by inhibiting the production of IL-2 by antigen-activated T cells, whereas others (eg, rapamycin) block IL-2R signaling.

IFN-γ

The most important cytokine responsible for cell-mediated immunity is IFN-γ.[36] It is the signature cytokine produced by T_H1 cells but is also derived from cytotoxic T cells and NK cells. IFN-γ mediates increased MHC class I and II expression and stimulates antigen presentation and cytokine production by APCs. IFN-γ stimulates mononuclear phagocytic functions, including adherence, phagocytosis, secretion, respiratory burst, and nitric oxide production. The net result is the accumulation of macrophages at the site of cellular immune responses, with their activation into macrophages capable of killing intracellular pathogens. In addition to its effects on mononuclear phagocytes, IFN-γ stimulates killing by NK cells and neutrophils. It stimulates adherence of granulocytes to endothelial cells through the induction of ICAM-1, an activity shared with IL-1 and TNF. As with other interferons, IFN-γ inhibits viral replication. IFN-γ is critical for many aspects of innate and adaptive immunity, but its singular importance in the immune response to intracellular pathogens is shown by the enhanced susceptibility to tuberculosis observed in patients with mutations that result in defects in its synthesis or responsiveness.[37] IFN-γ is an inhibitor of T_H2-mediated allergic inflammatory responses through its capacity to suppress many IL-4–mediated effects.

Cellular responses to IFN-γ are activated through its interaction with a heterodimer receptor consisting of IFN-γ receptor (IFNGR) 1 and IFNGR2. Binding of IFN-γ to the receptor activates the JAK-STAT pathway. JAK1 and JAK2 constitutively associate with IFNGR1 and IFNGR2, respectively, and binding ultimately leads to phosphorylation of 2 STAT1 molecules, as discussed in greater detail below.[38]

IL-16

IL-16 is a T cell–derived product that is chemotactic for $CD4^+$ lymphocytes, eosinophils, and monocytes and uses the CD4 molecule as its receptor.[39] The product of this gene undergoes proteolytic processing by caspase-3 and yields 2 functional proteins. The cytokine function is exclusively attributed to the secreted C-terminal peptide, whereas the N-terminal product might play a role in cell-cycle control.[40]

IL-17

Whereas IFN-γ is important in orchestrating the cellular immune response to intracellular pathogens, IL-17 generates T cell–mediated immune responses to extracellular pathogens. It is produced by a unique family of T_H lymphocytes, termed T_H17 cells. IL-17 comprises a structurally related family of 6 proteins (IL-17A through IL-17F) having no sequence similarity to any other cytokine.[41] Because of its unique spectrum of activities, IL-17E is now termed IL-25 and is discussed separately. IL-17A (generally referred to as IL-17) is mainly expressed in $CD4^+$ T_H (T_H17) cells and, to a lesser extent, neutrophils, eosinophils, and CD8 T cells. Similar to IL-17A, its most closely structurally related family member, IL-17F, is expressed by T_H17 cells but also activated basophils and mast cells.[41] The primary cellular sources for IL-17B and IL-17C have not been determined. IL-17D is expressed in resting CD4 T and B cells.

IL-17 induces expression of a variety of cytokines and chemokines from stromal cells, fibroblasts, endothelium, and epithelium, including IL-6, IL-11, granulocyte colony-stimulating factor, GM-CSF, CXCL8, CXCL10 (IFN-inducible protein 10), and TGF-β, cytokines important to both fibroblast activation and neutrophil recruitment. Activation of fibroblasts by IL-17 might contribute to fibrotic autoimmune diseases, and a role for

IL-17 has been proposed in inflammatory bowel disease and multiple sclerosis. IL-17 family members are also expressed in patients with asthma.[42] The tendency to induce neutrophil, but not eosinophil, migration makes it plausible that IL-17 plays a role in severe persistent asthma, in which accumulation of neutrophils is a hallmark. Both IL-17 and IL-17F induce goblet cell hyperplasia and mucus hypersecretion and activate epithelial innate immune responses. IL-17 could therefore plausibly contribute to the development of airway hyperreactivity (AHR), remodeling, neutrophilic infiltration, and subepithelial fibrosis.

Induction of cytokines responsible for PMN recruitment and activation is central to its role in driving cellular immune responses to extracellular pathogens, as suggested by increased susceptibility to infection by *Staphylococcus aureus* and *Citrobacter* and *Klebsiella* species in IL-17–deficient mice.[43] In human subjects hyper-IgE syndrome has been characterized by a genetic deficiency in T_H17 cell differentiation.[44,45] Increased susceptibility of these patients to infections with *Candida* species and *S aureus* is consistent with T_H17 cells' role in immunity against these pathogens.[46]

The IL-17 receptor (IL-17R) family consists of 5 broadly distributed receptors that have individual ligand specificities. IL-17RA is the best described and binds both IL-17A and IL-17F. IL-17RB binds both IL-17B and IL-17E, whereas the less well-described IL-17RC and IL-17RD might undergo alternate splicing to produce soluble (decoy) forms.[41] The least described of these receptors, IL-17RE, is expressed in the pancreas, brain, and prostate.

IL-34

IL-34 is a newly discovered interleukin also having no homology to other cytokines.[47] It is expressed in numerous tissues but is most abundant in the spleen. The receptor for IL-34 is colony-stimulating factor 1 receptor (CD115), a receptor also used by macrophage colony-stimulating factor (M-CSF), and like M-CSF, IL-34 stimulates monocyte proliferation and function.

ALLERGIC IMMUNITY

An additional outcome of proinflammatory T-cell activation is the development of allergic (and presumably antiparasitic) immunity. Several features specifically associated with the allergic state are regulated by cytokines, including the regulation of IgE, eosinophilia, and mast cell proliferation, and these will be discussed separately.

Regulation of IgE

The inappropriate production of IgE in response to allergen defines atopy and is primarily mediated by IL-4 and IL-13.

IL-4. In addition to T_H2 lymphocytes, IL-4[48] is derived from basophils, NK T cells, eosinophils, and mast cells. In both eosinophils and basophils, IL-4 exists as a preformed, granule-associated peptide that can be rapidly released in allergic inflammatory responses. IL-4 stimulates MHC class II, B7 (CD80/CD86), CD40, surface IgM, and low-affinity IgE receptor (CD23) expression by B cells, thereby enhancing the antigen-presenting capacity of B cells. IL-4 induces the immunoglobulin isotype switch from IgM to IgE.[49,50] IL-4 can be identified in the sera, bronchoalveolar lavage fluid, and lung tissue of asthmatic subjects and in nasal polyp tissue and nasal mucosa of subjects with allergic rhinitis.

In addition to these effects on B cells, IL-4 has important influences on T lymphocytes. As will be discussed later, IL-4 contributes to the differentiation of naive T_H0 lymphocytes toward a T_H2 phenotype. IL-4 is also important in maintaining allergic immune responses by preventing apoptosis of T_H2 lymphocytes.[51,52] IL-4 renders T_H2 cells refractory to the anti-inflammatory influences of corticosteroids.

Another important activity of IL-4 in allergic inflammation is its ability to induce expression of VCAM-1. This produces enhanced adhesiveness of endothelium for T cells, eosinophils, basophils, and monocytes, but not neutrophils, as is characteristic of T_H2-mediated allergic reactions.[53] IL-4 interacts with mast cells to stimulate IgE receptor expression and regulates expression of leukotriene C_4 synthase, thereby determining their capacity to produce cysteinyl leukotrienes.[54] IL-4 contributes to the excessive mucous production in the asthmatic airway. Functional IL-4 receptors are heterodimers consisting of the IL-4 receptor (IL-4R) α chain interacting with either the shared γ chain or the IL-13 receptor (IL-13R) $\alpha 1$ chain.[55] This shared use of the IL-4Rα chain by IL-13 and IL-4 explains many of the common biologic activities of these cytokines.

In contrast to these proinflammatory effects, IL-4 downregulates antibody-dependent cellular cytotoxicity by mononuclear phagocytes, inhibits their expression of Fcγ receptors and differentiation into macrophages, and downregulates production of nitric oxide, IL-1, IL-6, and TNF-α while stimulating production of IL-1ra and IL-10.[56]

IL-13. IL-13 shares much of IL-4's biologic activities on mononuclear phagocytic cells, endothelial cells, epithelial cells, and B cells. Thus IL-13 induces the IgE isotype switch and VCAM-1 expression.[57] Functional IL-13 receptors are heterodimers containing the IL-4Rα chain and a unique IL-13Rα chain. The 2 IL-13Rα chains include the active form (IL-13Rα1) and a decoy (IL-13Rα2), which lacks the motif required for initiating intracellular signaling cascades.[58] IL-13Rα1 expression is more limited than IL-4 receptors and includes endothelial cells, B cells, mononuclear phagocytes, and basophils but not mast cells or T cells. This more limited distribution of IL-13Rα1 explains the unique ability of IL-4 to induce T_H2 lymphocyte differentiation and mast cell activation. However, IL-13 is more widely produced than IL-4 and is more readily identified in allergic inflammatory tissue.[59] In murine studies IL-13 has a singularly important role in causing mucus hypersecretion and nonspecific AHR, and its expression results in the characteristic airway metaplasia of asthma with the replacement of epithelial cells with goblet cells.[59]

Eosinophilia

Another characteristic feature of allergic diseases is the presence of increased numbers of activated eosinophils.

IL-5. IL-5 is the most important eosinophilopoietin.[60] In addition to stimulating eosinophil production and release from the bone marrow,[61] IL-5 is chemotactic for eosinophils and activates mature eosinophils, inducing eosinophil secretion and enhanced cytotoxicity. Another mechanism by which IL-5 promotes accumulation of eosinophils is through its ability to upregulate chemokine receptors and $\alpha D\beta 2$ integrins, thereby promoting their adherence to VCAM-1–expressing endothelial cells. IL-5 prolongs eosinophil survival by blocking apoptosis.[62] Administration of IL-5 causes mucosal eosinophilia and an increase in bronchial hyperreactivity. IL-5–dependent activation of eosinophils is now

thought to be less central to the pathophysiology of asthma as a result of the disappointing results in trials using IL-5 antagonists, perhaps because of redundant cytokine profiles involving GM-CSF and heterogeneous presentations of asthma that are less dependent on eosinophils. Thus in asthmatic patients screened for sputum eosinophils, anti–IL-5 does have increased therapeutic benefit.[63,64] Other activities of IL-5 include basophil differentiation. In addition to T_H2-like lymphocytes, other sources for IL-5 include mast cells, NK T cells, and eosinophils themselves. IL-5 interacts with specific IL-5 receptors (IL-5Rs) that consist of a heterodimer containing IL-5Rα and a β chain (CD131) shared with GM-CSF receptor and IL-3 receptor (Table I).[65]

IL-3 and GM-CSF. In addition to IL-5, IL-3[66] and GM-CSF[67] also strongly contribute to the activity of eosinophils in allergic inflammation through their capacities to prolong eosinophil survival and to generate activated eosinophils. IL-3 is an important factor that supports the growth of precursors for a variety of hematopoietic cells, including DCs, erythrocytes, granulocytes (especially basophils), macrophages, mast cells, and lymphoid cells. The major source of IL-3 is T lymphocytes, but in patients with allergic inflammation, it is also derived from eosinophils and mast cells.

GM-CSF supports the maturation of DCs, neutrophils, and macrophages. GM-CSF synergizes with other colony-stimulating factors to support the production of platelets and erythrocytes. GM-CSF is an activating factor for mature granulocytes and mononuclear phagocytic cells. In the lungs GM-CSF is uniquely important in the maturation of alveolar macrophages, including their expression of matrix metalloproteinases and reactive oxygen species and their processing of surfactant proteins.[68,69] The role of GM-CSF in allergic immunity is derived from its shared ability with IL-3 and IL-5 to inhibit apoptosis of eosinophils and thereby prolong the survival of eosinophils at sites of allergic inflammation. GM-CSF is particularly important in the allergic airway because mature activated eosinophils lose their expression of IL-5Rs and responsiveness to IL-5 but instead upregulate GM-CSF receptors. Thus GM-CSF, and not IL-5, might be responsible for the persistent survival and function of eosinophils in the asthmatic airway. These observations provide one explanation for the failure of IL-5 antagonism in asthma trials. GM-CSF activates mature eosinophils, increasing their degranulation, cytotoxicity, and response to chemoattractants.

Mast cell proliferation and activation

Increased numbers of mast cells characterize allergic diseases, and this is a T cell–dependent process. The most important cytokine responsible for mast cell growth and proliferation from hematopoietic precursors is stem cell factor (SCF; or c-kit ligand).[70] SCF is derived from bone marrow stromal cells, endothelial cells, and fibroblasts. SCF induces histamine release from mast cells but inconsistently from basophils and remains the only cytokine with this property. In addition to being essential for mast cell differentiation, SCF interacts with other hematopoietic growth factors to stimulate myeloid, lymphoid, and erythroid progenitor cells. Several cytokines, including and especially IL-3, IL-5, IL-6, IL-9, IL-10, IL-11, and nerve growth factor, also contribute to mast cell proliferation.[71-75] In addition to the factors that stimulate mast cell proliferation, several cytokines induce histamine release from basophils, including several members of the chemokine family (discussed later).

Other T_H2 cell–derived cytokines involved in allergic inflammation: IL-9, IL-25, and IL-31

IL-9 was originally described as a mast cell growth factor[76] and contributes to mast cell–mediated allergic responses through its ability to stimulate production of mast cell proteases. In addition, IL-9 increases expression of the IgE high-affinity receptor on mast cells. IL-9 synergizes with IL-4 to enhance the production of IgE and IL-5 to enhance the production of eosinophils. IL-9 supports the growth and survival of T lymphocytes. IL-9 has other important activities in allergic inflammation, including inducing expression of CCL11 (eotaxin-1), IL-5 receptors, and chemokine receptor 4. IL-9 is derived from eosinophils and T_H2-like lymphocytes. Its selective production by T_H2 cells supports a role in allergic inflammation. It appears to be primarily produced by a unique subfamily of T_H2 cells termed T_H9 lymphocytes (discussed below).[77,78]

IL-25 was originally described as a member of the IL-17 family (IL-17E) but has now been given its distinct nomenclature because of its unique spectrum of activities. Similar to IL-4, IL-5, IL-9, and IL-13, it is derived in part from T_H2-like lymphocytes. It stimulates release of IL-4, IL-5, and IL-13 from nonlymphoid cells and from T_H lymphocytes themselves, contributing to T_H2 immune deviation. IL-25 enhances IgE secretion through its ability to stimulate IL-4 and IL-13 production.[79] IL-25 stimulation of IL-5 production promotes eosinophilopoiesis. IL-25 increases expression of CCL5 (RANTES) and CCL11, which further contribute to the homing of eosinophils to the lungs.[41]

IL-31 is a member of the subfamily of hematopoietin cytokines that also includes IL-3, IL-5, and GM-CSF. It is primarily expressed by T_H2 lymphocytes. Its activities include induction of chemokines that are involved in recruitment of neutrophils, monocytes, and T cells. Overexpression of IL-31 in mice produces an inflammatory infiltrate suggestive of atopic dermatitis.[80-82] Similarly, the murine model of AHR demonstrates increased expression of the IL-31 receptor.

ANTI-INFLAMMATORY CYTOKINES

In addition to cytokines that stimulate cytotoxic, cellular, humoral, and allergic inflammation, several cytokines have predominantly anti-inflammatory effects, including, as previously discussed, IL-1ra, but also TGF-β and members of the IL-10 family.

TGF-β

TGF-β represents a family of peptides that are arguably the most pleiotropic of the cytokines, including having both stimulatory and inhibitory effects on numerous cell types.[83] TGF-β is synthesized as an inactive precursor that requires cleavage for activation. It is produced by numerous cell types, including eosinophils, monocytes, and T cells. TGF-β is an important stimulant of fibrosis, inducing formation of the extracellular matrix and promoting wound healing and scar formation. In immunity it is largely inhibitory for B cells and T_H/cytotoxic lymphocytes. In general, it inhibits proliferation and induces apoptosis. The production of TGF-β by apoptotic cells creates an immunosuppressive milieu and is one explanation for the absence of inflammation and autoimmunity as a consequence of apoptotic cell death.[84] It inhibits cytotoxicity of mononuclear phagocytes and NK cells. The primary TGF-β–producing T_H lymphocytes are Treg cells (discussed below), and the expression of

membrane-bound TGF-β mediates much of their suppressive activity. TGF-β production by mucosal (T_H3) cells supports the α isotype switch and secretory IgA production by B cells[85] and is also critical for the maintenance of immune nonresponsiveness to otherwise benign gut pathogens and food allergens. TGF-β is constitutively produced in the healthy lung and helps promote B- and T-cell nonresponsiveness and lessens allergic inflammation through inhibition of IgE synthesis and mast cell proliferation. In established allergic inflammation, eosinophils comprise the most important source of TGF-β,[86] and their expression of TGF-β is a cause of the fibrosis observed in patients with asthma.

In contrast to these largely anti-inflammatory influences, TGF-β is central to the differentiation of T_H17 and IL-9–producing T_H2 (T_H9) lymphocytes. These conflicting proinflammatory and anti-inflammatory effects reflect the distinctive actions of TGF-β as a function of which cells are producing it, the stage of the immune response during which it is acting, different signaling pathways it engages, and other divergent influences.

IL-10 family

IL-10 is an important immunoregulatory cytokine with multiple biologic effects on different cell types. Although the primary T-cell source for IL-10 is regulatory T lymphocytes, monocytes and B cells are the major sources of IL-10 in human subjects.[87] IL-10 forms a homodimer and exerts its biologic function through IL-10R1 and IL-10R2 receptor complex. IL-10 inhibits production of IFN-γ by T_H1 lymphocytes; IL-4 and IL-5 by T_H2 lymphocytes; IL-1β, IL-6, CXCL8, IL-12, and TNF-α by mononuclear phagocytes; and IFN-γ and TNF-α by NK cells. MHC class II expression by APCs is inhibited by IL-10, as is CD23 (low-affinity IgE receptor [FcεRII]) and ICAM-1. IL-10 inhibition of expression of the costimulatory molecules CD80 and CD86 by DCs and other APCs eliminates the ability of the APC to provide the accessory signals necessary for T_H cell activation,[88] which is primarily responsible for the inhibition of cytokine production. However, IL-10 also functions directly on T cells to inhibit their cytokine production by suppressing expression of CD28 and inducible T-cell costimulator.[89] Constitutive expression of IL-10 in the respiratory tract of healthy subjects has a role in the maintenance of tolerance to allergens, whereas asthma and allergic rhinitis are associated with diminished IL-10 expression.[90] This diminished IL-10 expression contributes to the development of an inflammatory milieu, reflecting in part the presence of mature DCs.

Other members of the IL-10 family: IL-19, IL-20, IL-22, IL-24, IL-26, IL-28, and IL-29

These newer members of the IL-10 family cytokines and their receptors loosely share homologies with interferons/interferon receptors, and many display antiviral activity.[91] In contrast to IL-10, none of these cytokines significantly inhibit cytokine synthesis, an activity that remains unique for IL-10. Features of the IL-10 superfamily are summarized in Table III.

IL-19 shares 21% amino acid identity with IL-10, but as with other members of the IL-10 superfamily, it is the exon-intron structure that primarily defines their homology. Within the immune system, IL-19 is primarily produced by monocytes, and its expression can be induced by LPS, IL-4, and GM-CSF. IL-19 signals through a receptor complex composed of the IL-20 receptor (IL-20R) 1 and IL-20R2 chains and activates monocytes to release IL-6, TNF-α, and reactive oxygen species. IL-19 contributes to T_H2 immune deviation, as well as the development of airway inflammation, in murine models, and its increased expression has been observed in asthmatic patients.[92]

Similar to IL-19, IL-20[93] signals through the IL-20R1/IL-20R2 heterodimer; however, IL-20 also binds to the receptor complex composed of IL-22 receptor (IL-22R) 1/IL-20R2. IL-20 is predominantly expressed by monocytes and skin keratinocytes, and it is overexpressed in patients with psoriasis. It induces keratinocyte proliferation, and overexpression in mice is lethal, secondary to defective skin formation.

IL-22 is derived from T lymphocytes, mast cells, and, at lower levels, activated NK cells.[94] Among T-lymphocyte subsets, IL-22 is preferentially expressed by T_H17 cells. Notably, patients with psoriasis, Crohn disease, interstitial lung diseases, and rheumatoid arthritis all have evidence of increased levels of IL-22 that correlate with disease severity.[95-97] The IL-22 receptor complex is a heterodimer consisting of IL-22R1/IL-10R2 chains. Neither resting nor stimulated immune cells express IL-22R1, and therefore despite its structural similarity to IL-10, immune cells are not the target cells of IL-22. The predominant biologic activity described for IL-22 is induction of acute-phase proteins by hepatocytes, including serum amyloid A protein, and it likely provides a protective role in liver injury. In addition, IL-22 leads to the production of antimicrobial peptides, and consistent with its expression by T_H17 cells, it is presumed to play an important role in defense against extracellular pathogens.

IL-24 is produced by both monocytes and T_H2 lymphocytes in an IL-4–inducible fashion. Originally identified as a tumor-suppressor molecule (melanoma differentiation-associated gene 7) that was expressed in healthy melanocytes but not metastatic melanoma cells, it was subsequently discovered to share structural homology with IL-10 and to be located within the same locus on chromosome 1. IL-24 signals through a heterodimer consisting of IL-20R1/IL-20R2. Its potential role as a cancer therapeutic is derived from evidence that IL-24 induces antitumor immune responses with significant independent "bystander" antitumor effects.[98,99] Given the apparently ubiquitous apoptotic effect on malignant cells, the lack of an effect on normal cells, and the absence of significant side effects (eg, cytokine storm), IL-24 is a potential cancer therapeutic.

IL-26 is located in a chromosomal cluster with IL-22 and IFN-γ in an area thought to contribute to allergic and autoimmune diseases; in contrast, IL-10, IL-19, IL-20, and IL-24 cluster separately. IL-26 is primarily generated by monocytes and T memory cells. IL-26 has a unique receptor consisting of a heterodimer of IL-20R1/IL-10R2.[100] Binding of the IL-26 receptor leads to induction of CXCL8, IL-10, and ICAM-1.

As previously discussed, the type III interferons IL-28 and IL-29 are closely related to the type I interferons, but their genomic organization and receptor use is more similar to that of members of the IL-10 family.

IL-35

IL-35 is a dimer composed of IL-12p35 and IL-27p28 (IL-30) chains. It is primarily secreted by Treg cells and suppresses inflammatory responses by causing proliferation of Treg cells while reducing the activity of T_H17 cells.[101] Studies using a murine model show that the absence of either IL-35 chain from Treg cells reduces their ability to suppress inflammation.[102]

TABLE IV. T_H lymphocyte families

Family	Cytokine repertoire	Cytokines involved in differentiation	Transcription factors involved in differentiation
T_H1	IFN-γ, TNF-α, TNF-β, GM-CSF, IL-2, IL-3	**IL-12:** activates STAT4, leading to expression of T-bet; induces IL-18R expression **IL-18:** upregulates IL-12R, further induces IFN-γ expression **IL-27:** activates STAT4, leading to increased expression of T-bet and IFN-γ **IFN-γ:** increases expression of T-bet by increasing expression of STAT1; negative regulator of T_H17 and T_H2	**T-bet:** master regulator of T_H1 cells; potentiates production of IFN-γ and IL-12Rβ2; suppresses T_H2 and T_H17 differentiation **STAT4:** produced in response to IL-12 and potentiates production of IFN-γ **STAT1:** increases expression of T-bet; negative regulator of T_H17
T_H2	IL-2, IL-3, IL-4, IL-5, IL-9, IL-13, IL-24, IL-25, IL-31, TNF-α, GM-CSF	**IL-4:** activates STAT6, leading to expression of GATA-3; negative regulator of T_H17, IL-19, IL-25, IL-33 **TSLP:** promote differentiation and survival of T_H2-like cells	**GATA-3:** master regulator of T_H2 cells; potentiates IL-4 expression; suppresses expression of T_H1 differentiation and cytokines expression (IFN-γ) **MAF:** contributes to IL-4 production once a T_H2 program is established; inhibition of T_H17 differentiation **STAT6:** promotes T_H2 cell differentiation; negative regulator of T-bet expression and T_H1 differentiation **NFAT:** increases transcription of IL-4
T_H9	IL-4, IL-9	**TGF-β:** induces the high IL-9 phenotype of T_H2-like lymphocytes	
T_H17	IL-17 (IL-17A), IL-17F, IL-21, IL-22	**IL-6:** differentiation factor for the generation of T_H17 cells TGF-β, IL-21 **IL-23:** support the differentiation and function of T_H17 cells in the additional presence of IL-6	**RORγt** (retinoic acid–related orphan nuclear receptor) is the master regulator of T_H17 cell differentiation **STAT3:** activated by IL-6 and essential for T_H17 differentiation
nTreg/iTreg	IL-10	**TGF-β:** differentiation factor for the generation of nTreg cells **IL-10:** important for differentiation of peripheral iTreg cells, role in nTreg development uncertain **IL-2:** promotes survival, proliferation, and survival of nTreg cells through their constitutive expression of CD25	**FOXP3:** master regulator of thymus-derived nTreg cells
T_H3	TGF-β, IL-10		

T_H LYMPHOCYTE FAMILIES

T_H1, T_H2, and T_H17 lymphocytes

Subclasses of T_H lymphocytes can be identified based on their repertoire of cytokines (Table IV).[103] Naive T_H0 cells produce primarily IL-2 but might also synthesize cytokines characteristic of effector T lymphocytes. In contrast to murine studies, categorically distinct T_H cytokine profiles are seldom apparent in human cells, although there remains an inverse relationship between the tendency of T lymphocytes to produce IFN-γ as opposed to IL-4/IL-5 or IL-17. In human subjects T_H1 cells primarily produce IFN-γ and TNF-β but not IL-4 and IL-5. T_H2 cells more prominently produce IL-4, IL-5, IL-9, and IL-13 but not IFN-γ. T_H1 lymphocytes promote cell-mediated immune responses and are important in antibody-dependent immunity. T_H17 cells are more important in the T cell–mediated immune response to extracellular pathogens and likely contribute to autoimmune diseases. T_H2 lymphocytes produce IL-4, IL-5, and IL-13, which induce antiparasitic and allergic immune responses. A subclass of T_H2 cells characterized by prominent IL-9 production has recently been described (T_H9 cells).[77,78]

Cytokines involved in T_H1 differentiation

One of the more important questions in understanding the cause of immune disorders is to determine the basis for effector T-cell differentiation in response to antigen. The most critical element in determining T_H differentiation is the cytokine milieu in which the T lymphocyte is activated (Table IV). T_H1 differentiation is induced and maintained through the influences of IL-12, IL-18, and IL-27, with IL-12 providing the most important role.[104] IL-12 interacts with naive T_H lymphocytes to activate STAT4, leading to expression of the transcription factor T-box expressed in T cells (T-bet). T-bet is a nuclear transcription factor that is the master regulator responsible for the differentiation of T_H1 cells. Actions of T-bet include production of IFN-γ and IL-12R. Simultaneously, it blocks alternative T_H differentiation pathways by suppressing expression of T_H2 cytokines, such as IL-4, and acting as a negative regulator of T_H17 differentiation. Similar to IL-12, IL-27 also activates STAT4, leading to increased expression of T-bet and IFN-γ. Addition of recombinant IL-27 to naive T cells in culture under T_H2-polarizing conditions results in decreased expression of GATA-3, the transcription factor that is the master regulator for T_H2 development, along with a decrease in production of IL-4 and other T_H2 cytokines.[105] Once T_H1 cells become differentiated, newly synthesized IFN-γ, acting through STAT-1, also increases expression of T-bet and functions as a negative regulator of T_H17 and T_H2 differentiation. IL-18 upregulates IL-12R expression and is a growth factor for T_H1 cells. IL-12–producing DCs are the most important mediator of T_H1-like immune deviation. In addition, insofar as mononuclear phagocytes are an additional

source of IL-12, this suggests a mechanism whereby antigens likely to be processed by macrophages, including obligate intracellular bacteria (eg, mycobacteria), produce T_H1 responses.

Cytokines involved in T_H2 differentiation: IL-4, IL-19, IL-25, IL-33, and thymic stromal lymphopoietin

One determinant of T_H2 differentiation is IL-4 itself.[106] IL-4 activates STAT6, which in turn promotes expression of GATA-3, the master regulator of T_H2 cells, and suppresses expression of T-bet. GATA-3 potentiates IL-4 expression and suppresses expression of T_H1 differentiation and cytokine (IFN-γ) production. IL-4 and GATA-3 similarly inhibit differentiation of T_H17 lymphocytes. Other transcription factors, including especially MAF and NFAT, contribute to IL-4 and other T_H2 signature cytokine production once T_H2 differentiation is established. The original source of the IL-4 responsible for T_H2 differentiation can be the naive T_H0 lymphocytes themselves. Basophils, NK T cells, and mast cells are also capable of robust IL-4 secretion.[107,108] Whatever the source is for the IL-4, the end result is that in a milieu in which allergic inflammation is present (eg, bronchial lymphatic tissue), more and more extensive allergenic responses against bystander antigens develop.

IL-19, a member of the IL-10 family, is primarily produced by mononuclear phagocytic cells, and its expression is upregulated by IL-4 and downregulated by IFN-γ. IL-19 promotes T_H2 immune deviation.[109] IL-19 expression is important to the development of airway inflammation in murine models, and its increased expression has been observed in asthmatic patients.[92]

As discussed, IL-25 induces expression of T_H2 signature cytokines from numerous cell types but also specifically contributes to T_H2 immune deviation.[110] Its production by T_H2 lymphocytes suggests a positive feedback cascade.

Currently, the 2 most important cytokines responsible for T_H2 immune deviation are considered to be IL-33 and thymic stromal lymphopoietin (TSLP). Similar to IL-18, IL-33[111] is an IL-1–like cytokine that signals through an IL-1 receptor–related protein.[112] As with IL-1 and IL-18, IL-33 is produced as an inactive precursor, and its secretion and activation are dependent on cleavage by caspase-1. IL-33 is expressed by bronchial epithelial cells, fibroblasts, smooth muscle cells, keratinocytes, macrophages, and DCs. IL-33 receptors are expressed on T cells (specifically nascent and mature T_H2 cells), macrophages, hematopoietic stem cells, mast cells, and fibroblasts. Administration of IL-33 induces T_H2 immune deviation and cytokine production, causes increased IgE levels, and generates profound mucosal eosinophilic inflammation in the lung and gastrointestinal tract.[111,113] Administration of an IL-33 receptor antagonist reduces production of T_H2 cytokines and airway inflammation in murine asthma models.[112,114] Its primary production by epithelial cells suggests a mechanism whereby the respiratory tract can generate a "danger signal" that will drive a subsequent T_H2 immune response, arguably the initial trigger of asthma.

The cytokine TSLP has also been suggested as a primary instigator of T_H2 immune deviation.[115] TSLP is expressed by epithelial cells of the skin, gut, and lung and activates DCs in such a way as to promote T_H2 cytokine production by their subsequently engaged effector T cells. The expression of TSLP in the lungs of mice produces severe AHR,[116,117] and similarly, expression in the skin produces skin inflammation suggestive of atopic dermatitis.[118] TSLP is highly expressed in the keratinocytes of patients with atopic dermatitis and the lungs of asthmatic patients.[119] The TSLP receptor is a heterodimer composed of a unique TSLP-specific receptor and the IL-7 receptor α chain (CD127). TSLP receptors are primarily expressed by DCs, but their expression by mast cells also promotes secretion of T_H2 signature cytokines. As with IL-33, its prominent expression by epithelium suggests an initial triggering event plausibly central to the development of allergic diseases of the skin and airways.

T_H9 lymphocytes are a recently described proposed subfamily of T_H2 cells characterized by prominent production of IL-9 and relatively less IL-4. They result from the differentiation of T_H2 cells in the concomitant presence of TGF-β.[77,78]

T_H17 lymphocytes

The selective production of IL-17 by clonal T_H lymphocytes has led to the recognition of the T_H17 cell as a distinct lymphocyte subset.[120] The presence of distinct pathways involved in differentiation of IL-17–producing T lymphocytes (Table IV) and that counterregulate development of the alternative T_H1- and T_H2-like pathways further supports the concept that these T_H17-producing T_H lymphocytes comprise a distinct lineage. The mechanisms underlying T_H17 differentiation in human subjects are not fully established. In mice IL-6 acting in the additional presence of TGF-β is the most important cytokine responsible for differentiation of T_H17 cells.[121] IL-21 and IL-23 further contribute to T_H17 differentiation and expansion of established T_H17 cells.[122] Only in the absence of IL-6 does TGF-β promote differentiation into Treg cell pathways, as previously described. The highly pleiotropic cytokine TGF-β is therefore involved in the differentiation of Treg cells or, in the additional presence of IL-6 or IL-4, can be switched to induced T_H17 or T_H9 cells, respectively.[123] The action of IL-6 in inducing T_H17 is mediated through its activation of STAT3. The net result is activation of retinoic acid receptor–related orphan receptor (ROR) γt, the master regulating transcription factor for T_H17 cells. Heterozygous mutations in STAT3 produce the hyper-IgE syndrome,[124,125] a condition characterized by deficient T_H17 lymphocytes.[44,46]

TREG LYMPHOCYTE FAMILIES: NATURAL TREG, INDUCED TREG, AND T_H3 CELLS

In addition to traditional T_H subclasses, much progress has been made in the past several years in identifying and clarifying the characteristics of several families of regulatory T lymphocytes (Table V).[126] These include peripherally differentiated (induced) IL-10–producing lymphocytes, termed induced Treg (iTreg) cells; thymic-derived $CD25^+$ natural Treg (nTreg) cells; and TGF-β–producing T_H3 cells. Thymus-derived nTreg cells are characterized by their constitutive expression of IL-2Rα chains (CD25) and the transcription factor FOXP3. Similar to the role assumed by T-bet in T_H1, GATA-3 in T_H2, and RORγt in T_H17 differentiation, FOXP3 serves as a master regulator of nTreg cells (Table IV). Although they secrete IL-10, membrane TGF-β appears to be primarily responsible for mediating their immune suppression, which is contact dependent. nTreg cells are produced in response to expression in the thymus of self-antigens and are thereby important for the prevention of autoimmunity. These nTreg cells are unlikely to be involved in tolerance to antigens not presented in the thymus (eg, in either tolerance to allergens in healthy subjects or in the immune benefits associated with

TABLE V. $CD4^+$ T cells with regulatory activity

Treg cell subtype	Characteristics
nTreg (natural Treg cells)	$CD25^+Foxp3^+$ thymus derived. Not dependent on IL-10 for their biologic activity. Mediate self-tolerance/prevent autoimmune disease. Not likely to be relevant to acquired tolerance to allergens.
T_H3	Characterized by TGF-β (± IL-10) production. Mediate mucosal tolerance/antigen-specific IgA production. Not relevant to inhalant allergy or immunotherapy.
iTreg (induced Treg cells)	Peripheral-derived Treg cells. IL-10 responsible for their biologic activity (± TGF-β). Thought to be derived from T_H1/T_H2-like effector lymphocytes ±CD25 expression (reflecting their effector function/activation) ± FOXP3 expression. Induced in contact-dependent fashion by membrane TGF-β. Proposed mechanism of immunotherapy

allergen immunotherapy). T_H3 cells are primarily gut derived and generate mucosal tolerance. Reflecting their prominent production of TGF-β, in addition to tolerance, they are relevant to secretory IgA production. In contrast to thymus-derived nTreg cells, an additional, less well-characterized class of adaptive Treg cells has been described that can develop in the periphery. These iTreg cells differentiate from pre-existing effector T lymphocytes or possibly circulating naive T_H0 cells and are characterized by their prominent production of IL-10. iTreg expression of FOXP3 and CD25 is controversial but does occur. For example, it is unclear whether CD25 expression reflects the constitutive expression of this component of IL-2R, the signature characteristic of nTreg cells, or the derivation of iTreg cells from activated effector T cells that are transiently expressing CD25. The induction of IL-10–producing iTreg cells plays a key role in reducing allergen-specific T-cell responsiveness after immunotherapy.[127,128]

SIGNAL TRANSDUCTION BY CYTOKINE RECEPTORS

Two key events are required to initiate the intracellular signaling pathways activated by cytokines. First, binding of a cytokine to its receptor mediates the transduction of signals from the extracellular environment into the cytoplasm. Second, activation of tyrosine kinases results in phosphorylation of the receptor and signaling molecules, events that ultimately lead to delivery of intracellular signals. With the notable exceptions of the receptors for SCF (c-kit or CD117) and M-CSF (colony-stimulating factor 1 receptor [also used by IL-34]), cytokine receptors generally do not have cytoplasmic domains with intrinsic tyrosine kinase activity; however, cytokine receptors do activate cytoplasmic tyrosine kinases.

Although numerous biochemical cascades are involved in cytokine signaling, this discussion will primarily focus on 2 families of protein tyrosine kinases, termed JAKs and STATs, which uniquely function in cytokine signaling (Fig 1, *A*).[129,130] The role for JAK family members in the pathway to gene activation was largely deduced from studies of signal transduction by the interferon receptors. The 2 chains of the IFN-α receptor bind JAK1 and TYK2, respectively, whereas the 2 chains of the IFN-γ receptor bind JAK1 and JAK2. The receptors and the JAKs themselves become phosphorylated, and this phosphorylated complex becomes the catalyst for the phosphorylation of cytoplasmic substrates. There are 4 JAKs, JAK1, JAK2, JAK3, and TYK2, and as such, receptor signaling is mediated by a surprisingly limited number of highly redundant tyrosine kinases. For example, JAK2 is involved in GM-CSF, granulocyte colony-stimulating factor, IL-6, and IL-3 signaling.

JAK1 and JAK3 are tyrosine phosphorylated in response to IL-2, IL-4, and all the other cytokines whose receptors are members of the shared γ chain family. This use of JAK3 by the shared γ chain is consistent with JAK3 deficiency sharing the severe combined immunodeficiency syndrome phenotype with γ chain deficiency. Once engagement of a cytokine receptor has led to tyrosine phosphorylation of the receptor and of receptor-associated JAKs, the next step in signal transduction involves the tyrosine phosphorylation of the STATs (Table VI).[129,130] After their activation, these proteins migrate to the nucleus, where they bind to specific regulatory sequences in the promoters of cytokine-responsive genes, thereby initiating gene transcription. As with the JAKs, the function of STATs was originally characterized with studies involving the biochemical events of interferon-induced gene transcription. Ligand binding of IFN-α/β induces the formation of a trimer composed of STAT1, STAT2, and a non-STAT protein, interferon regulatory factor protein p48. Evidence suggests that STAT2 is the crucial STAT in establishing type I interferon activity because it is specifically recruited to DNA sequences comprising interferon-stimulated response elements present in the promoters of type I interferon-responsive genes.[131] In contrast, the stimulation of cells with IFN-γ results in the tyrosine phosphorylation of STAT1 by JAK1 and JAK2 but not of STAT2. These homodimers of STAT2 recognize IFN-γ activation site DNA sequences in the promoters of IFN-γ–responsive genes. Similar to type I interferons, IL-28 and IL-29 (IFN-λs) induce the activation of the JAK/STAT signaling pathways.[26,132,133] JAK1 in particular is critical in mediating IFN-λ–induced STAT phosphorylation.[132] IFN-λ induces homodimers of STAT2 capable of recognizing both interferon-stimulated response element and IFN-γ activation site sequences. It is therefore not surprising that many genes whose expression is classically induced by both type I interferons and IFN-γ are also induced by IFN-λs.

There are 5 additional members of the STAT family. STAT3, STAT4, and STAT6 were identified as IL-6–, IL-12–, and IL-4–inducible peptides, respectively. Although important in cytokine signaling, STAT5 (consisting of 2 homologous genes, STAT5A and STAT5B) was originally defined as a prolactin-activated peptide. Engagement of the IL-4 receptor leads to the activation of JAK1, which in turn phosphorylates STAT6. STAT6 is necessary for IL-4–dependent expression of IL-4 receptor (IL-4R) α, the ϵ heavy chain, MHC class II, CD23, and mucin.[134] An endogenous inhibitor of STAT6 is referred to as the suppressor of cytokine signaling 1.[135] Suppressor of cytokine signaling 1 inhibits IL-4–induced activation of JAK1 and STAT6 and thereby effectively inhibits IL-4 signaling.

Compared with the number of cytokines, relatively few STATs exist, and therefore the signaling pathways of numerous distinct cytokines share common STAT proteins. For example, epidermal growth factor, platelet-derived growth factor, M-CSF, IL-6, IL-11, and the interferons all activate STAT1α. Mechanisms

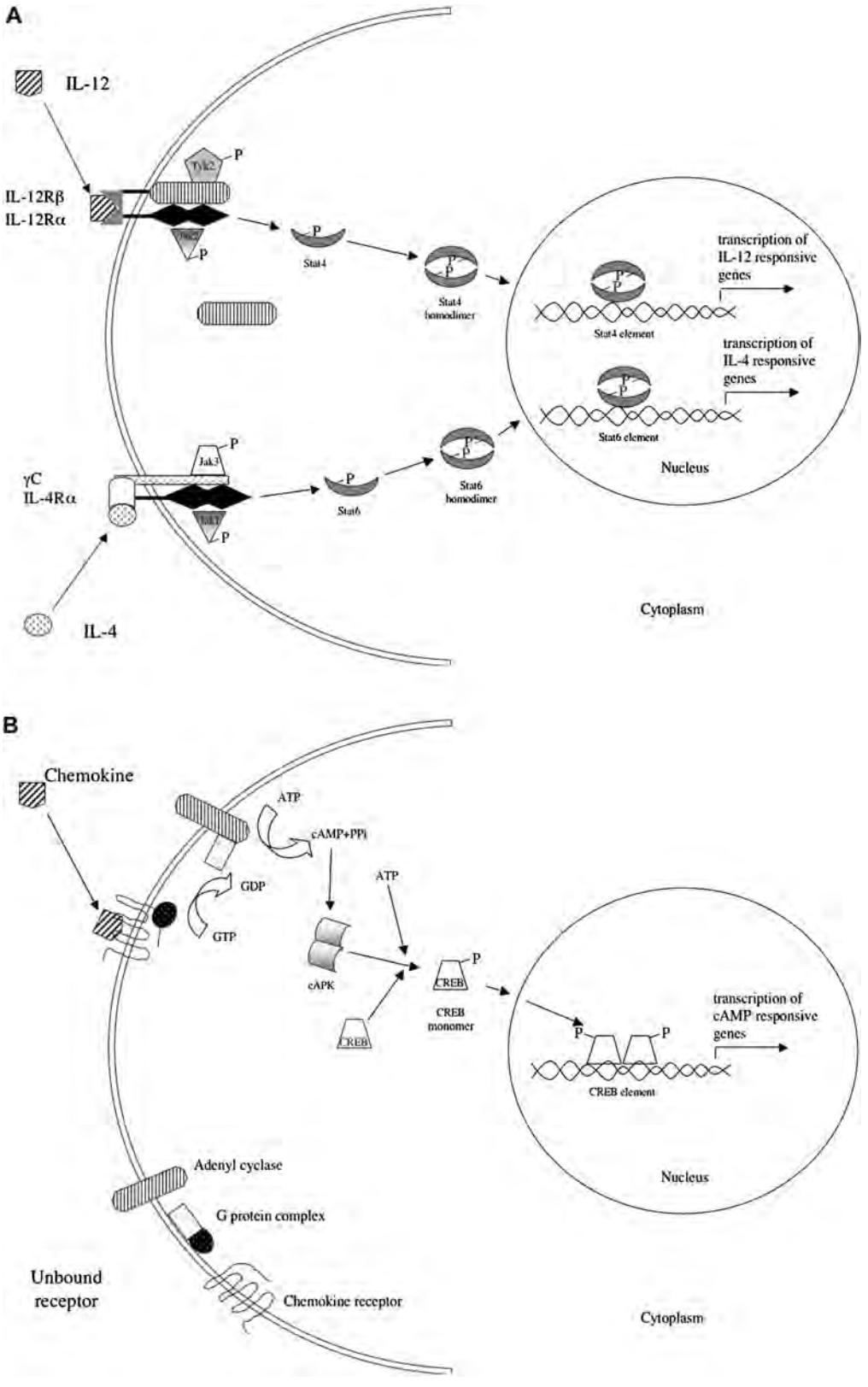

FIG 1. Comparison of cytokine and chemokine signaling. **A,** Generalized cytokine signaling: a model of intracellular signaling pathways leading to transcription modulation by IL-4 and IL-12 (see text for details). **B,** Generalized chemokine signaling: a model of chemokine binding and activation of G proteins leading to induction of transcription factors and gene expression (see text for details). *cAPK*, cAMP-dependent protein kinase; *CREB*, cAMP response element binding protein.

TABLE VI. STAT family

STAT protein	Cytokine
STAT1	IFN-α/β* IFN-γ* Epidermal growth factor, platelet-derived growth factor, M-CSF, IL-6, IL-11
STAT2	IFN-α/β* IFN-λ
STAT3	IL-6 (IL-6 family cytokines, including IL-6, oncostatin M, and LIF) trigger STAT3 though the gp130 receptor) IL-5, IL-10, epidermal growth factor, human growth factor
STAT4	IL-12 (essential endogenous mediator of T_H1 differentiation)
STAT5A and STAT5B†	Prolactin IL-2, IL-3, IL-7, GM-CSF, erythropoietin, thrombopoietin
STAT6	IL-4 (essential endogenous mediator of T_H2 differentiation)

*IFN-α/β signaling complex (interferon-stimulated gene factor 3) consists of trimers of STAT1 (alternatively spliced α [p91] or β [p84] peptides), STAT2, and the non-STAT protein p48. IFN-γ signaling complex consists of dimers of STAT1.
†Two distinct genes that are 90% identical.

must exist that lead to the distinct responses to different cytokines. In part these reflect the activities of other signaling pathways stimulated by cytokine receptors. For example, the Ras-dependent pathway is also activated by members of the cytokine receptor families. In this cascade Ras, Raf-1, Map/Erk kinase kinase, and mitogen-activated protein kinases (MAPKs) are sequentially phosphorylated and activated. The MAPK pathway is associated with induction of several transcription factors, such as c-myc, c-fos, and nuclear factor–IL-6. This ras pathway is activated by several growth factors, as well as by the cytokines IL-2, IL-3, IL-5, and erythropoietin. An example of another complementary distinct pathway used for cytokine signaling is provided by IL-4, which activates the signaling protein insulin response substrate (IRS) 1 and its homologue, IRS-2. IRS-1 and IRS-2 regulate cellular proliferation and protection from apoptosis.

CHEMOKINES

Chemokines are a group of small (8-12 kd) proteins that posses the ability to induce cell migration or chemotaxis in numerous cell types, including neutrophils, monocytes, lymphocytes, eosinophils, fibroblasts, and keratinocytes. Activity is regulated through binding to members of the 7-transmembrane, G protein–coupled receptor superfamily. This section uses the systematic nomenclature with the common names listed in parentheses the first time the chemokine is described. To date, 52 chemokines and 20 chemokine receptors have been described, which are listed in Table VII[136,137] along with the known chromosomal location and physiologic properties of each. Many of the chemokine receptors can bind more than 1 ligand, allowing extensive overlap and redundancy of chemokine function.

Originally, chemokines were described as inflammatory mediators produced at sites of infection or injury or in response to proinflammatory stimuli. Inflammatory chemokines recruit and activate leukocytes to mount an immune response and initiate wound healing. Although chemotaxis stands as the cardinal feature of chemokines, their physiologic role is more complex than originally described, with many having additional homeostatic or housekeeping functions. These functions range from trafficking of lymphocytes during hematopoiesis, antigen sampling in secondary lymphoid tissue, immune surveillance, and organ development.[136] In general, homeostatic chemokines are expressed in specific tissues or organs, whereas inflammatory chemokines are produced by many cell types in multiple locations.

Classification

Chemokines are characterized by the presence of 3 to 4 conserved cysteine residues and can be subdivided into 4 families based on the positioning of the N-terminal cysteine residues (Table VII). Within a subfamily, there exists 30% to 90% amino acid identity between members; however, across subfamilies, the amino acid identity decreases to less than 30%. The C-X-C subfamily is characterized by the separation of the first 2 cysteines by a variable amino acid. The CXC chemokines can be broken into 2 general subgroups: ELR and non-ELR containing. ELR is a conserved amino acid motif (Glu-Leu-Arg) immediately preceding the first cysteine residue. The ELR chemokines (CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, and CXCL8) are angiogenic and act mainly through the CXCR2 receptor. In contrast, the non-ELR chemokines (CXCL4, CXCL9, CXCL10, CXCL11 and CXCL17) are angiostatic and act mainly through the CXCR3B receptor. This non-ELR group of chemokines can be induced by a variety of interferons. The exception to this is CXCL12, which is a non-ELR chemokine but is angiostatic and binds to the CXCR4 on endothelial cells. In the C-C subfamily the cysteine residues are adjacent to each other. The majority of the known chemokines are contained in these 2 families. Additionally, these groups can be distinguished by their primary target cell, with the C-X-C subfamily targeting neutrophils and the C-C family targeting eosinophils, monocytes, and T cells. A third family of chemokines, referred to as the C subfamily, lacks the first and third cysteines, containing only a single cysteine residue in the conserved position. This subfamily includes the lymphocyte-specific chemotactic peptide XCL1 (lymphotactin).[138] A fourth subfamily (CX3C) has the 2 N-terminal cysteine residues separated by 3 variable amino acids.[139] In human subjects this family only has 1 member, CX3CL1 (fractalkine), and it is unique in that it is has a mucin-like glycosylated stalk that allows it to exist as a soluble or membrane-bound chemokine.

Receptors and signal transduction

Receptor number on the cell surface varies from 3000 per cell on monocytes and lymphocytes for CCR1 and CCR2 to 40,000 to 50,000 per cell on eosinophils for CCR3.[140-142] Receptor numbers can be altered depending on the environmental milieu and the signals a cell receives. A given cell can express multiple chemokine receptors, each of which can induce specific signals, suggesting that each receptor can signal through different pathways. Additional complexities of receptor use are emerging through the recent demonstration that CXCR4 and CCR5 can heterodimerize and transmit a compound signal when stimulated with their respective ligands.[143] The ability to signal through different pathways is due in part to the heptahelical transmembrane property of the receptors. A large surface area, allowing interactions with the α and βγ subunits of the heterotrimeric G proteins and other effector molecules, is created by looping of the receptor

TABLE VII. CC, C, CXC, and CX3C chemokine/receptor families

Systematic name	Human chromosome	Common name	Receptor	Physiologic features
CC chemokine/receptor family				
CCL1	17q11.2	I-309	CCR8, R11	Inflammation
CCL2	17q11.2	MCP-1, MCAF	CCR2	Inflammation
CCL3	17q11-q21	MIP-1α/LD78α	CCR1, R5	Inflammation, homeostasis
CCL3L1	17q21.1	LD78β	CCR5	Inflammation
CCL4	17q11.2	MIP-1β	CCR5	Inflammation
CCL4L1	17q12	None	CCR5	Inflammation
CCL4L2	17q12	None	CCR5	Inflammation
CCL5	17q11.2	RANTES	CCR1, R3, R4, R5	Inflammation
CCL6	(mouse)	C-10	CCR1, R2, R3	Unknown
CCL7	17q11.2	MCP-3	CCR1, R2, R3	Inflammation
CCL8	17q11.2	MCP-2	CCR1, R2, R5, R11	Inflammation
CCL9	(mouse)	MRP-2/MIP-1γ	CCR1	Unknown
CCL10	(mouse)	MRP-2/MIP-1γ	CCR1	Unknown
CCL11	17q11.2	Eotaxin	CCR3	Inflammation, homeostasis
CCL12	(mouse)	MCP-5	CCR2	Unknown
CCL13	17q11.2	MCP-4	CCR1, R2, R3, R11	Inflammation
CCL14	17q11.2	HCC-1	CCR1	Inflammation
CCL15	17q11.2	HCC-2, Lkn-1	CCR1, R3	Inflammation
CCL16	17q11.2	HCC-4, LEC	CCR1	Inflammation
CCL17	16q13	TARC	CCR4	Inflammation, homeostasis
CCL18	17q11.2	DC-CK1, PARC	Unknown	Homeostasis
CCL19	9p13	MIP-3β, ELC	CCR7, R11	Homeostasis
CCL20	2q33-q37	MIP-3α, LARC	CCR6	Inflammation, homeostasis
CCL21	9p13	6Ckine, SLC	CCR7, R11	Homeostasis
CCL22	16q13	MDC, STCP-1	CCR4	Inflammation, homeostasis
CCL23	17q11,2	MPIF-1	CCR1	Inflammation
CCL24	7q11.23	MPIF-2, Eotaxin-2	CCR3	Inflammation
CCL25	19p13.2	TECK	CCR9, R11	Homeostasis
CCL26	7q11.23	Eotaxin-3	CCR3	Inflammation
CCL27	9p13	CTACK, ILC	CCR2, R3, R10	Homeostasis
CCL28	5p12	MEC	CCR3, R10	Inflammation, homeostasis
C chemokine/receptor family				
XCL1	1q23	Lymphotactin	XCR1	Inflammation
XCL2	1q23	SCM1-b	XCR1	Inflammation
CXC chemokine/receptor family				
CXCL1 (ELR)	4q12-q13	GROα, MGSA-α	CXCR2>R1	Inflammation, homeostasis
CXCL2 (ELR)	4q12-q13	GROβ, MGSA-β	CXCR2	Inflammation
CXCL3 (ELR)	4q12-q13	GROγ, MGSA-γ	CXCR2	Inflammation
CXCL4 (non-ELR)	4q12-q13	PF4	CXCR3	Inflammation
CXCL4L1 (non-ELR)	4q12-q21	PF4V1	CRCR3	Inflammation
CXCL5 (ELR)	4q12-q13	ENA-78	CXCR1, R2	Inflammation
CXCL6 (ELR)	4q12-q13	GCP-2	CXCR1, R2	Inflammation
CXCL7 (ELR)	4q12-q13	NAP-2	CXCR2	Inflammation
CXCL8 (ELR)	4q12-q13	IL-8	CXCR1, R2	Inflammation
CXCL9 (non-ELR)	4q21.21	Mig	CXCR3	Inflammation
CXCL10 (non-ELR)	4q21.21	IP-10	CXCR3	Inflammation
CXCL11 (non-ELR)	4q21.21	I-TAC	CXCR3	Inflammation
CXCL12 (non-ELR)	10q11.1	SDF-1α/β	CXCR4, R7	Inflammation, homeostasis

(Continued)

Table VII. *(Continued)*

Systematic name	Human chromosome	Common name	Receptor	Physiologic features
CXCL13 (non-ELR)	4q21	BLC, BCA-1	CXCR3, R5	Inflammation, homeostasis
CXCL14 (non-ELR)	5q31	BRAK, bolekine	Unknown	Homeostasis
CXCL15 (ELR)	(mouse)		Unknown	Unknown
CXCL16 (non-ELR)	17p13	SR-PSOX	CXCR6	Inflammation
CXCL17 (non-ELR)	19q13.2	VCC1, DMC	Unknown	Inflammation, homeostasis
CX3C chemokine/ receptor family				
CXCCL1	16q13	Fractalkine	CR3CR1	Inflammation

This table is an adaptation of the tables presented by Zlotnik and Yoshie[137] and Moser and Loetscher.[136] The terms *inflammation* and *homeostasis* under the "Physiologic features" heading refer to inflammatory chemokines and homeostatic chemokines, respectively. The most common names for the human ligands are listed but are not all inclusive of ligand names found in the literature. ELR is a conserved amino acid motif (Glu-Leu-Arg) immediately preceding the first cysteine amino acid in the CXCL chemokine family.

along the inner plasma membrane and the lateral orientation of the carboxy terminus.[144]

Signaling is initiated after binding of the chemokine to the receptor, which activates guanine exchange factors, allowing replacement of guanine diphosphate with guanine triphosphate on the Gα subunit (Fig 1, *B*). The result is dissociation of the heterotrimeric G protein complex from the receptor and separation of the Gα and Gβγ subunits. The Gα subunit is able to directly activate the Src family kinases, leading to activation of the MAPKs and protein kinase B.[145] Signaling through the Gβγ subunit is more complex, involving at least 3 separate pathways. Gβγ can activate protein kinase B and the MAPKs through phosphatidylinositol 3–kinase γ and PKC through phospholipase C and Pyk-2.[146-148] Activation of phospholipase C increases the intracellular calcium ion concentration. Calcium influx activates many cellular processes, including degranulation of neutrophils, eosinophils, and basophils. Other pathways activated by chemokines include phospholipases A_2 and D, protein tyrosine kinases, low-molecular-weight guanine triphosphatases, Rho, and Rac. Several other reviews cover chemokine signaling in more extensive detail.[144,149] Signaling through the G proteins ends when a phosphate group is removed from the guanine triphosphate bound to the Gα subunit reforming guanine diphosphate. This allows the Gα and Gβγ subunits to rejoin and terminate downstream signaling events. Chemokines can also activate signaling pathways, such as MAPK and protein tyrosine kinase, through G protein–independent mechanisms. Signaling through chemokine receptors can be dampened through several processes, including homologous and heterologous desensitization.

Homologous desensitization occurs when G protein–coupled receptor kinases selectively phosphorylate chemokine-occupied receptors, leading to endocytic uptake of chemokine receptor complexes. Heterologous desensitization occurs when non–G protein–coupled receptor kinases phosphorylate ligand-free (non-engaged) chemokine receptors, preventing future G protein coupling and receptor activation.

In addition to the receptors that activate cellular responses to chemokines, 3 other receptors bind chemokines: duffy antigen receptor for chemokines, D6, and CCX-CKR. These receptors bind chemokines but do not signal, leading to their designation as decoy receptors. Decoy receptors bind ligand and prevent the ligand from being able to act. In terms of chemokine action, decoy receptors play a role in dampening the immune response, leading to resolution of inflammation. Recently, this concept has been challenged by the finding that duffy antigen receptor for chemokines can mediate chemokine transcytosis, leading to apical retention of the chemokine and enhanced leukocyte migration across monolayers.[150]

Chemokine function

The original description of chemokines focused on their primary role in directing lymphocytes to sites of inflammation. A detailed examination of cell migration and recruitment is beyond the scope of this review and is covered elsewhere.[151] Briefly, in a process known as rolling, lymphocytes interact transiently with the vascular endothelium, searching for activating signals from chemokines. On binding of a chemokine to its receptor, integrins are expressed, which mediate high-affinity interactions and lead to firm arrest of the leukocytes. This has been demonstrated for the chemokines CCL19 (ELC), CCL21 (SLC), and CXCL12 (SDF-1), which rapidly induce a high-affinity state for the β_2-intergrin lymphocyte function–associated antigen 1.[152] Once the cell has ceased rolling, it can cross the endothelium and will continue this process as it migrates along a concentration gradient and crosses the endothelial layer to the source of the generated chemokine. It is the expression of particular chemokines, receptors, and adhesion molecules that contribute to the selective migration and tissue specificity of leukocytes.

Chemokines perform a variety functions aside from chemotaxis. Chemokines can have direct effects on T-cell differentiation through ligand-receptor interactions on the developing cell or indirectly by altering APC trafficking or cytokine secretion. Functioning through the CCR5 receptor, CCL3 (macrophage inflammatory protein [MIP] 1α), CCL4 (MIP-1β), and CCL5 can directly promote development of IFN-γ T_H1 cells or indirectly by increasing IL-12 production from APCs. In contrast, CCL2 (monocyte chemoattractant protein [MCP] 1), CCL7 (MCP-3), CCL8 (MCP-2), and CCL13 (MCP-4) can inhibit IL-12 production from APCs and enhance IL-4 production from activated T cells, leading to a T_H2 phenotype.[153] Chemokine receptor expression can serve as a marker for maturation and differentiation of lymphocytes. When monocytes and immature DCs exit blood in tissues and begin immune surveillance, they express the CCR1, CCR2,

CCR5, CCR6, and CXCR2 receptors, which are classified as inflammatory receptors. As antigen is encountered and the DCs mature, the inflammatory receptors are downregulated and replaced by expression of CCR7. CCR7 expression allows the DCs to accumulate in the draining lymphatics and T-cell areas of the lymph nodes.[154] Expression of CXCR5 has been demonstrated on a distinct memory T-cell subset that displays B helper cell function. These cells respond to CXCL13 (BLC) and are directed to the B-cell follicle to help support production of antibodies.[155,156] Release of mature neutrophils from the bone marrow is regulated by binding of CXCL12 with its receptor, CXCR4.[157] Other examples include a role for CXCL1, CXCL12, and CCL3 in brain development; a role for CCL2 and CXCL8 in wound healing; and a role for CXCL12 in organogenesis.

Clinical relevance

Aberrant regulation of chemokine expression has been implicated in many diseases (Table VIII); however, the focus of this section will be on the role that chemokines play in allergic disorders. Many studies have demonstrated increased chemokine levels in asthmatic patients compared with control subjects, as measured in bronchoalveolar lavage and biopsy samples.[158,159] These include CCL2, CCL3, CCL5, CCL7, CCL11, CCL13, CCL24, CXCL8, and CXCL10. Investigators have used murine models of asthma to understand the role that chemokines play in inducing AHR. CCL2, CCL5, CCL11, CXCL10, and CXCL12 all contribute to AHR and cellular emigration in these models of airway inflammation.[160-162]

The C-C chemokine family has been extensively studied in allergic diseases because of its members' ability to recruit eosinophils, T cells, and monocytes to regions of inflammation. CCL5 and CCL11 are the most important eosinophil chemoattractants in allergic inflammation.[163] This has been demonstrated in mice, in which instillation of CCL5 or CCL11 into the lungs results in an eosinophilic and mononuclear cell infiltrate in the absence of neutrophils.[164] Aside from production by eosinophils, macrophages, mast cells, and T cells, CCL5 and CCL11 are produced by structural cells of the airway, including airway smooth muscle and fibroblasts. In addition to lymphoid tissue, nasal epithelial cells express CCL17 (TARC), and expression of this chemokine and its receptor, CCR4, was higher in patients with allergic rhinitis compared with that seen in nonallergic control subjects. Both IL-4 and IL-13 promote CCL17 expression, leading to a T_H2 response.[165] This is relevant in allergic bronchopulmonary aspergillosis (ABPA), in which increased serum levels of CCL17 predict ABPA exacerbations better than IgE levels.[165] CCL17 levels might serve as a marker of ABPA in patients with cystic fibrosis.[166]

CXCL8 is derived primarily from mononuclear phagocytes and endothelial and epithelial cells but also from T cells, eosinophils, neutrophils, fibroblasts, keratinocytes, hepatocytes, and chondrocytes. CXCL8 synthesis can be induced by LPS, IL-1, TNF, or viral infection.[167,168] On a molar basis, CXCL8 is one of the most potent chemoattractants for neutrophils in addition to stimulating the neutrophil respiratory burst and adherence to endothelial cells through CXCR1.[169] CXCL10 and CXCL13 are induced at different times after allergen exposure. CXCL10 is produced in the early phases after allergen exposure, whereas CXCL13 is only induced after secondary and subsequent allergen exposures.[170] This might have to do with the cellular sources of these cytokines.

TABLE VIII. Chemokine/chemokine receptor involvement in human disease

Chemokine/chemokine receptor	Disease
CCR5, CCL3L1, CCL4L1, CXCR4	HIV/AIDS
CXCR4	WHIM syndrome
CX3CR1, CX3CL1, CXCL1, CXCL8, CXCR2, CCL2	Atherosclerosis
CCL2, CCL5, CCL7, CCL11, CXCL8	Asthma, allergic diseases
CXCR4, CXCL1, CXCL12	Cancer metastases
CXCL4	Heparin-induced thrombocytopenia
CCL26	Eosinophilic esophagitis
CCR5	Rheumatoid arthritis
CCR5	Renal allograft rejection
CCR5	West Nile virus infection
Duffy antigen receptor for chemokines	Malaria (*Plasmodium vivax* infection)

WHIM, Warts, hypogammaglobulinemia, infection, and myelokathexis.

Airway epithelial cells produce CXCL10, and contact with allergen might induce expression and thus explain the high levels early after allergen exposure. CXCL13 is produced by T_H17, but not T_H1 or T_H2, cells. It is tempting to speculate that T_H17 cells might play a role in asthma in later exposures after the allergic phenotype has already been established.

T-cell subsets that might have regulatory activity are being identified, and chemokines and their receptors appear to have important roles in mediating activity and migration of these cells. Among $CD4^+CD25^+Foxp3^+$ nTreg cells, there appears to be at least 2 subgroups that can be distinguished based on CCR6 expression. Those that are high in CCR6 seem to have regulatory activity, whereas those low in CCR6 secrete T_H2 cytokines on stimulation with bacterial superantigen.[171] Another group has demonstrated low levels of XCR1 on the surface of $CD4^+CD25^{hi}CD127^{low}$ T cells isolated from allergic asthmatic subjects compared with those from healthy control subjects.[172] Although in the early stages, this emerging field of chemokine response and expression by Treg cells will hopefully clarify many of the questions about how these cells work.

CONCLUSIONS

It has been almost 25 years since the cloning of the first cytokine was described. Since that time, more than 300 cytokines, chemokines, and growth factors have been described, with varying functions on not just the immune system but on every organ system in the body. Despite the large number of articles concerning the role of these proteins, we are still in our infancy in understanding how these factors alone and in concert with other factors influence homeostatic and inflammatory events. Abnormal production of these factors can lead to diseases such as asthma and atopy, and continued research is needed to piece together how these can be balanced to eliminate disease processes without compromising the individual to other deleterious outcomes.

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IgE, mast cells, basophils, and eosinophils

Kelly D. Stone, MD, PhD, Calman Prussin, MD, and Dean D. Metcalfe, MD *Bethesda, Md*

IgE, mast cells, basophils, and eosinophils are essential components of allergic inflammation. Antigen-specific IgE production, with subsequent fixation of IgE to FcεRI receptors on mast cells and basophils, is central to the initiation and propagation of immediate hypersensitivity reactions. Mast cells, basophils, and eosinophils are central effector cells in allergic inflammation, as well as in innate and adaptive immunity. This review highlights what is known about these components and their roles in disease pathogenesis. (J Allergy Clin Immunol 2010;125:S73-80.)

Key words: *IgE, mast cells, basophils, allergy, mastocytosis, hypereosinophilic syndromes*

Abbreviations used

AID: Activation induced deaminase
CD40L: CD40 ligand
CEL: Chronic eosinophilic leukemia
CysLT: Cysteinyl leukotriene
ECP: Eosinophil cationic protein
EDN: Eosinophil-derived neurotoxin
EPO: Eosinophil peroxidase
ITAM: Immunoreceptor tyrosine–based activation motif
LT: Leukotriene
MBP: Major basic protein
PAF: Platelet-activating factor
PG: Prostaglandin
SCF: Stem cell factor
SM: Systemic mastocytosis
STAT6: Signal transducer and activator of transcription 6
TLR: Toll-like receptor

IgE

IgE concentration in the serum is the lowest of the 5 immunoglobulin subtypes, has the shortest half-life (approximately 2 days), and expression is tightly regulated in the absence of disease. IgE shows no transplacental transfer. In the absence of disease, IgE levels in cord blood are low (<2 kIU/L; < 4.8 mg/L), gradually increase throughout childhood with a peak at 10 to 15 years of age, and then decrease throughout adulthood. Total IgE levels are also influenced by genetic makeup, race, immune status, and environmental factors (eg, pollen exposure).[1]

IgE synthesis

Isotype switching in general requires transcription through switch regions upstream of the new constant region, DNA cleavage of single-stranded DNA at the site of transcription, and DNA repair to recombine the VDJ domain with the new C domain. Isotype switching to IgE requires 2 signals. Signal 1 is provided by IL-4 or IL-13, acting through the IL-4 and IL-13 receptors by means of signal transducer and activator of transcription 6 (STAT6), which activates transcription at the IgE isotype-specific, Sε switch region. Signal 2 is provided by CD40 ligand (CD40L) on T cells acting through CD40 on B cells, which activates DNA switch recombination. In addition to activating transcription at the Cε locus, IL-4 and CD40L also induce expression of activation-induced deaminase (AID), which is involved in DNA repair, leading to class switch and somatic hypermutation.[2] Patients with mutations in the genes encoding CD40, CD40L, and AID have all been shown to have defective class switching, with hyper-IgM syndrome.

The process of class switching is initiated when allergen is taken up by antigen-presenting cells, including allergen-specific B cells that take up allergen through the cell-surface immunoglobulin receptor. Processed fragments are then presented in the context of MHC class II to T_H2 cells recognizing the allergen–MHC II complex. Activation of the allergen-specific T_H2 cells leads to expression of IL-4, IL-13, and CD154 and induction of class switching to IgE. At the initiation of class switching, T cells are the source of both signals. However, basophils express high levels of IL-4, IL-13, and CD154 after activation and have been suggested to play a role in polyclonal amplification of IgE production and in the differentiation of T_H2 cells.[2] IL-4 production by human mast cells is minimal, likely making their role in the amplification less important.

Although class switching is generally thought to occur in the germinal center of lymphoid tissues, class switching to IgE has also been reported to occur in the respiratory mucosa of patients with allergic rhinitis and atopic asthma and in the gastrointestinal tract in patients with food allergy.[3] These findings might have implications for patients with negative skin prick test responses or RAST results for allergens but with a history consistent with allergy, although the significance of these findings and clinical application is still not clear.

IgE receptors

There are 2 receptors for IgE: the low-affinity IgE receptor (FcεRII; CD23) expressed on the surface of B cells, as well as other hematopoietic cells, and the high-affinity IgE receptor (FcεRI). FcεRI is expressed on mast cells and basophils as tetramers ($\alpha\beta\gamma2$) and on antigen-presenting cells, at much lower levels, as trimers ($\alpha\gamma2$). Expression of the β chain in mast cells and basophils results in increased FcεRI surface expression and amplifies signaling through the receptor. FcεRI not occupied by

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Reprint requests: Dean Metcalfe, MD, National Institutes of Health, National Institute of Allergy and Infectious Diseases, 10 Center Dr, Bldg 10, Rm 11C205, MSC 1881, Bethesda, MD 20892-1881. E-mail: dmetcalfe@niaid.nih.gov.

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IgE has a half-life on the mast cell surface of 24 hours *in vitro*, whereas receptors bound to IgE appear to be expressed for the life of the cell.[4] The density of human basophil FcεRI expression correlates directly with serum IgE levels, where binding of IgE stabilizes the receptor at the cell surface. Similarly, the density of human mast cell FcεRI levels correlates with free IgE levels *in vitro*.[5]

The FcεRI subunits have no known enzymatic activity but rather signal through associated cytoplasmic tyrosine kinases. The α chain of FcεRI binds to the Fc portion (C3 domain) of IgE and consists of an extracellular domain, a transmembrane domain, and a short cytoplasmic tail with no signaling motifs. The β subunit consists of 4 transmembrane domains with a single immunoreceptor tyrosine–based activation motif (ITAM) and is associated with Lyn kinase. The γ subunits form a disulfide-linked dimer, and each subunit contains an ITAM. After aggregation of FcεRI by multivalent antigen recognized by bound IgE, Lyn phosphorylates tyrosine residues in the ITAMs of the β and γ subunits. The tyrosine-phosphorylated γ subunit then recruits Syk kinase. Syk activates a number of downstream signaling events associated with mast cell or basophil activation.[6,7] Syk-deficient basophils and mast cells do not degranulate after FcεRI aggregation. Syk is the target for a number of experimental therapeutic agents.

The low-affinity IgE receptor FcεRII (CD23) is a Ca-dependent lectin that is expressed on B cells, as well as T cells, Langerhans cells, macrophages, monocytes, eosinophils, and platelets. The receptor consists of a large extracellular domain with the lectin head that binds IgE, a single transmembrane domain, and a short cytoplasmic tail. Like the FcεRI receptor, expression of CD23 is upregulated by IgE and IL-4.[8] CD23 can be shed from the membrane into a soluble form, sCD23, by endogenous proteases (a disintegrin and metallopeptidase 10-ADAM10)[9] and exogenous proteases, including the dust mite major allergen Der p 1. CD23 activation mediates IgE regulation, differentiation of B cells, activation of monocytes, and antigen presentation. Increased expression of membrane-bound CD23 on B cells and resultant soluble CD23 is seen in patients with allergic disorders. CD23 expression on B cells is reduced with allergen immunotherapy. Polymorphisms in the gene encoding CD23 have been reported to be associated with the risk of asthma exacerbation.[10] An α-CD23 mAb, lumiliximab, has been tested *in vitro*, where it leads to a reduction in T_H2 responses and reduced IgE synthesis. Lumiliximab has been studied in a phase I trial in allergic asthma and is undergoing a phase II trial for the treatment of chronic lymphocytic leukemia.[8,11]

Measurement of total and specific IgE

Total IgE is measured with a 2-site, noncompetitive immunometric assay. Anti-IgE antibody directed at the Fc region of IgE is fixed to a solid surface and is used to capture IgE from serum. After washing, a different α-IgE antibody linked to an enzyme, fluorophore, or radionuclide is added to detect captured IgE.[12] The minimum amount of IgE detectable in serum with these methods is usually 0.5 to 1 μg/L, where 1 kIU/L equals 2.4 μg/L IgE.

Methods for detection of free IgE are also important in some situations, specifically to determine the effectiveness of omalizumab (humanized anti-IgE mAb) treatment in decreasing free IgE levels in patients with suboptimal clinical responses. Total IgE levels generally increase by up to 5-fold after omalizumab treatment because of the increased stability of omalizumab-IgE complexes, whereas free IgE levels decrease by up to 95%. There is great variability in the accuracy of different systems for total IgE measurements in the presence of omalizumab, although some tests perform well in this setting.[13] By using an mAb in the solid phase to capture IgE, followed by labeled FcεRI α chain for detection of captured IgE, free IgE levels can be accurately measured[14] as an indication of the mechanistic effectiveness of omalizumab in decreasing free IgE levels.

Measurement of allergen-specific IgE is determined by means of skin testing or measurement of allergen-specific IgE in serum. Assays to detect allergen-specific IgE are particularly useful to identify and monitor food allergy and when skin testing cannot be performed because of diffuse skin disease, significant dermatographism, inability to wean off medications interfering with the testing, or use of an extract believed to have a high probability of inducing a systemic reaction in the subject to be tested. The general principle used in such assays is to detect IgE that will bind to allergen fixed on a solid surface. The assays are influenced by the amount and quality of allergen bound to the solid support, the degree of nonspecific IgE binding, the affinity of the IgE antibody, and the degree of blocking of allergen-specific IgE binding by allergen-specific IgG. As a result, there is variability of levels of allergen-specific IgE detected by using different techniques and different reagents, making comparison between systems difficult.[15] In addition, IgE concentration, clonality, specific activity, and affinity all influence biological activity, but are not measured by current *in vitro* assays.[16]

Role in health and disease

Increased IgE levels are seen in patients with atopic diseases, with the highest levels generally being seen in patients with atopic dermatitis, followed by those with atopic asthma, perennial allergic rhinitis, and seasonal allergic rhinitis. For seasonal allergens, peak IgE levels occur 4 to 6 weeks after the peak of the pollen season. An increased total IgE level (>1,000 ng/mL) is one of the major diagnostic criteria for allergic bronchopulmonary aspergillosis, and unlike other diseases associated with increased IgE levels, the level of total IgE in patients with allergic bronchopulmonary aspergillosis can used to monitor disease activity and response to therapy.

Increased IgE levels are also seen in other disorders, including parasitic infections (eg, strongyloidiasis, ascariasis, and schistosomiasis), nonparasitic infections (eg, EBV, cytomegalovirus, HIV, and *Mycobacterium tuberculosis*), inflammatory diseases (eg, Kimura disease, Churg-Strauss vasculitis, and Kawasaki disease), hematologic malignancies (eg, Hodgkin lymphoma and IgE myeloma), cutaneous diseases (eg, Netherton syndrome and bullous pemphigoid), cystic fibrosis, nephrotic syndrome, and primary immunodeficiency diseases.[1,17] Primary immunodeficiency diseases associated with increased IgE levels include hyper-IgE syndrome, Wiskott-Aldrich syndrome, Omenn syndrome, immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance (IPEX), and atypical complete DiGeorge syndrome.[18] Increased IgE levels are also detected after hematopoietic stem cell transplantation, in smokers (particularly male smokers), and in those with alcoholism.

Because IgE plays a central role in the pathogenesis of atopic diseases, therapies directed at decreasing total IgE levels with anti-IgE mAbs (eg, omalizumab) have been developed. Omalizumab binds to the C3 region of the IgE Fc fragment and results in

complexes that decrease the level of free IgE available to bind IgE receptors. Omalizumab is approved for the treatment of atopic asthma and allergic rhinitis in patients older than 12 years with perennial allergen sensitization who are refractory to standard therapy. Reports have also been published describing the use of omalizumab in the treatment of other diseases, including idiopathic anaphylaxis, chronic urticaria, and eosinophilic gastrointestinal disorders.[19] Episodes of anaphylaxis associated with administration of omalizumab have been reported and have led the US Food and Drug Administration to place a black box warning on this medication. Recommendations for administration are available from the American Academy of Allergy, Asthma & Immunology and American College of Allergy, Asthma & Immunology Joint Task Force.[20]

MAST CELLS

Mast cells are tissue-based inflammatory cells of hematopoietic origin that respond to signals of innate and adaptive immunity with immediate and delayed release of inflammatory mediators. They are located primarily in association with blood vessels and at epithelial surfaces. Mast cells are central to the pathogenesis of diseases of immediate hypersensitivity and mastocytosis, but are also implicated in host responses to pathogens, autoimmune diseases, fibrosis, and wound healing.

Morphology and phenotype

Mast cells are up to 20 μm in diameter, are ovoid or irregularly elongated cells with an ovoid nucleus, and contain abundant metachromatic cytoplasmic granules. The metachromatic granule staining occurs as a result of abundant sulfated proteoglycans (eg, heparin and chondroitin sulfates) in the granules. The granule contents are crystalline by means of electron microscopy, but become amorphous after activation of the mast cell and before release of contents.[21,22]

Human mast cells are divided into 2 major subtypes based on the presence of tryptase (MC_T cells) or tryptase and mast cell-specific chymase (MC_{TC} cells), each predominating in different locations.[23] Tryptase staining identifies all mast cells and is the primary method for identifying tissue mast cells. MC_T cells are the prominent mast cell type within the mucosa of the respiratory and gastrointestinal tracts and increase with mucosal inflammation. MC_T cells appear selectively attenuated in the small bowels of patients with end-stage immunodeficiency diseases. MC_{TC} cells are localized within connective tissues, such as the dermis, submucosa of the gastrointestinal tract, heart, conjunctivae, and perivascular tissues.[24]

Mast cells are KIT (CD117) positive (receptor for stem cell factor [SCF]) and FcϵRI$^+$; they express other cell-surface receptors, depending on their location and stage of differentiation and activation. Mast cells express the activating IgG receptor FcγRIIa (CD32a) in the resting state and, in the presence of IFN-γ, the high affinity activating FcγRI (CD64). Inhibitory G protein–coupled receptors can also be expressed on mast cells, including the β_2-adrenergic receptor, the adenosine receptor A2B, and the prostaglandin (PG) E_2 receptor EP_2. Mast cells might also express the following receptors: C3a and C5a receptors, IL-3R, IL-4R, IL-5R, IL-9R, IL-10R, GM-CSFR, IFN-γR, CCR3, CCR5, CXCR2, CXCR4, nerve growth factor receptor, and Toll-like receptors (TLRs), among others.[21,22,24]

Development and trafficking

Human mast cells arise from CD34$^+$ pluripotent progenitor cells. Mast cell precursors circulate in the blood and then home to tissues, where they mature. Maturation of precursors in the tissues is dependent on SCF expressed on the surface of fibroblasts, stromal cells, and endothelial cells through binding to KIT on mast cells. The mechanisms of homing to specific tissues remains poorly understood, although the precursors express multiple chemokine receptors and integrins. Mast cell phenotype and behavior is altered by cytokines, such as IL-4, IL-5, and IFN-γ. For example, IL-4 upregulates expression of FcϵRI, IL-5 promotes proliferation in the presence of SCF, and IFN-γ decreases mast cell numbers. Homing receptors, tissue-specific expression of SCF, and the cytokine milieu are all likely involved in the heterogeneity of differentiation and distribution of mast cells in specific tissues.

Mast cells increase in number several-fold in association with IgE-dependent immediate hypersensitivity reactions, including rhinitis, urticaria, and asthma; connective tissue disorders, such as rheumatoid arthritis; infectious diseases, such as parasitic infection; neoplastic diseases, such as lymphoma and leukemia; and osteoporosis, chronic liver disease, and chronic renal disease. The most striking increase in mast cells occurs in parasitic diseases and in mastocytosis (associated with gain-of-function mutations in KIT). Loss-of-function mutations in KIT result in piebaldism (white forelock and hypopigmented patches of skin) caused by defective melanocyte migration, but do not result in significant pathology in most patients, such as an increase in susceptibility to infection or autoimmune disease.

Activation

Aggregation of FcϵRI by polyvalent antigen recognized by bound IgE activates mast cells and is the basis for anaphylaxis and other allergic diseases. FcϵRI density on the surface of mast cells is upregulated in the presence of increased free IgE levels and in the presence of IL-4, thus enhancing activation. In addition, mast cells are activated by C3a and C5a through C3aR and C5aR (CD88), nerve growth factor through TRKA, and IgG through FcγRI. Mast cells are also activated by TLR ligands. For example, activation through TLR3 by double-stranded RNA induces human mast cells to produce IFN-γ. The extent and pattern of mediators released depends on the signal, its intensity, and the cytokine milieu. Mediator release, for example, is enhanced in the presence of SCF.[6,7]

Mediators and effector function

Mediators produced by mast cells are divided into preformed mediators, newly synthesized lipid mediators, and cytokines/chemokines. These categories are not absolutely exclusive because at least 1 cytokine, TNF-α, occurs both preformed and as a newly synthesized molecule.

Preformed mediators, including histamine, serine proteases (tryptase and chymase), carboxypeptidase A, and proteoglycans are stored in cytoplasmic granules. Proteoglycans, including heparin and chondroitin sulfates, are abundant in the granules and, because of their negative charge, form complexes with histamine, proteases, and other granule contents. On activation of mast cells, the granules fuse with the plasma membrane, and the contents are released into the extracellular environment within minutes. Histamine in the granules dissociates from the proteoglycans in the extracellular fluid by exchanging with sodium ions. Histamine has

effects on smooth muscle (contraction), endothelial cells, nerve endings, and mucous secretion. Histamine has a half-life of around 1 minute in the extracellular fluid and is degraded by histamine N-methyltransferase to tele-methylhistamine (degraded to tele-methylimidazole acetaldehyde and tele-methylimidazole acetic acid) and by diamine oxidase to imidazole acetaldehyde (degraded to imidazole acetic acid and then ribosylated). Although histamine is difficult to measure in serum because of its short half-life, histamine and its metabolites can be measured in urine.

The majority of protein in the granules is made up of neutral proteases: tryptase in MC_T cells and tryptase, chymase, cathepsin G, and carboxypeptidase in MC_{TC} cells. Human mast cell α- and β-tryptases are derived from 2 adjacent genes on chromosome 16p13.3. Mature β-tryptase is the predominant form stored in secretory granules of all human mast cells (10-35 pg per human mast cell). It consists of 4 monomers stabilized in the tetrameric form by heparin proteoglycan. Tryptase is also constitutively secreted from human mast cells. Secreted tryptase consists largely of β-protryptase (immature β-tryptase) and α-protryptase. When mast cells are activated, there is a marked increase in tryptase that consists of mature β-tryptase. Commercial clinical assays for tryptase recognize both α- and β-tryptases, either total tryptase (protryptases and mature forms of α- and β-tryptases) or mature α- and β-tryptases. The α- and β- tryptases have 90% sequence homology. Baseline serum consists primarily of secreted protryptases that have been constitutively secreted from mast cells; their level is believed to reflect the mast cell burden and is increased in patients with systemic mastocytosis (SM). The marked increase in total tryptase level after an anaphylactic event is due to the additional release of mature β-tryptase. Tryptase levels after anaphylaxis peak in serum at around 1 hour, and increased levels can persist for several hours after a precipitating event, unlike histamine, which decreases to the baseline level by 1 hour. Anaphylaxis to parenteral agents (drugs and insect venom) is associated with increased tryptase levels, whereas anaphylaxis to oral agents, particularly foods, is often not accompanied by increased tryptase levels in the serum. The function of tryptase *in vivo* is unknown, but *in vitro* it will digest fibrinogen, fibronectin, prourokinase, pro–matrix metalloproteinase 3, protease-activated receptor 2, and complement component C3. Tryptase can activate fibroblasts, promote accumulation of inflammatory cells, and potentiate histamine-induced airway bronchoconstriction.

Mast cells activated through FcεRI or KIT rapidly synthesize eicosanoid mediators from endogenous membrane arachidonic acid stores. Arachidonic acid released by phospholipase A_2 is converted by COX and PGD synthase enzymes to PGD_2 (not produced by basophils) or by the 5-lipoxygenase pathway in cooperation with the 5-lipoxygenase activating protein to leukotriene (LT) A_4, which is converted to LTB_4 or conjugated with glutathione to form LTC_4, the parent compound to the cysteinyl leukotrienes (CysLTs), which also include LTD_4 and LTE_4. LTB_4 works through at least 2 G protein–coupled receptors, BLT1 and BLT2, for chemotaxis of neutrophils and effector T cells. CysLTs work through at least 2 G protein–coupled receptors, CysLT1 and CysLT2 as potent bronchoconstrictors, to promote vascular permeability, induce mucus production, and attract eosinophils. PGD_2 is also a bronchoconstrictor and attracts eosinophils and basophils, and its active metabolite (9α,11β-PGF_2) is a constrictor of coronary arteries.

TNF-α is a major cytokine stored and released by mast cells. It upregulates endothelial and epithelial adhesion molecules, increases bronchial responsiveness, and has antitumor effects. Other cytokines produced by mast cells include IL-3, GM-CSF, and IL-5, which are critical for eosinophil development and survival, and IL-6, IL-10 and IL-13. Human mast cells also produce several chemokines, including CXCL8 (IL-8) and CCL3 (macrophage inflammatory protein 1α).[21,22,24]

Role in health and disease

Mast cells are thought to function in homeostasis, including wound healing, and in innate and adaptive immunity based on animal studies and *in vitro* models. Diseases associated with mast cells include those caused by extrinsic mechanisms, such as IgE-mediated diseases acting through FcεRI receptors on mast cells or direct mast cell activators acting through other receptors and those caused by intrinsic mast cell disorders, most notably mastocytosis and the recently described monoclonal mast cell activation syndrome.

Mast cell activation through FcεRI is central to the pathogenesis of allergic diseases, including anaphylaxis, allergic rhinitis, and allergic asthma. Activation of FcεRI by polyvalent allergen recognized by bound IgE leads to the initiation of an immediate hypersensitivity reaction, as well as a late-phase reaction. The immediate reaction is determined by preformed mediators and rapidly synthesized lipid mediators and results in erythema, edema, and itching in the skin; sneezing and rhinorrhea in the upper respiratory tract; cough, bronchospasm, edema, and mucous secretion in the lower respiratory tract; nausea, vomiting, diarrhea, and cramping in the gastrointestinal tract; and hypotension. Late-phase reactions are mediated by cytokines and chemokines and can occur 6 to 24 hours after the immediate reaction. Late-phase reactions are characterized by edema and leukocytic influx and can play a role in persistent asthma.

Pathologic excess of mast cells, most notably in the skin, bone marrow, gastrointestinal tract, spleen, liver, and lymph nodes, usually caused by activating mutations in KIT, leads to mastocytosis.[24] This disease can occur in any age group and in the majority of cases is first suspected because of the appearance of fixed pigmented skin lesions that urticate with stroking (Darier sign), termed urticaria pigmentosa. The clinical presentation can also include unexplained flushing and hypotension. Mastocytosis varies from indolent forms of mastocytosis to mastocytosis associated with bone marrow pathology, including myelodysplasia. Diagnostic criteria for the disease have been established and include characteristic skin findings, an increased baseline serum total tryptase level, and specific bone marrow findings.[21] Cutaneous mastocytosis is diagnosed based on typical skin lesions with multifocal or diffuse infiltrates of mast cells on biopsy and the absence of diagnostic criteria sufficient for the diagnosis of SM. SM is diagnosed based on the presence of major and minor criteria.[25] The major criterion is the presence of multifocal dense infiltrates of 15 or more mast cells per high-power field in the bone marrow, other extracutaneous organs, or both. The minor criteria are as follows: (1) in biopsy sections of bone marrow or other extracutaneous organs, greater than 25% of mast cells in the infiltrate are spindle shaped or have atypical morphology, or of all mast cells in bone marrow aspirate smears, greater than 25% are atypical or mature; (2) an activating point mutation at codon 816 of KIT in the bone marrow, blood, or another extracutaneous site is detected; (3) mast cells in bone marrow, blood, or other extracutaneous organs expressing CD2, CD25, or both in addition to normal mast cell markers are present; and (4) serum total tryptase

levels persistently exceed 20 ng/mL (unless there is an associated clonal myeloid disorder, in which case this parameter is not valid).[25] The presence of the major criterion and 1 minor criterion or the presence of at least 3 minor criteria is diagnostic for SM.

Monoclonal mast cell activation syndrome is a recently described syndrome characterized by patients with idiopathic anaphylaxis or systemic anaphylaxis to bee stings who are found based on bone marrow biopsy to have at least 2 minor criteria for SM but lack cutaneous findings.[26-28] Aberrant, clonal mast cell populations are characteristic of this disorder. Although optimal treatment is not determined, consideration of this diagnosis should be made in patients with idiopathic anaphylaxis.

BASOPHILS

Basophils share many features with mast cells, including expression of FcεRI, secretion of T_H2 cytokines, metachromatic staining, and release of histamine after activation, but constitute a distinct lineage with many unique features (Table I). A notable feature of basophils is their rapid and potent expression of IL-4 and IL-13. Although basophils have been viewed as having functions similar to mast cells, recent work has highlighted the unique functions of basophils and their role in allergic responses and immune regulation.[29-31]

Morphology and phenotype

Basophils are 5 to 8 μm in diameter, exhibit a segmented condensed nucleus, and are identified by means of staining with basic dyes, such as toluidine blue or Alcian blue. There are fewer but larger granules in basophils compared with those seen in mast cells. Unlike mast cells, basophils have little proliferative capacity. Basophils express a variety of cytokine receptors (eg, IL-3R, IL-5R, and GM-CSFR), chemokine receptors (CCR2 and CCR3), complement receptors (CD11b, CD11c, CD35, and CD88), PG receptors (CRTH2), immunoglobulin Fc receptors (FcεRI and FcγRIIb), and TLRs.[22,32]

Development and trafficking

Basophils develop from $CD34^+$ progenitors, differentiate and mature in the bone marrow, and circulate in the periphery, where they constitute less than 1% of peripheral blood leukocytes and are thought to have a half-life of a few days. IL-3 is the dominant cytokine driving basophil differentiation and is sufficient to differentiate stem cells into basophils. Although not predominantly a tissue-dwelling cell, basophils express integrins and chemokine receptors and are able to infiltrate inflamed tissues, particularly in the skin of patients with atopic dermatitis and the airway of patients with respiratory allergies.

Activation

Basophils express a complete FcεRI ($\alpha\beta\gamma2$), the surface expression of which directly correlates with free IgE concentration. Aggregation of FcεRI bound to IgE by multivalent antigen leads to basophil activation, granule exocytosis, and mediator release. C3a and C5a also activate basophils through their receptors on the surface of basophils. IL-3, IL-5, GM-CSF, and histamine-releasing factor, as well as several chemokines, prime basophils, leading to enhanced degranulation and IL-4 and IL-13 secretion after FcεRI activation, but do not fully activate basophils alone.[33] TLR2 and TLR4 are also expressed on basophils, and activation leads to IL-4 and IL-13 secretion and potentiation of IgE- and non–IgE-induced activation. Similarly, IL-33, a member of the IL-1 superfamily, activates basophils through the ST2 receptor, resulting in IL-4 and IL-13 expression and potentiation of IgE-mediated degranulation.[34,35] The gp120 protein from HIV is reported to act as a superantigen binding IgE, leading to secretion of IL-4 and IL-13.

TABLE I. Major features of mast cells and basophils

	Mast cells	Basophils
Origin	Hematopoietic stem cells	Hematopoietic stem cells
Site of maturation	Connective tissues	Bone marrow
Lifespan	Months	Days
Primary location	Tissues	Intravascular circulation
Size	6-12 μm	5-7 μm
Nucleus	Oval or round	Segmented
Granules	Smaller and more numerous compared with basophils	Larger and fewer compared with mast cells
Peptidoglycans	Heparin and chondroitin sulfates	Predominantly chondroitin sulfates
Tryptase content	High	Low
Lipid mediators	PGD_2, LTB_4, LTC_4, LTD_4, LTE_4, PAF	LTC_4, LTD_4, LTE_4

Mediators and effector function

Like mast cells, mediators produced by basophils are divided into preformed mediators, newly synthesized lipid mediators, and cytokines/chemokines.[33]

The major preformed mediator in storage granules of basophils is histamine. Histamine in these granules complexes with proteoglycans, most notably chondroitin sulfate, and dissociates after exocytosis by ion exchange and changes in pH. Basophil granules appear to contain less heparin than do mast cell granules. Tryptase levels in basophil granules are thought to be much lower than those in mast cells; however, there can be variability.

Basophils rapidly produce LTC_4 and its peptidolytic products, LTD_4 and LTE_4, after activation. All 3 CysLTs are potent bronchoconstrictors and increase vascular permeability. Unlike mast cells, basophils do not produce PGD_2.

Cytokines expressed by activated basophils include IL-4, IL-13, and GM-CSF. IL-4 in particular is rapidly secreted after activation and at high levels. In several model systems, rapid, non–IgE-mediated IL-4 production by basophils is the source of early IL-4 that "primes the pump" for subsequent T_H2 cell differentiation.[31] Basophils expressing IL-4, IL-13, and CD154 (CD40L) have been suggested to be important for amplification of IgE synthesis. The protease granzyme B is produced by activated basophils after IL-3 treatment and is secreted after inhalation allergen challenge of asthmatic subjects.[36]

Role in health and disease

The physiologic role of basophils remains unknown, although they are thought to play a role in host defense, particularly against parasites. A role for basophils in innate immunity is suggested by their expression of a functional TLR2 receptor, as well as their non–IgE-dependent activation by multiple proteases, including Der p 1 and hookworm. Basophils are the predominant source of IL-4 in allergen- and helminth parasite–activated PBMCs, as well as in corresponding murine models. Basophils have been

identified in cutaneous and pulmonary late-phase allergic responses and are found in increased numbers in the lungs of patients who die of asthma.[29-32] Recent data from murine models (immunized with protease allergen, ovalbumin, and helminth infection) have suggested a direct role for basophils in antigen presentation for induction of T_H2 responses, with expression of MHC class II molecules and IL-4 production.[37-39]

EOSINOPHILS

Eosinophils are granulocytes that were first described to stain with acid aniline dyes, such as eosin. Blood and tissue eosinophilia are hallmark signs of helminth infection, allergy, asthma, eosinophilic gastrointestinal disorders, and a number of other rare disorders.

Morphology and phenotype

Human eosinophils have a bilobed nucleus with highly condensed chromatin and 2 major types of granules, specific and primary. Specific granules have a distinctive ultrastructural appearance with an electron-dense core and contain cationic proteins that give eosinophils their unique staining properties. The major cationic proteins in the specific granules are major basic protein (MBP), eosinophil peroxidase (EPO), eosinophil cationic protein (ECP), and eosinophil-derived neurotoxin (EDN). Primary granules are similar to those found in other granulocyte lineages, are formed early in eosinophil development, and are enriched in Charcot-Leyden crystal protein. Eosinophils also contain lipid bodies, which are cytoplasmic structures lacking a surrounding membrane that contain eicosanoid synthetic enzymes and are the major site of eicosanoid synthesis. Lipid bodies are formed rapidly after activation of eosinophils.[40-42]

Eosinophils express an array of cell-surface molecules, including immunoglobulin receptors for IgG (FcγRII/CD32) and IgA (FcαRI/CD89); complement receptors (CR1/CD35, CR3, and CD88); cytokine receptors (IL-3R, IL-5R, and GM-CSF that promote eosinophil development, as well as receptors for IL-1α, IL-2, IL-4, IFN-α, and TNF-α); chemokines (CCR1 and CCR3); adhesion molecules (very late antigen 4, $\alpha4\beta7$, and siglec-8); leukotriene receptors (CysLT1R and CysLT2R; LTB_4 receptor); PG receptors (PGD_2 type 2 receptor); platelet-activating factor (PAF) receptor; and TLRs (particularly TLR7/8). Eosinophil expression of FcϵRI is minimal, does not activate eosinophils, and is of unclear functional significance. Eosinophils also express several inhibitory receptors.[43]

Development and trafficking

IL-5, IL-3, and GM-CSF all promote the development of eosinophils from $CD34^+$ hematopoietic progenitor cells, although only IL-5 is specific for eosinophil development and differentiation. Pluripotent hematopoietic stem cells differentiate into an eosinophil/basophil progenitor before commitment to the eosinophil lineage. Progenitors committed to the eosinophil lineage are identified based on expression of CD34, IL-5 receptor, and CCR3. Eosinophils develop in the bone marrow and are released into the circulation, most notably after stimulation by IL-5, although there is a large pool of mature eosinophils that remains in the bone marrow. IL-5 produced at sites of allergic inflammation or helminth infection acts distally on the bone marrow to release eosinophils.[44] Additionally, allergen challenge or the experimental administration of CCL11 (eotaxin-1), acting through the CCR3 receptor, causes bone marrow release of mature eosinophils and eosinophil precursors.

Once released from the bone marrow, after stimulation with IL-5, eosinophils enter the circulation and traffic to tissue. The half-life of eosinophils in the circulation is 8 to 18 hours. The vast majority of eosinophils are located in the tissues, particularly at mucosal surfaces in the gastrointestinal tract in homeostasis and at sites of T_H2-dominated inflammation. IL-4 and IL-13 play a central role in promoting eosinophil trafficking to mucosal tissue by upregulating eotaxin (CCL11 and CCL26) and endothelial cell vascular cell adhesion molecule 1 expression. In contrast to eotaxins, IL-5 does not have a major role in promoting eosinophil entry into tissues. PAF, LTD_2, C5a, and CCL5 (RANTES) are also potent eosinophil chemotactic factors. Survival of eosinophils in the tissues might be enhanced by IL-3, IL-5, GM-CSF, IL-33, and IFN-γ.

Activation

There is no consensus on the major signaling mechanism for eosinophil activation. Eosinophils can be activated by cross-linking of IgG or IgA Fc receptors by agarose beads with IgG, IgA, or secretory IgA, with the latter being most potent. Eosinophils can be primed for activation by a number of mediators, including IL-3, IL-5, GM-CSF, CC chemokines, and PAF. The outcome of activation is variable, with 4 mechanisms of eosinophil degranulation reported: exocytosis, compound exocytosis, piecemeal exocytosis, and cytolysis. Different mediators of activation can differentially affect the type of degranulation and factors expressed in the activated state. The details of this remain unknown.

Mediators and effector function

Eosinophils release proinflammatory mediators, including granule-stored cationic proteins, newly synthesized eicosanoids, and cytokines.[40-42]

MBP accounts for more than 50% of the eosinophil granule protein mass and is the major component of the crystalloid cores of specific granules. MBP is highly cationic and lacks enzymatic activity, and toxicity is believed to be mediated by enhanced membrane permeability resulting from interactions of the cationic protein with the plasma membrane. MBP has *in vitro* activity against parasites, including helminths and schistosomula. In patients with asthma, serum and bronchoalveolar lavage fluid MBP correlate with bronchial hyperresponsiveness.

EDN and ECP, both of which have RNAse activity, are localized to the matrix of specific granules and demonstrate *in vitro* toxicity to parasites and single-stranded RNA pneumoviruses, including respiratory syncytial virus. Although both proteins exhibit RNAse activity (EDN > > ECP), the RNAse activity does not appear to be required for toxicity. Genes encoding EDN and ECP both show exceedingly high rates of mutations, suggesting the molecules are under extraordinary selective pressure, as might be expected of genes responding to the rapid evolution of microbial pathogens. EPO is a highly cationic protein localized to the matrix of specific granules and makes up approximately 25% of granule protein. EPO catalyzes the oxidation of halides, pseudohalides, and nitric oxide to oxidant products that are toxic to microorganisms and host cells.

Charcot-Leyden crystal protein (galectin-10) is a hydrophobic protein of unknown function that is produced in high levels in

eosinophils. The protein is stored in primary granules and is released with eosinophil activation. Crystals of this protein can be detected in the stool or sputum of patients with gastrointestinal or respiratory eosinophilia.

Eosinophils are also a source of lipid-derived mediators, including LTC_4, PGE_2, thromboxane, and PAF. Although granule proteins are the major eosinophil effector molecules, eosinophils are capable of producing a number of cytokines and chemokines, including TGF-β, IL-3, IL-4, IL-5, IL-8, IL-10, IL-12, IL-13, IL-16, IL-18, TNF-α, CCL5, and CCL11. Eosinophil cytokines are stored preformed in granules and can be rapidly released on degranulation. However, eosinophils generally produce lower amounts of cytokines than other leukocytes, and no essential role for eosinophil cytokine expression in disease or host defense has been demonstrated. Eosinophils demonstrate immunomodulatory activity through multiple mechanisms, including secretion of cytokines, antigen presentation, or expression of indolamine 2,3 dioxygenase, leading to kynurenine production, which has anti-T_H1 activity.

Role in health and disease

Peripheral blood eosinophil counts up to 500/mm^3 are normal, and there is significant diurnal variation, with lowest levels in the morning and highest levels in the evening. An increase in peripheral blood and tissue eosinophil numbers is typical of a number of diseases, such as allergic diseases, including atopic asthma (usually mild eosinophilia), drug reactions, helminth infections, and hypereosinophilic syndromes, among other disorders. Eosinophilia can also be seen in specific primary immunodeficiency diseases, most notably Omenn syndrome and hyper-IgE syndrome. Eosinopenia is typically seen in patients with acute bacterial or viral infections and with systemic corticosteroid treatment. The presence of eosinophilia in a febrile patient should raise the suspicion of possible adrenal insufficiency.[45]

Allergic diseases, including allergic rhinitis, atopic asthma, and atopic dermatitis, can be associated with a mild peripheral blood eosinophilia, although tissue eosinophil numbers and numbers of eosinophils in nasal secretions, sputum, and bronchoalveolar lavage fluid can be more significantly increased. Studies in murine models support a role for eosinophils in airway remodeling, airway hyperreactivity, and mucous production.[40] Anti–IL-5 treatment of a diverse population of asthmatic patients demonstrated a 90% decrease in peripheral eosinophil counts but only a 50% decrease in tissue eosinophil counts and minimal improvement in asthma control. There is now a greater appreciation that there are multiple phenotypes of asthma, including phenotypes based on inflammatory mechanisms (eg, eosinophilic, neutrophilic, and paucigranulocytic).[46] More recent studies of anti–IL-5 treatment focusing on patients with "eosinophilic asthma" refractory to treatment with corticosteroids demonstrated significant improvement in peripheral blood and sputum eosinophil counts and improved asthma control.[47,48] Identifying phenotypes of diseases susceptible to specific treatment is an important goal in therapeutic trials. In this case eosinophils appear to play a particularly important role in those with primary eosinophilic inflammation.

Hypereosinophilic syndromes are a heterogeneous group of disorders characterized by a marked increase in eosinophil counts in the peripheral blood (>1,500/mm^3); persistent eosinophilia, evidence of end-organ damage, or both; and exclusion of known causes of eosinophilia, including parasitic infections and drug reactions. These disorders have been classified into one of 6 groups: (1) myeloproliferative variant (includes FIP1L1/PDGFR fusion-positive and fusion-negative chronic eosinophilic leukemia [CEL]); (2) lymphocytic variant (clonal expansion of T cells secreting IL-5); (3) familial (family history of persistent eosinophilia with no identifiable cause); (4) undefined (includes benign eosinophilia with no end-organ involvement and eosinophilia associated with recurrent angioedema); (5) overlap (hypereosinophilia with organ-restricted eosinophilic disorders, such as eosinophilic gastrointestinal disorders or eosinophilic pneumonia), and (6) associated (hypereosinophilia associated with Churg-Strauss syndrome, mastocytosis, sarcoidosis, HIV, and other disorders).[49] Treatment for these disorders is initiated early to prevent end-organ damage. Systemic corticosteroids are the first-line treatment for most forms of hypereosinophilic syndromes. FIP1L1/PDGFR-positive CEL is treated with the tyrosine kinase inhibitor imatinib as first-line therapy.[50,51] In non–FIP1L1/PDGFR-positive CEL, anti–IL-5 treatment with mepolizumab has been shown to reduce the dose of systemic corticosteroid required to maintain reduced peripheral eosinophil counts.[52]

CONCLUSION

Mast cells, basophils, and eosinophils express many of the same receptors and cytokines yet have different effector functions. Mast cells are tissue resident cells and uniquely required for immediate hypersensitivity. Basophils are largely circulating cells but home to areas of allergic inflammation during the late-phase response. Eosinophils are resident to the gastrointestinal tract but also home to allergic inflammatory sites. The dominant cytokines produced by these cells differ: basophils express abundant IL-4 and IL-13 but little IL-5, whereas mast cells produce IL-5 and IL-13 but little IL-4. Although eosinophils can express a range of cytokines, their production of cytotoxic granule proteins is thought to be their major effector function. Differences in trafficking, activation, and mediator production contribute to each cell's unique role.

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Genetics of allergic disease

John W. Holloway, PhD,[a,b] Ian A. Yang, MBBS, PhD,[c,d] and Stephen T. Holgate, MD, DSc, FMed Sci[a]

Southampton, United Kingdom, and Brisbane, Australia

Allergic diseases are complex genetic diseases resulting from the effect of multiple genetic and interacting environmental factors on their pathophysiology. Recent years have seen considerable progress in unraveling the contribution of these factors to an individual subject's susceptibility to, subsequent development of, and severity of disease. This has resulted in increasing insight into novel areas of allergic disease pathophysiology, for example the significant role played by locally acting tissue susceptibility factors like epithelial/epidermal barrier function and remodeling, such as filaggrin, *ADAM33*, and *GSDML*/*ORMDL3*, in patients with atopic dermatitis and asthma. Furthermore, studies of gene-environment interactions and Mendelian randomization approaches have led to increased insight into the importance of environmental triggers for allergic disease. Studies of the timing of action of genetic variants in determining disease susceptibility have highlighted the importance of *in utero* development and early life in determining susceptibility to allergic disease. In the future, genetic discoveries in allergic disease will potentially lead to better endophenotyping, prognostication, prediction of treatment response, and insights into molecular pathways to develop more targeted therapy for these conditions. (J Allergy Clin Immunol 2010;125:S81-94.)

Key words: *Heritability, genetics, genetic testing, pharmacogenetics, epigenetics*

Abbreviations used

ADAM33:	A disintegrin and metalloprotease 33 gene
ADRB2:	β_2-Adrenoceptor gene
ARG:	Arginase gene
BHR:	Bronchial hyperresponsiveness
CNV:	Copy number variant
CRHR1:	Corticotropin-releasing hormone receptor 1 gene
CTNNA3:	α-T-catenin gene
FLG:	Filaggrin gene
GSDML:	Gasdermin like
GWAS:	Genome-wide association study
LD:	Linkage disequilibrium
ORMDL3:	ORM1-like 3 (s. cerevisiae)
PCDH1:	Protocadherin 1 gene
PDE4D:	Phosphodiesterase 4D gene
SNP:	Single nucleotide polymorphism
STAT6:	Signal transducer and activator of transcription 6 gene
TDI:	Toluene diisocyante
TLR:	Toll-like receptor

THE HERITABILITY OF ALLERGIC DISEASE

In 1860, Henry Hyde Salter, in his magnus opus, *On Asthma: Its Pathology and Treatment*, wrote, "Is asthma hereditary? I think there can be no doubt that it is."[1] Subsequent to this, many studies have now conclusively shown that susceptibility to asthma and other allergic diseases has a heritable component. Although there are rare monogenic diseases whose phenotypes include aspects of allergic disease, such as high serum IgE levels and atopic dermatitis,[2-5] common forms of these conditions are thought to be determined by the actions and interactions of multiple genetic and environmental factors. This is evidenced by the lack of concordance for allergic disease between monozygotic twins[6,7] and the lack of segregation in families with any clear inheritance pattern.[8,9] Thus allergic diseases can be termed complex genetic diseases involving both genetic and environmental factors influencing not only the development of IgE-mediated sensitivity but also the subsequent development of clinical symptoms in a range of tissues, including skin, nose, and lung tissue.[10] Since the first report of linkage between chromosome 11q13 and atopy in 1989,[11] there have been more than a thousand published studies of the genetics of asthma and other allergic diseases. Our knowledge of how genetic variation between subjects determines susceptibility, severity, and response to treatment has expanded considerably, providing intriguing insights into the pathophysiology of these complex disorders.

GENETIC STUDIES OF ALLERGIC DISEASE

The nature of the individual genes that have been identified as susceptibility factors for allergic disease have been comprehensively reviewed elsewhere,[10,12] and the list of these genetic factors is likely to expand considerably in the coming months and years with the recent advent of genome-wide association approaches (see below). However, it is important to recognize the different approaches undertaken to identify these genetic factors and their advantages and disadvantages.

Candidate gene/gene region studies

Single nucleotide polymorphisms (SNPs) in the promoter and coding regions of a wide range of candidate genes have been studied for association with a range of atopy-related phenotypes. Candidate genes are selected for analysis based on a wide range of evidence, such as biological function, differential expression in disease, involvement in other diseases with phenotypic overlap, affected tissues, cell type or types involved, and findings from animal models. The advantage of this approach is that candidate

From the Divisions of [a]Infection, Inflammation and Immunity and [b]Human Genetics, School of Medicine, University of Southampton, Southampton, and [c]the Department of Thoracic Medicine, the Prince Charles Hospital, and [d]the School of Medicine, the University of Queensland, Brisbane, Australia.

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Reprint requests: John W. Holloway, PhD, III Division (MP810), School of Medicine, Southampton General Hospital, Southampton SO16 6YD, United Kingdom. E-mail: J.W.Holloway@southampton.ac.uk.

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BOX 1. Key concepts: Explanations for association (or lack of association) between polymorphisms and allergic disease phenotypes

POSITIVE ASSOCIATION

Causal link
: The polymorphism tested directly affects gene expression or protein function, resulting in crease susceptibility.

Linkage Equilibrium (LD)
: The polymorphism tested is not directly casual but is in LD with an adjacent polymorphism that is directly causal. LD refers to the nonrandom association of alleles at 1 (or more) loci; the allele of one polymorphism in an LD block (haplotype) can predict the allele of an adjacent (not genotyped) polymorphism. The size of the LD blocks depends on the recombination rate in that region and the time since the first disease-contributing variant arose in an ancestral subject in that population.

Population stratification
: Population stratification is the presence of a systematic difference in allele frequencies between subpopulations in a population caused by different ancestry. Allele frequencies often differ between populations of different ancestry; hence if case and control populations are not adequately matched for ancestry, this can lead to false-positive associations. This can be controlled for by the assessment of ancestry by using polymorphisms known to differ in allele frequency between populations (ancestry informative markers) or through the use of family-based association.

Type I error
: A positive association might represent a false-positive observation. Especially in studies of multiple SNPs, phenotypes, or both, it is important to consider the strength of *P* values observed in the context of the number of statistical tests undertaken.

NO OBSERVED ASSOCIATION

Variants assessed do not contribute to phenotype.
: The variants assessed do not contribute to the heritability of the phenotype assessed. It is important to recognize that this does not exclude the encoded protein from playing an important role in the pathogenesis of the disease; rather, it only indicates that genetic variation in the gene does not contribute to it.

Type II error
: No association is observed because of lack of power. The effect size for common variants on susceptibility to complex disease is typically small (odds ratio, <1.5). The majority of studies are not adequately powered to detect an effect of this size.

Failure to replicate previous report of positive association
: There are a number of reasons why a study might fail to replicate a previous report of a positive association between a polymorphism and a phenotype. Apart from the consideration of whether either of the studies represents a false-negative or false-positive association, it is important to determine whether the studies truly replicate one another. For example, were they carried out in populations of similar genetic ancestry or with similar environmental exposures? Were exactly the same polymorphisms studied in the gene, and was the phenotype tested the same?

genes have biological plausibility and often display known functional consequences that have potentially important implications for the disease of interest. Disadvantages are the limitation to genes of known or postulated involvement in the disease, thereby excluding the discovery of novel genes that influence the diseases.

There are almost 1,000 studies published that examine polymorphisms in several hundred genes for association with asthma and allergy phenotypes.[10,12] When assessing the significance of association studies, it is important to consider several things. For example, was the size of the study adequately powered if negative results were reported? Were the cases and control subjects appropriately matched? Could population stratification account for the associations observed? In the definitions of the phenotypes, which phenotypes have been measured (and which have not)? How were they measured? Regarding correction for multiple testing, have the authors taken multiple testing into account when assessing the significance of association?[13]

It is also important to note that positive association does not necessarily imply that the genetic variant in question has a direct effect on gene expression or protein function (Box 1). Genetic variants showing association with a disease are not necessarily causal because of the phenomenon of linkage disequilibrium (LD), meaning that a variant displaying association with a phenotype might only represent a proxy marker for another indentified genetic variant. Positive association might also represent a type I error. Candidate gene studies have suffered from nonreplication of findings between studies, which might be due to poor study design, population stratification, different LD patterns between subjects of different ethnicities, and differing environmental

exposures between study cohorts. Unfortunately, the genetic association approach can also be limited by underpowered studies and loose phenotype definitions.[14] A good example of the complexity of interpreting candidate gene association studies is provided by the study of Rogers et al,[15] who used genome-wide SNP array data to investigate the association of 39 previously reported asthma candidate genes in a large family-based sample. Despite using strict criteria for selecting the genes for replication, including selecting genes with (1) significant association with asthma affection status (and not other related phenotypes, such as atopy, IgE levels, or lung function) reported in at least 2 populations, (2) at least 1 significant association study that has no fewer than 150 cases and 150 control subjects or 150 trios, and (3) asthma association with SNPs (as opposed to haplotypes, microsatellite markers, or structural genetic variants) in at least 1 population, they were only able to find clear evidence for replication of association with 6 of 39 genes and limited evidence for replication for a further 15 of 39 genes.

Positional cloning by linkage

Positional cloning is a hypothesis-independent approach and starts with the investigation of families. Markers randomly spaced throughout the entire genome are tested for linkage (ie, coinheritance) with the disease phenotype of interest. If linkage is found between a particular marker and the phenotype, then further typing of genetic markers aid in more accurately defining the critical region of the causative gene. After this, the genes positioned in this region can be examined for possible involvement in the disease process and the presence of disease-causing mutations in affected subjects. This approach is often termed *positional cloning* or *genome scanning* if the whole genome is examined in this manner. Although this approach requires no assumptions to be made as to the particular gene involved in genetic susceptibility to the disease in question, it does require considerable molecular genetic analysis to be undertaken, involving considerable time and expense. Many genome-wide screens for atopy and atopic disorder susceptibility genes have been completed.[12,16] The results of the genome-wide screens for allergy and allergic disease susceptibility genes reflect the genetic and environmental heterogeneity seen in allergic disorders. Multiple regions of the genome have been observed to be linked to varying phenotypes, with little replication between cohorts recruited from both similar and different populations. This illustrates the difficulty of identifying susceptibility genes for complex genetic diseases. Different genetic loci will show linkage in populations of different ethnicities and different environmental exposures (stratification). In studies of complex disease, the real challenge has not been identification of regions of linkage but rather identification of the precise gene and genetic variant underlying the observed linkage. To date, several genes have been identified as the result of positional cloning with a genome-wide scan for allergic disease phenotypes, including a disintegrin and metalloprotease 33 (ADAM33),[17] Chitinase 3 Like-1 (*CHI3L1*),[18] Dipeptidyl-peptidase 10 (*DPP10*),[19] Major histocompatibilty complex, class I, G (*HLA-G*),[20] PHD finger protein 11 (*PHF11*),[21] Prostaglandin D2 receptor (*PTGDR*),[22] and plasminogen activator, urokinase receptor (*PLUAR*)[23] for asthma; the protocadherin 1 gene (*PCDH1*) for bronchial hyperresponsiveness (BHR)[24]; and Collagen, type XXIX, alpha 1 (*COL29A1*)[25] for atopic dermatitis. The identification of these positional candidates, many of the protein products of which had not been implicated in allergic disease previously, has revealed the importance of using hypothesis-independent approaches to identify susceptibility genes. Furthermore, unlike many candidate gene studies, the susceptibility genes identified through positional cloning have, in general, been more likely to be replicated in subsequent studies of additional cohorts,[15,26,27] although even positionally cloned genes might prove difficult to replicate at times.[28] Despite the success of such positional cloning studies, in general, linkage analysis for allergic disease phenotypes has proved to be slow and expensive, and the majority of studies, despite recruiting several hundred families, have proved to be underpowered to identify susceptibility genes for complex disease. A meta-analysis of linkage analyses in asthma has demonstrated susceptibility loci for BHR, allergen skin prick test positivity, and total serum IgE levels but no consistent statistically significant loci for asthma as a phenotype,[29] indicating heterogeneity in outcomes.

Genome-wide association studies

In recent years, the study of the genetic basis of complex disease has been revolutionized by technologic advances in array-based SNP genotyping technologies and the characterization of millions of SNP variants in the human genome.[30] This has made possible the simultaneous determination of the genotype of >500,000 SNPs throughout the genome of a subject. This has allowed the use of genome-wide, hypothesis-independent association studies that, unlike positional cloning by linkage, do not require the recruitment and phenotyping of large family-based samples and achieve much greater statistical power for the same number of subjects. Genome-wide association studies (GWASs) have now revolutionized the study of genetic factors in complex common diseases.[31,32] For more than 150 phenotypes, from common diseases to physiologic measurements, such as height and body mass index, and biological measurements, such as circulating lipid levels and blood eosinophil levels, GWASs have provided compelling statistical associations for hundreds of different loci in the human genome.[33]

To date, several GWASs have been performed with great success in allergic diseases, such as asthma, eczema, and allergic sensitization; Table I summarizes the findings of these studies.[34-44] The first novel asthma susceptibility locus to be identified by using a GWAS approach contains the ORM1-like 3 (s. cerevisiae) (*ORMDL3*) and Gasdermin like (*GSDML*) genes on chromosome 17q12-21.1.[34] In this study 317,000 SNPs were genotyped in 994 subjects with childhood-onset asthma and 1,243 nonasthmatic control subjects. After adjustments for quality control, 7 SNPs remained above the 1% false discovery rate threshold, and all mapped to a 112-kb region at 17q21. Replication of the findings was achieved by genotyping 9 of the associated SNPs (>5% false discovery rate) in the 17q21 locus in 2,320 subjects (200 asthmatic cases and 2,120 control subjects), and 5 SNPs were found to be significantly associated with disease ($P < .01$). Although several genes were within the LD block in which the associated SNPs lay, the authors used data from gene expression levels measured in EBV-transformed lymphoblastoid (B cell)–derived cell lines, showing transcript levels from one gene, *ORMDL3*, were strongly associated with disease-associated markers ($P < 10 \times 10^{-22}$ for rs7216389) identified by the GWAS, to conclude that the casual variant was likely to alter the expression of this gene. Considerable work is still required

TABLE I. Summary of genome-wide association studies for atopy and allergic disease phenotypes as of October 2009

Initial study	Gene name (HGNC ID)	Chromosome	Associated phenotype	Gene product: possible functional role in asthma or allergic disease	Associated variant	Size of study	Population	Replication of association*
Weidinger et al[42]	*FCERIA* (147140)	1q23	Total IgE	α Subunit of the high-affinity IgE receptor	rs2251746, rs2427837	1,530 subjects	European	SAME: replication in 4 independent samples (n = 9,769)[42]
	RAD50 (604040)	5q23	IgE levels Atopic eczema and asthma	RAD50 homolog (*Saccharomyces cerevisiae*): This protein is important for DNA double-strand break repair, cell-cycle checkpoint activation, telomere maintenance, and meiotic recombination. The gene is also adjacent to the *IL4/IL13* locus.	rs2706347, rs3798135, rs2040704, rs7737470			SAME: replication in 4 independent samples (n = 9,769)[42]
Esparza-Gordillo et al[44]	*EMSY* (608574)	11q13	Atopic dermatitis	EMSY: This is a nuclear protein shown to interact with BRCA2 and with a role in chromatin remodeling. It is also a susceptibility locus for Crohn disease. Increases in *EMSY* copy number is reported in epithelium-derived cancer of the breast and ovary.	rs7927894	939 atopic dermatitis cases, 975 control subjects, and 270 nuclear families with 2 affected siblings	European	SAME: replication in 2 samples (n = 2,637 cases and 3,957 control subjects)[44]
Gudbjartsson et al[41]	*IL1RL1* (601203)	2q12	1. Blood eosinophil counts 2. Asthma	Interleukin 1 receptor–like 1 is Induced by proinflammatory stimuli and might be involved in the function of helper T cells.	rs1420101	1. 9,392 subjects 2. Then tested as candidate gene for asthma in 7,996 cases and 44,890 control subjects	Icelandic	SAME: replication for eosinophils in 12,118 Europeans and 5,212 East Asians
	WDR36 (606669)	5q22	1. Blood eosinophil counts 2. Asthma	WD repeat domain 36 might facilitate formation of heterotrimeric or multiprotein complexes. Members of this family are involved in a variety of cellular processes, including cell-cycle progression, signal transduction, apoptosis, and gene regulation.	rs2416257	1. 9,392 subjects 2. Then tested as candidate gene for asthma in 7,996 cases and 44,890 control subjects	Icelandic	SAME: replication for eosinophils in 12,118 Europeans and 5,212 East Asians
	MYB (189990)	6q23	1. Blood eosinophil counts 2. Asthma	v-myb myeloblastosis viral oncogene homolog is a nuclear transcription factor implicated in proliferation, survival, and differentiation of hematopoietic stem and progenitor cells.	rs9494145	1. 9,392 subjects 2. Then tested as candidate gene for asthma in 7,996 cases and 44,890 control subjects	Icelandic	SAME: replication for eosinophils in 12,118 Europeans and 5,212 East Asians

(Continued)

TABLE I. *(Continued)*

Initial study	Gene name (HGNC ID)	Chromosome	Associated phenotype	Gene product: possible functional role in asthma or allergic disease	Associated variant	Size of study	Population	Replication of association*
	IL33 (606678)	9q24	1. Blood eosinophil counts 2. Asthma	IL-33 is an IL-1–like cytokine ligand for the IL-1 receptor–related protein ST2, activating mast cells and T_H2 lymphocytes.	rs3939286	1. 9,392 subjects 2. Then tested as candidate gene for asthma in 7,996 cases and 44,890 control subjects	Icelandic	SAME: replication for eosinophils in 12,118 Europeans and 5,212 East Asians
Kim et al[43]	*CTNNA3* (607667)	10q22.2	TDI–induced asthma	Catenin (cadherin-associated protein), α 3, is a key molecule in the E-cadherin–mediated cell–cell adhesion complex. Genetic polymorphisms might disturb the defense systems of the airway epithelium, increasing airway hyperresponsiveness to environmental toxins, such as TDI.	rs1076205, rs7088181, rs4378283	84 TDI asthma cases and 263 unexposed healthy control subjects	Korean	NO
Moffatt et al[34]	*ORMDL3* (610075)/ *GSDMB* (611221)	17q12-17q21.1	Childhood-onset asthma	ORMDL3 is a transmembrane protein anchored in the endoplasmic reticulum with an unknown function. Gasdermin B (gasdermin like) is an epithelially expressed, unclear function, related protein possibly involved in TGF-β signaling.	rs7216389 and *ORMDL3*/ *GSDML* mRNA expression	994 asthmatic subjects and 1,243 control subjects; replicated in 2,320* and 3,301† subjects	White *Germany †United Kingdom	MULTIPLE[35-38]
Himes et al[39]	*PDE4D* (600129)	5q12	Childhood asthma	Phosphodiesterase E3 dunce homolog, Drosophila) gene *(PDE4D)* is a regulator of airway smooth muscle contractility.	rs1588265, rs1544791	359 cases and 846 genetically matched control subjects from the Illumina ICONdb public resource; replication in 18,891 white and Hispanic subjects (4,342 cases)	US white	SAME
Hancock et al[40]	*TLE4* (605132)	9q21.31	Childhood asthma	Transducin-like enhancer of split 4 is a transcription factor with a possible role in B-cell differentiation.	Rs2378383	492 children and parents; replication in 177 trios	Mexican	SAME

MULTIPLE, Replication in multiple independent populations after initial report; *NO*, no replication; *SAME*, replication in independent populations in initial report.

to fully characterize this region of the genome before accepting *ORMDL3* as the causal gene[45]; for example, expression of the *GSDML* gene also appears to be coregulated by these SNPs of interest (personal communication cited in Bouzigon et al[35]).

Importantly, subsequent studies have replicated the association between variation in the chromosome 17q21 region (mainly rs7216389) and childhood asthma in ethnically diverse populations.[36-38,46,47] A further asthma susceptibility gene has been discovered in a GWAS of 359 asthma cases from the Childhood Asthma Management Program study and 846 matched control subjects from the Illumina database.[39] Using a microarray platform of more than 500,000 SNPs, the strongest region of association was at chromosome 5q12 at the region of the phosphodiesterase 4D gene *(PDE4D)*, which is involved in airway smooth muscle contraction. Pooling of data from independent replication studies in 7 white or Hispanic populations confirmed the positive associations observed.

More recently, Hancock et al,[40] in a GWAS study, studied 492 Mexican asthmatic children and their parents, together with a replication cohort of 117 trios. Although a number of loci were

significantly associated with asthma in the initial cohort, only one, an SNP (rs2378383) in the gene *TLE4* on chromosome 9q21.31, showed significant association in the replication cohort (P = .03, P combined = 6.79×10^{-7}). Although this observation will require further validation in independent populations, it shows that even the relatively small (in GWAS terms) family-based cohorts previously extensively used for linkage studies might be of value in identifying novel disease susceptibility loci in the era of genome-wide association approaches.

Intermediate phenotypes of allergic disease, such as IgE levels, skin prick test responses, or measures of lung function as continuous measures are statistically more powerful than affection status for genetic association studies. A recent example of genome-wide association applied to an allergic intermediate phenotype is the study of blood eosinophil counts in an Icelandic population; the study revealed that sequence variants in genes affecting eosinophil numbers, including Interleukin 1 receptor-like 1 (*IL1RL1*), WD repeat domain 36 (*WDR36*), Interleukin 33 (*IL33*), and v-myb myeloblastosis viral oncogene homolog (*MYB*), associate with asthma and myocardial infarction.[41] *IL1RL1* had already been proposed as a candidate gene for eczema[48] and asthma.[49] A GWAS approach has also been taken to identify variants regulating serum IgE levels. This study identified functional variants in the gene encoding the α chain of the high-affinity receptor for IgE *(FCER1A)* on chromosome 1q23 as being associated with serum IgE levels and allergic sensitization, as well as confirming previous candidate gene studies that implicated variants in the signal transducer and activator of transcription 6 *(STAT6)* gene in regulating total IgE levels and atopy.[42]

Genome-wide association has also been used to better understand the genetic mechanisms of occupational asthma. A study of workers from spray-painting and polishing departments of the furniture and musical instrument industries from Korea uncovered multiple polymorphisms of the α-T-catenin gene *(CTNNA3)* that might be determinants of susceptibility to toluene diisocyante (TDI)–induced asthma.[43] These polymorphisms were associated with increased BHR; increased specific IgG levels to kertain 19 (CK19), which might be an intermediate phenotype of TDI-induced asthma[50]; and lower *CTNNA3* mRNA expression. The authors speculated that genetic polymorphism might downregulate *CTNNA3* and disturb barrier systems of the airway epithelium in stressful environments.

Finally, using a GWAS approach to identify susceptibility genes for atopic dermatitis, Esparza-Gordillo et al[44] recently highlighted a role for an SNP adjacent to a gene of unknown function (*C11orf30* encoding a nuclear protein, EMSY) on chromosome 11q13 in susceptibility to atopic dermatitis. This locus has previously been identified as a susceptibility locus for Crohn disease, another disease involving epithelial inflammation and defective barrier function, and increases in copy number of the *C11orf30* locus have been reported in epithelium-derived cancer of the breast and ovary. Together, this suggests that the 11q13 locus represents another gene for an allergic disease that acts at the mucosal surface rather than by modulating the level or type of immune response.

These studies show the power of the GWAS approach for identifying complex disease susceptibility variants, and the number is likely to rapidly increase in the near future. However, as for other complex diseases, such as Crohn disease and diabetes mellitus (which have been extensively studied with GWAS approaches), the results from studies performed to date do not fully explain the heritability of common complex disease. However, many geneticists remain optimistic that we can account for this "missing heritability."[51,52] It is thought that this inability to find genes could be explained by limitations of GWASs, such as the presence of other variants in the genome not captured by the current generation of genome-wide genotyping platforms, analyses not adjusted for gene-environment and gene-gene (epistasis) interactions, or epigenetic changes in gene expression.[32]

The unexpected missing heritability after assessing common genetic variation in the genome has led, in part, to the proposal that rare variants (less than the frequency of SNPs included in GWAS studies, typically 5% minor allele frequency) of high genetic effect or common copy number variants (CNVs) might be responsible for some of the genetic heritability of common complex diseases.[53] The discovery of rare, high-penetrance loss-of-function mutations in the filaggrin gene *(FLG)* predisposing such subjects to ichthyosis vulgaris, atopic dermatitis, and asthma in the presence of atopic dermatitis is supporting evidence for the rare variant hypothesis. The identification of rare variants contributing to allergic disease will be aided by efforts such as the 1000 Genomes project, which aims to create the most detailed and medically useful picture to date of human genetic variation through complete sequencing of 1,200 individual genomes.[54] However, in regard to CNV polymorphisms, recent work indicates that most of the common diallelic CNVs are in strong LD with SNPs, and hence any contribution to disease susceptibility would have been detected by using GWAS approaches.[55]

WHAT HAVE GENETIC STUDIES OF ALLERGIC DISEASE TAUGHT US?

Susceptibility to allergic disease is likely to result from the inheritance of many mutant genes. Unfortunately, as in many other complex disorders, in allergic diseases any specific biochemical defect or defects at the cellular level that cause the disease are unknown, even though considerable knowledge has been accrued on molecular pathways involved in pathogenesis. By undertaking research into the genetic basis of these conditions, these mutant genes and their abnormal gene products can be identified solely by the anomalous phenotypes they produce. Identifying the genes that produce these disease phenotypes has provided a greater understanding of the fundamental mechanisms of these disorders. The results of studies of the genetic basis of allergic disease have increased our understanding of these conditions in a number of ways (Box 2).

Importance of environmental triggers: Gene-environment interactions

Allergic disease is likely to result from the effects of environmental stimuli in genetically susceptible subjects. Inhaled and ingested environmental factors have been hypothesized to contribute to the development of asthma, including allergens, diet, respiratory viruses, air pollutants, environmental tobacco smoke, endotoxin, and occupational exposures. Recent gene-environment studies have focused on functional SNPs in candidate genes that are predicted to play a role in sensing these environmental agents and mediating the effects of exposure. To this end, the study of gene-environment interactions enables us to further understand the pathogenesis of an allergic disease such as asthma and the determinants of its severity and progression.[56]

BOX 2. Key concepts: What insights can genetic studies of allergic disease provide?

Greater understanding of disease pathogenesis
- Identification of novel genes and pathways leading to new pharmacologic targets for developing therapeutics

Identification of environmental factors that interact with a subject's genetic makeup to initiate disease and confirmation of causality of environmental factors through Mendelian randomization
- Prevention of disease by environmental modification

Identification of susceptible subjects
- Early-in-life screening and targeting of preventative therapies to at-risk subjects to prevent disease

Targeting of therapies
- Subclassification of disease on the basis of genetics and targeting of specific therapies based on this classification
- Identification of subjects at risk of severe disease and targeting of preventative treatments
- Determination of the likelihood of a subject responding to a particular therapy (pharmacogenetics) and individualized treatment plans

Pattern-recognition receptors, such as CD14 and Toll-like receptor (TLR) 4, are involved in the recognition and clearance of bacterial endotoxin (LPS) by activating a cascade of host innate immune responses. SNPs alter the biology of these receptors and could influence the early-life origins of asthma, when the immune system is developing. In case-control and family-based studies, Smit et al[57] found that in atopic subjects the presence of SNPs in the *CD14*, *TLR4*, and other TLR genes modified the associations with the risk of asthma, particularly in the presence of country living. In a study on farm living, Bieli et al[58] observed that certain alleles in the *CD14* promoter region might be associated with protection against asthma and allergic disease in the presence of farm milk consumption.

Exposure and sensitization to house dust mite antigen (eg, Der p 1) is a well-recognized risk factor for atopy and asthma. Sharma et al[59] found an association between SNPs in the TGF-β1 gene *(TGFB1)* and asthma phenotypes (BHR and asthma exacerbations), and these associations were modified by the presence of dust mite exposure, possibly because of differential immune modulation by the *TGFB1* SNPs. Other studies have found modification by house dust mite exposure for associations of *IL10* SNPs with asthma[60] and dendritic cell–associated nuclear protein 1 *(DCNP1)* SNPs with house dust mite–specific IgE.[61] Although these observations are yet to be replicated, they provide initial evidence of gene-environment interaction with allergens.

The effects of air pollution on asthma susceptibility are also likely to be modified by SNPs in genes encoding inflammatory cytokines and metabolizing enzymes.[62] Recently, Salam et al[63] studied SNPs in arginase *(ARG)* genes (involved in the response to nitrosative stress) and observed an *ARG1* haplotype interaction between ozone exposure during childhood and risk of asthma. Glutathione-S-transferase polymorphisms might also influence the effects of ambient air pollution on asthma risk during childhood, particularly when controlled for levels of ozone[64] and diesel exhaust particles.[65] Gene-environment interaction has also been observed with environmental tobacco smoke and risk of childhood asthma in relation to the TNF-α gene *(TNFA)*[66] and SNPs in the chromosome 17q21 region.[35]

Although data are constantly emerging for gene-environment effects in asthma, the translational research challenge now is to integrate molecular, clinical, and epidemiologic studies of asthma to discover robust mechanisms of gene–environment interaction that would facilitate personalized interventions for persons with asthma. Furthermore, the use of genetic epidemiology is likely to present real opportunities for solving problems of casual inference in observational epidemiology. Epidemiologic studies of environmental exposures might identify spurious causes of disease caused by confounding by behavioral, physiologic, and socioeconomic factors related both to exposures and to disease end points. For example, the epidemiologic findings that hormone replacement therapy protects against coronary heart disease and that vitamin E and vitamin C reduce the risk of cardiovascular disease have all been refuted by randomized controlled trials and have raised concerns about the value of epidemiologic studies.[67] One solution to this is the use of Mendelian randomization. This approach is based on Mendel's second law, which states that inheritance of one trait is independent of inheritance of other traits. It uses common genetic polymorphisms that are known to influence exposure patterns (eg, availability of dietary nutrients, such as vitamins E or D) or have effects equivalent to those produced by modifiable exposures (eg, increased blood cholesterol concentration). Associations between genetic variants and outcome are not generally confounded by behavioral or environmental exposures. Thus if a genetic factor that modulates exposure to the environment (eg, apolipoprotein E for cholesterol or vitamin D receptor polymorphisms) modulates the effect of the exposure on outcome, it strengthens casual inference for the exposure of interest.[67,68] For example, in trying to assess the relationship between dietary calcium intake and osteoporosis, measuring exposure is difficult and potentially confounded by other factors, such as socioeconomic status. Lactase persistence is an autosomal dominant condition in part determined by a polymorphism near the lactase gene *(LCT)* that results in a sustained ability to digest the milk sugar lactose throughout adulthood. As a consequence, subjects with lactase persistence have a higher dietary intake of dairy products. Obermayer-Pietsch et al[69] have shown that in

postmenopausal women the CC genotype is strongly associated with low dietary intake of calcium from milk, lower bone mineral density at the hip and spine, and a greater risk of nonvertebral fractures. This provides strong evidence that milk drinking improves bone health, especially because directly studying milk intake is potentially beset with problems of confounding, reverse causation (persons with bone problems might be told to drink more milk), and measurement error. The use of the Mendelian randomization approach is likely to be of value in the future for increasing evidence for causality for a range of environmental exposures shown to be associated with increased risk of allergic disease from farm exposure and diet to aeroallergen and air pollution exposure.

Identification of new models of pathogenesis

It is clear from genetic studies of allergic disease that the propensity toward atopy is influenced by factors different than those that influence disease progression. However, these disease factors require interaction with atopy (or something else) to trigger disease. For example, in patients with asthma, bronchoconstriction is triggered mostly by an allergic response to inhaled allergen accompanied by an eosinophilic inflammation in the lungs, but in some persons who might have "asthma susceptibility genes" but not atopy, asthma is triggered by other exposures, such as TDI. This grouping of genes into atopic immune response genes and tissue-specific factors also applies equally to other clinical manifestations of atopy, such as rhinitis and atopic dermatitis. It is possible to group the genes identified as contributing to allergic disease into 4 broad groups (Fig 1).[1]

First, there is a group of genes that are involved in directly modulating response to environmental exposures. These include genes encoding components of the innate immune system that interact with levels of microbial exposure to alter the risk of allergic immune responses, such as the genes encoding components of the LPS response pathway, such as CD14[79] and TLR4.[79] Other environmental response genes include detoxifying enzymes, such as the glutathione S-transferase genes that modulate the effect of exposures involving oxidant stress, such as tobacco smoke and air pollution.[62]

The second major group, which includes many of the genes identified through hypothesis-independent genome-wide approaches, is a group of genes involved in maintaining the integrity of the epithelial barrier at the mucosal surface and signaling of the epithelium to the immune system after environmental exposure. For example, polymorphisms in *FLG* that directly affect dermal barrier function are associated not only with increased risk of atopic dermatitis but also with increased atopic sensitization (see below). Genes encoding chitinases, such as AMCase[80] and YKL-40,[18] appear to play an important role in modulating allergic inflammation and are produced in increased levels by the epithelium and alternatively activated macrophages in patients with asthma.[81] The gene *PCDH1* a member of a family of cell adhesion molecules and expressed in the bronchial epithelium, has also been identified as a susceptibility gene for BHR.[24]

The third group of genes are those that regulate the immune response, including *IL13*, *IL4RA*, *STAT6*, *TBX21* (encoding T-box transcription factor), *HLAG*, and *GATA3*, which regulate T_H1/T_H2 differentiation and effector function, but also others, such as *IRAKM* and *PHF11*, that might regulate the level of inflammation that occurs at the end organ for allergic disease (eg, the airway, skin, and nose).

Finally, a number of genes appear to be involved in determining the tissue response to chronic inflammation, such as airway remodeling. They include genes such as *ADAM33* which is expressed in fibroblasts and smooth muscle; *PDE4D*, which is expressed in smooth muscle (and inflammatory cells); and *COL29A1*, encoding a novel collagen expressed in the skin and linked to atopic dermatitis.

Thus the insights provided by the realization that genetic variation in genes regulating atopic immune responses are not the only or even the major factor in determining susceptibility to allergic disease has highlighted the importance of local tissue response factors and epithelial susceptibility factors in the pathogenesis of allergic disease.[82] This is possibly the greatest contribution that genetic studies have made to the study of allergic disease and where the most effect in the form of new therapeutics targeting novel pathways of disease pathogenesis is likely to occur.

Sensitization and progression: *FLG* in atopic dermatitis and asthma

Atopic dermatitis often represents the first clinical manifestation of atopy in childhood and suggests a high risk for the development of persistent asthma in childhood. Studies of the gene *FLG* have now shown that the link between early childhood eczema and the subsequent development of asthma is, in part, due to defective epidermal barrier function leading to increased allergen sensitization. Filaggrin (filament-aggregating protein) has a key role in epidermal barrier function. The protein is a major component of the protein-lipid cornified envelope of the epidermis, which is important for water permeability and for blocking the entry of microbes and allergens.[83] *FLG* is located on chromosome 1q21 in the epidermal differentiation complex. In 2006, Smith et al[5] reported that loss-of-function mutations in *FLG* caused ichthyosis vulgaris, a severe skin disorder characterized by dry flakey skin and a predisposition to atopic dermatitis and associated asthma. The mutations in *FLG* appear to act in a semidominant fashion, with carriers of homozygous or compound heterozygous mutations (R501X and 2282del4) having severe ichthyosis vulgaris, whereas heterozygotes have mild disease. The combined carrier frequencies of null *FLG* mutations (5 in total) are around 9% in the European population.[84]

Subsequently, these mutations have also been linked to atopic dermatitis,[70,85,86] asthma,[87-89] and allergy,[90] although only in the presence of atopic dermatitis, and account for up to 15% of the population-attributable risk of atopic dermatitis.[87] Confirmation of the hypothesis that by conferring a deficit in epidermal barrier function *FLG* mutation could initiate systemic allergy by allergen exposure through the skin and start the "atopic march" in susceptible subjects has recently been provided by the analysis of the spontaneous recessive mouse mutant flaky-tail *(flt)*, the phenotype of which has been shown to result from a frame-shift mutation in the murine filaggrin gene. Topical application of allergen in mice homozygous for this mutation resulted in enhanced cutaneous allergen priming and resultant allergen-specific IgE and IgG antibody responses.[91]

[1]See references 14, 17, 21, 23-35, 34, 39, 41, 44, and 70-77.

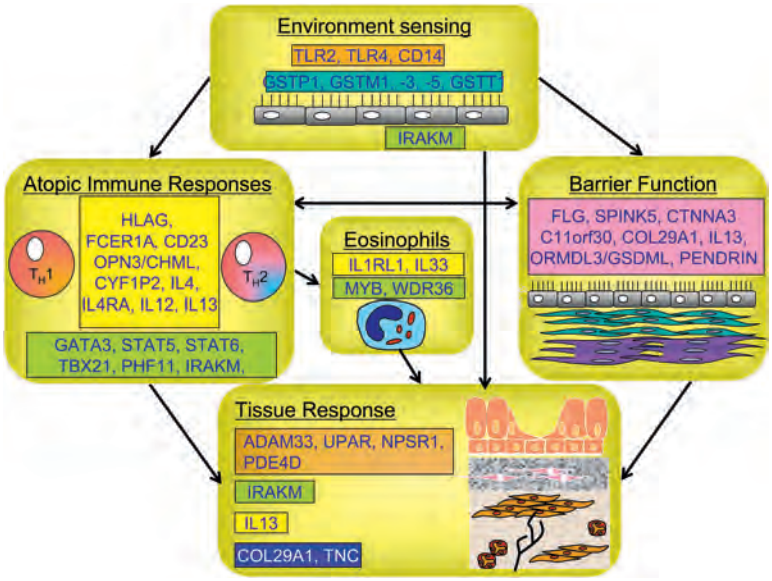

FIG 1. Susceptibility genes for allergic disease. Group 1: sensing the environment. The group of genes encodes molecules that directly modulate the effect of environmental risk factors for allergic disease. For example, genes such as *TLR2, TLR4,* and *CD14,* encoding components of the innate immune system, interact with levels of microbial exposure to alter the risk of allergic immune responses.[71] Polymorphisms of glutathione-S-transferase genes (*GSTM1*, *GSTM2*, GSTM3, *GSTM5*, *GSTT1*, and *GSTP1*[72,73]) have been shown to modulate the effect of exposures involving oxidant stress, such as tobacco smoke and air pollution on asthma susceptibility. Group 2: barrier function. A high proportion of the novel genes identified for susceptibility to allergic disease through genome-wide linkage and association approaches have been shown to be expressed in the epithelium. This includes genes such as *FLG,*[70] which directly affects dermal barrier function and is associated not only with increased risk of atopic dermatitis but also with increased atopic sensitization. Other susceptibility genes, such as *ORMDL3*/*GSDML,*[34] *PCDH1,*[24] and *C11orf30,*[44] are also expressed in the epithelium and might have a role in possibly regulating epithelial barrier function. Group 3: regulation of (atopic) inflammation. This group includes genes that regulate T_H1/T_H2 differentiation and effector function (eg, *IL13, IL4RA,* and *STAT6*[74]; *TBX21* [encoding T-box transcription factor][75]; and *GATA3*[76]), as well as genes such as *IRAKM,*[77] *PHF11,*[21] and *UPAR*[23] that potentially regulate both atopic sensitization and the level inflammation that occurs at the end-organ location for allergic disease. This also includes the genes shown to regulate the level of blood eosinophilia (*IL1RL1*, *IL33, MYB,* and *WDR36*).[41] Group 4: tissue response genes. This group includes genes that modulate the consequences of chronic inflammation (eg, airway remodeling), such as *ADAM33*[17] and *PDE4D,*[39] which are expressed in fibroblasts and smooth muscle, and *COL29A1,*[25] encoding a novel collagen expressed in the skin linked to atopic dermatitis. Some genes can affect more than 1 disease component. For example, *IL13* regulates both atopic sensitization through IgE isotype switching but also has direct effects on the airway epithelium and mesenchyme, promoting goblet cell metaplasia and fibroblast proliferation.[14] Adapted with permission from Rose-Zerilli MJ, Davis SA, Holgate ST, Holloway JW. The genetics of allergic disease and asthma. In: Leung DYM, Sampson H, Geha R, Szefler SJ, editors. Pediatric allergy: principles and practice. 2nd ed. St Louis: Mosby; 2009.

The importance of early life

It is well established that for both atopy and asthma, phenotypic measures, such as cord blood immune responses, airway function, and bronchial responsiveness, in the newborn period (and hence dependent on fetal immune and lung development) predict subsequent development of allergic disease.[92-95] Lower rates of fetal growth are also associated with impaired lung development in children.[96] Furthermore, there might also be interaction between atopy and lung development.[97] A number of genetic studies have now provided evidence to support a role for early-life developmental effects in allergic disease. For example, *ADAM33* was identified as an asthma susceptibility gene by using a genome-wide positional cloning approach in 2002.[17] The observed positive association between polymorphisms in this gene and asthma susceptibility and BHR, but not atopy or serum IgE levels, coupled with the selective expression of ADAM33 in airway smooth muscle cells and fibroblasts strongly suggests that alterations in its activity might underlie abnormalities in the function of these cells critical for both BHR and airway remodeling. As in adult airways, multiple ADAM33 protein isoforms exist in the human embryonic lung when assessed at 8 to 12 weeks of development,[98] and a polymorphism in *ADAM33* is associated with early-life measures of lung function (specific airway resistance at age 3 years).[99] Although replication studies are awaited, this suggests that variability in this gene is acting *in utero* or in early life to determine lung development. A recent replication study of the association between SNPs on chromosome 17q21 in the region of the gene encoding *ORMDL3* and asthma has also provided further support for a critical early-life period for the development of asthma. In this study Bouzigon et al[35] showed that 17q21 SNPs were associated particularly with early-onset asthma ($\leq$4 years of age), whereas no association was found for late-onset asthma. Furthermore, adjusting for early-life smoke exposure revealed a 2.9-fold increase in risk compared with that seen in unexposed patients with early-onset asthma.

WHAT WILL THE RESULTS OF FUTURE GENOMIC STUDIES REVEAL?

The progress in identification of complex disease susceptibility genes in the last few years has been remarkable. Use of this approach has, in the last 12 months alone, identified more novel susceptibility genes for allergic disease than almost a decade of efforts in positional cloning. The examples provided by other common inflammatory diseases show that there are likely to be 20 to 30 easily identifiable genes for disease susceptibility, with more to be identified through analysis of intermediate phenotypes, such as measures of lung function or immune function.

One limitation of the GWAS is the reliance on common haplotype blocks and genotyping of common variants. This restricts the ability to detect rare risk alleles that might be contributing to the disease.[53] CNVs are segmentally duplicated sequences in the genome that contribute a sizeable effect on the variability of gene expression.[100] Although fewer in number, CNVs cover a larger proportion of the genome sequence compared with SNPs and are also not well captured by currently available genome-wide genotyping arrays. GWASs in asthma should therefore be interpreted in this light, and further methods for fine resequencing and replication studies to find causal alleles are required. However, as for other complex diseases, such as Crohn disease and diabetes (which have been extensively studied with GWAS approaches), the results from GWASs of allergic disease are unlikely to fully explain the heritability of allergic disease given the limitations of this approach, which is best suited to identifying common variants for common diseases.

In addition to the presence of rare variants in the genome and copy number variation discussed above, another potential mechanism for explaining the heritability of common diseases that is not accounted for by loci identified with the GWAS approach is epigenetics.[51,101] Epigenetics refers to biochemical changes to DNA that do not alter the DNA sequence but might be induced by environmental factors and transmitted through generations. Epigenetic factors include modification of histones by means of acteylation and methylation and DNA methylation. Modification of histones, around which the DNA is coiled, alters the rate of transcription, altering protein expression. DNA methylation involves adding a methyl group to specific cytosine bases in the DNA to suppress gene expression. Importantly, both changes to histones and DNA methylation can be induced in response to environmental exposures, such as tobacco smoke and alterations in the early-life environment (eg, maternal nutrition).[102]

Evidence as to the importance of epigenetic factors in allergic disease include studies that have linked altered birth weight, head circumference at birth, or both (proxy markers for maternal nutrition) to an increase in adult IgE levels and risk of allergic disease.[103-105] A recent study has also shown that increased environmental particulate exposure from traffic pollution results in a dose-dependent increase in peripheral blood DNA methylation.[106] Observations, such as grandmaternal smoking increasing the risk of childhood asthma in grandchildren,[107] support the concept that transgenerational epigenetic effects (mediated by DNA methylation) might also be operating in allergic disease. Other support comes from the study of animal models; for example, mice exposed *in utero* to supplementation with methyl donors exhibit enhanced airway inflammation after allergen challenge.[108] It is likely in the near future that studies of large prospective birth cohorts with information on maternal environmental exposures during pregnancy are likely to provide important insights into the role of epigenetic factors in the heritability of allergic disease.[109]

WHAT IS THE POTENTIAL CLINICAL UTILITY OF GREATER UNDERSTANDING OF ALLERGIC DISEASE GENETICS?

Although undoubtedly the greatest effect of studies of the genetics of allergic disease has been in increasing our understanding of disease pathogenesis, there are a number of other ways in which greater understanding of the genetic basis of allergic disease will improve diagnosis and treatment in the future.

Predicting disease onset

One question that is often asked is whether identification of genetic factors can enable more accurate prediction of the likelihood a subject will develop allergic disease. In some respects the clinical use of family history is a surrogate measure for heritable risk, and this has been shown to have some validity.[110] However, at present, we are not in a position to use the rapidly accumulating knowledge of genetic variants that influence allergic disease progression in clinical practice. This simply reflects the complex interactions between different genetic and environmental factors required both to initiate disease and determine progression to a more severe phenotype in a subject, meaning that the predictive value of variation in any one gene is low, with a typical genotype relative risk of 1.1 to 1.5.[111]

However, it is possible that as our knowledge of the genetic factors underlying disease increase, the predictive power of genetic testing will increase sufficiently to enable its use in clinical decision making. For example, simulation studies based on the use of 50 genes relevant for disease development demonstrated that an area under the curve of 0.8 can be reached if the genotype relative risk is 1.5 and the risk allele frequency is 10%.[111,112] Whether this is likely to improve on diagnostics using traditional risk factor assessment is a separate issue. Recent analyses of the power of genetic testing to predict the risk of type II diabetes (for which many more genetic risk factors have been identified through genome-wide approaches than for allergic disease at this stage) demonstrate that the inclusion of common genetic variants has only a small effect on the ability to predict development of the condition.[113,114] This has led to some questioning the "disproportionate attention and importance of resources" focused on genetic studies in the prevention of common diseases.[115] However, the identification of further risk factors and the development of better methods for incorporating genetic factors into risk models are likely to substantially increase the value of genotypic risk factors and may also provide a means for predicting progression to severe disease and targeting of preventative treatment in the future.[116] The potential utility of such an approach for allergic disease has been highlighted by the recent observation that in infants with eczema and sensitization to food allergens, *FLG* mutations predict subsequent development of childhood asthma with 100% positive predictive value.[117]

Predicting asthma subtypes

A simplistic view of asthma or any other allergic disorder that focuses entirely on T_H2 polarization and activation of allergy

related cells, such as mast cells, basophils, and eosinophils, fails to take account of locally acting genetic and environmental factors that are required to translate the atopic phenotype in a specific organ to create disease.[116] In addition, the limited efficacy of biologic agents targeting individual T-cell receptors, such as CD25,[118] IL-5,[119,120] and TNF-α,[121] indicate that although individual patients might benefit from such therapies, they form only a small subgroup of the whole disease spectrum. Thus the concept is emerging of subphenotypes of asthma driven by differing gene-environment interactions.[122,123] Thus gene-environment interactions are likely to be crucial in driving such subphenotypes and are leading us toward stratified medicine.

Predicting severe disease

One area in which genetics might play an important role in prediction is in disease severity. The ability to identify those who are most likely to have severe persistent disease would allow targeting of preventative treatments and be of significant clinical utility. There is increasing evidence that many genetic disorders are influenced by "modifier" genes that are distinct from the disease susceptibility locus. The identification of such modifier genes in allergic diseases such as asthma is difficult because of the complex interactions among susceptibility, environment, and treatment. However, despite these difficulties, a number of studies have identified genes that are associated with measures of asthma severity. Identification of such markers of severe disease might, in the future, allow targeting of health care resources to those subjects who are likely to have severe disease and exhibit the greatest morbidity and mortality.[124]

Allergic disease and personalized medicine

There is an increasingly important role for pharmacogenetics, the study of genetic influences on interindividual variability in treatment responses. The main areas of focus for pharmacogenetic studies in patients with asthma have been the clinical response to bronchodilators, inhaled steroids, and leukotriene modifiers, as recently reviewed in detail.[125]

Naturally occurring polymorphisms in the β_2-adrenoceptor gene *(ADRB2)* might alter the function and expression of the β_2-adrenoceptor and therefore affect response to short- and long-acting bronchodilators. A number of nonsynonymous SNPs have been shown to be functional *in vitro*, including at amino acids 16, 27, and 164 and in the promoter region. For example, the arginine (Arg) to glycine (Gly) substitution at amino acid 16 is associated with downregulation in transfected cells. Recently, the study of *ADRB2* pharmacogenetics has been applied to longer-term clinical studies of long-acting bronchodilators. Although some studies have shown that Arg/Arg16 subjects have reduced peak expiratory flow rates compared with Gly/Gly16 subjects in response to salmeterol (with or without concomitant inhaled corticosteroid treatment),[126] subsequent studies have failed to confirm these findings.[127,128] Variation in study design (eg, sample size and use of combination inhalers) might explain some of the difference in results between these clinical studies. Given the discordant results, further work is required to fully evaluate the exact role of *ADRB2* polymorphisms in the response to bronchodilators in asthmatic subjects.[129] Furthermore, there are likely to be other genetic determinants of response to bronchodilator treatment. For example, Litonjua et al,[130] assessing the effect of 844 SNPs in 111 candidate genes, recently identified the *ARG1* gene encoding arginase 1 as a predictor of acute response to albuterol.

Polymorphisms in steroid pathways might also be clinically important in asthma management. Tantisira et al[131] screened 131 SNPs in 14 candidate genes involved in steroid biology in a large clinical study of 470 adult asthmatic subjects and then went on to further validate SNPs of interest in other clinical trials involving 311 children with asthma and 336 adults with asthma. They observed that SNPs in the corticotropin-releasing hormone receptor 1 gene *(CRHR1)* were associated with improved lung function (FEV_1) response to inhaled steroids after 6 to 8 weeks of treatment in the 3 clinical trials. Corticotropin-releasing hormone increases corticotrophin release from cells of the anterior pituitary by binding to its receptors, corticotropin-releasing hormone receptor 1 and 2. In biological terms SNPs in *CRHR1* could potentially reduce receptor function, leading to impaired cortisol release and greater response to exogenous steroids, such as inhaled steroids.[131] However, the association of *CRHR1* SNPs with inhaled steroids' effects on lung function decrease was not replicated in a long-term cohort study of 164 adult asthmatic subjects,[132] and hence the effect of the *CRHR1* polymorphism in the response of inhaled steroids in asthmatic subjects has yet to be definitively defined. In addition to variation in genes that determine cortisol synthesis, an obvious candidate for corticosteroid response is the glucocorticoid receptor gene *NR3C1*. Although common polymorphisms of *NR3C1* do not appear to be important in determining interindividual corticosteroid resistance and response, Hawkins et al[133] have recently shown that variation in another component of the large heterocomplex of proteins that cooperatively function to activate the glucocorticoid receptor *STIP1* is associated with the magnitude of FEV_1 improvement in response to inhaled corticosteroid treatment.

A number of SNPs in genes involved in the leukotriene pathway have been associated with response to leukotriene modifiers.[134] In a clinical study of montelukast in 252 adult asthmatic subjects, Lima et al[135] found associations of FEV_1 response with SNPs in the 5-lipoxygenase *(ALOX5)* and multidrug resistance protein 1 *(MRP1)* genes and changes in exacerbation rates with SNPs in the leukotriene C_4 synthase *(LTC4S)* and leukotriene A_4 hydrolase *(LTA4H)* genes. Associations with some of these leukotriene pathway genes were also replicated in a different study of montelukast[136] and also with zileuton.[137]

These studies show that pharmacogenetic effects have the potential to influence the efficacy of asthma therapies because SNPs can alter the expression and function of asthma pharmacologic targets and their metabolizing systems. Characterizing these effects at the candidate gene and genome-wide level might have clinical importance for individualizing asthma therapy, although to date, interpreting the effects and determining their clinical relevance has thus far been challenging.

CONCLUSIONS

The evidence to date from positional cloning studies, candidate gene studies, and GWASs has revealed a number of biologically plausible candidate genes for allergic disease. The challenge now is to identify robust susceptibility loci for asthma and then translate statistical significance from genetic and genomic studies to biological and clinical effect. The genetic epidemiologic observations for specific candidate genes in patients with asthma

and atopy require careful replication enhanced by international collaboration and the availability of large, well-characterized case-control populations for genotyping. The strongest candidates require intensive biological investigation of the functional consequences of the causal SNPs and experiments to apply these consequences to the biology of asthma pathogenesis. The testing of gene-environment interactions will also be important in asthma through *in vitro* and *in vivo* challenge studies and measurement of environmental exposures in longitudinal cohorts. Understanding the genetic discoveries in allergic disease will potentially lead to better molecular phenotyping, prognostication, prediction of treatment response, and insights into molecular pathways to develop more targeted therapies.

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Asthma: Clinical expression and molecular mechanisms

Robert F. Lemanske, Jr, MD,[a,b] and William W. Busse, MD[a] *Madison, Wis*

Asthma is a complex disorder that displays heterogeneity and variability in its clinical expression both acutely and chronically. This heterogeneity is influenced by multiple factors including age, sex, socioeconomic status, race and/or ethnicity, and gene by environment interactions. Presently, no precise physiologic, immunologic, or histologic characteristics can be used to definitively make a diagnosis of asthma, and therefore the diagnosis is often made on a clinical basis related to symptom patterns (airways obstruction and hyperresponsiveness) and responses to therapy (partial or complete reversibility) over time. Although current treatment modalities are capable of producing control of symptoms and improvements in pulmonary function in the majority of patients, acute and often severe exacerbations still occur and contribute significantly to both the morbidity and mortality of asthma in all age groups. This review will highlight some of the important clinical features of asthma and emphasize recent advances in both pathophysiology and treatment.
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Key words: *Asthma, respiratory syncytial virus, rhinovirus, allergen, prevention, exacerbation, inception, treatment*

Abbreviations used
API: Asthma predictive index
EBC: Exhaled breath condensate
EIB: Exercise-induced bronchospasm
GERD: Gastroesophageal reflux disease
ICS: Inhaled corticosteroid
LABA: Long-acting β-agonist
NSAID: Nonsteroidal anti-inflammatory drug
RBM: Reticular basement membrane
RSV: Respiratory syncytial virus

Asthma is a heterogeneous disorder that is characterized by variable airflow obstruction, airway inflammation and hyperresponsiveness, and reversibility either spontaneously or as a result of treatment. Multiple etiologies no doubt exist for both its inception and symptom exacerbation once the disease is established. Factors underlying inception can range from viral respiratory tract infections in infancy[1,2] to occupational exposures in adults.[3] Factors underlying asthma exacerbations include allergen exposure in sensitized individuals, viral infections, exercise, irritants, and ingestion of nonsteroidal anti-inflammatory agents, among others. Exacerbating factors can include one or all of these exposures and vary both among and within patients. Asthma treatment is determined to a large extent after an initial assessment of severity and subsequent establishment of control, both of which can be variable over time and assessed in 2 domains: impairment (current) and risk (long-term consequences).[4] Unfortunately, despite the availability of effective therapies, suboptimal asthma control exists in many patients on a worldwide basis.[5] The future development of novel therapies and treatment paradigms should address these disparities.

NATURAL HISTORY (INCEPTION AND PROGRESSION)

For many asthmatic subjects, the disease has its roots during infancy and early childhood. Viral respiratory tract infections produce wheezing episodes during the first 3 years of life in about 50% of children.[6] Some of these children will stop wheezing (transient wheezers), whereas others will go on to have persistent symptoms that will either dissipate before adolescence (primarily nonatopic subjects) or continue into adolescence (atopic wheezers).[7] Once in remission, the disease process might remain quiescent, or the subject could relapse in later life.[8,9] The phenotype of severe asthma has also been recently well described.[10]

The pattern and rate of loss of lung function in asthmatic subjects has been of interest and concern for many investigators. A number of groups have reported that the greatest absolute loss of lung function appears to occur very early in childhood.[8,11,12] Some have reported that the peak in lung function that is achieved at about 20 years of age in asthmatic subjects can be decreased[13] and that the rate of further loss during adulthood can be increased in asthmatic subjects.[14] About one fourth of children with asthma might experience greater rates of loss of lung function, and these children have certain phenotypic characteristics: younger age, male sex, higher postbronchodilator FEV_1 percent predicted, and greater airway eosinophilic inflammation.[15]

Molecular and cellular mechanisms in asthma

Children. The performance of invasive procedures in children to evaluate molecular and cellular mechanisms in asthma is

From the Departments of [a]Pediatrics and [b]Medicine, University of Wisconsin Medical School.
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Reprint requests: Robert F. Lemanske, Jr, MD, Departments of Pediatrics and Medicine, University of Wisconsin Hospital, 600 Highland Ave K4-916, Madison, WI 53792. E-mail: rfl@medicine.wisc.edu.
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obviously not as feasible from a variety of standpoints compared with adults. However, a few carefully and safely conducted studies in young children have provided insights into possible pathophysiologic features as they relate to developmental milestones and disease expression. When bronchoalveolar lavage has been performed in young wheezing children, a 3-fold increase in total cells, most significantly lymphocytes, polymorphonuclear cells, and macrophages/monocytes, compared with counts seen in healthy children has been noted. In addition, levels of leukotriene B_4 and C_4, prostaglandin E_2, and the potentially epithelium-derived 15-hydroxyeicosattetranoic acid were all increased.[16]

Several bronchial biopsy studies have been performed in children. In 53 infants with reversible airflow obstruction evaluated for severe wheezing or cough, bronchial biopsy specimens demonstrated no reticular basement membrane (RBM) thickening or the eosinophilic inflammation characteristic of asthma in older children and adults, even in the presence of atopic characteristics.[17] Conversely, children younger than 6 years with asthma had increased epithelial loss, basement membrane thickening, and eosinophilia compared with control subjects of the same age. However, similar pathologic changes were seen in atopic children without asthma.[18] Taken together, it appears that the inflammatory and structural changes associated with asthma occur sometime after infancy during the early preschool years, when children experience more persistent symptoms of airway dysfunction.

In older children 6 to 16 years of age with difficult asthma receiving high-dose inhaled corticosteroids (ICSs), RBM thickening to a similar extent to that seen in adult asthmatic subjects has been demonstrated.[19] Additionally, there was no association with RBM thickening and age, symptom duration, lung function, or concurrent eosinophilic airway inflammation. However, unlike adults with asthma, no relationship was observed between RBM thickness and bronchial wall thickening on high-resolution computed tomographic scanning in children with difficult asthma.[20] Finally, persistent airflow obstruction has been associated with a greater density of $CD4^+$ T lymphocytes in endobronchial biopsy specimens in 27 school-aged children with difficult asthma after treatment with systemic corticosteroids compared with that seen in control subjects.[21]

A number of biomarkers have been evaluated to avoid the invasive procedures of bronchial lavage, biopsy, or both in children. Exhaled nitric oxide might be useful as a diagnostic tool and in ongoing management of children with asthma. Exhaled nitric oxide levels have been demonstrated to differentiate young children with asthma from those without,[22] to identify children who are likely to respond to ICSs,[23] and to predict those children who will experience an asthma relapse after reduction of ICSs.[24] However, recent data indicate that when fraction of exhaled nitric oxide monitoring is used in conjunction with a National Asthma Education and Prevention Program guidelines–based asthma management program, it might result in excessive ICS dosing without any significant gains in achieving or maintaining asthma control.[25]

Exhaled breath condensate (EBC) is obtained by cooling exhaled air and is believed to reflect the contents of the airway lining fluid.[26] Hydrogen peroxide, isoprostanes, aldehydes, and nitrotyrosine are considered markers of oxidative stress, and their levels are increased in the EBC of children with asthma, suggesting an imbalance between oxidants and antioxidants. Conversely, levels of glutathione, a protective lung antioxidant, are decreased in children with acute asthma, suggesting a reduced antioxidant capacity.[27] Levels of the inflammatory mediators cysteinyl leukotrienes are increased in the EBC of children with atopic asthma, even while receiving corticosteroid treatment.[28] Finally, airway pH balance might have a role in asthma because a reduced EBC pH has been reported in children with acute or stable asthma.[26]

Levels of several other mediators of inflammatory cells have been found to be significantly higher in very young children with asthma, including the number of blood eosinophils, serum eosinophil cationic protein, eosinophil-derived neurotoxin, and urinary eosinophil-derived neurotoxin.[29] In addition, both increased eosinophil cationic protein and cysteinyl leukotriene levels[30] have been obtained from nasal washings in wheezing children less than 2 years of age.

Adults. Asthma for most, but not all, patients begins in early life. As noted above, the cellular and molecular patterns associated with airway inflammation in asthma are complex, interactive, redundant, and variable.[31] In adults, particularly those with established longstanding disease, the factors that contribute to the pathophysiology of airway abnormalities are dependent on the phases of asthma, such as acute, persistent, severe versus nonsevere, or during treatment.

An understanding of the immunopathology of airways in asthma has been markedly advanced with the use of bronchoscopy and biopsy. These airway samples can then be analyzed by using histologic and immunologic methods, and the identified features can be evaluated in relationship to clinical features of asthma to more fully understand the contribution of cellular and molecular events to the resulting physiology and response to treatment.[32] In addition, it is now appreciated that the regulation of airway inflammation is distinct in different phases of asthma (ie, early-onset disease largely related to allergic inflammation and in the persistent or chronic phase of the disease).[33] It is helpful to arbitrarily consider asthma in terms of the traditional T_H2 inflammatory processes and the more chronic inflammatory phase, in which resident airway cells assume the more dominant component contributing to airway dysfunction (Fig 1),[33] to appreciate the immunopathogenetic mechanisms associated with different phases of asthma.

In the acute inflammatory aspects of asthma, allergen-IgE–directed processes are predominant features of airway pathology, with mast cells, T_H2 lymphocytes, and eosinophils the predominant histologic features.[32] The cytokine network associated with these processes often includes IL-3, IL-4, IL-5, IL-9, and IL-13.[34] Mast cells are important contributors both to the initiation of asthma with release of acute-phase mediators, including cysteinyl leukotrienes, and also inflammatory cytokines, which serve to perpetuate inflammatory events in the airway.[35] Subpopulations of lymphocytes polarized toward a T_H2 profile further the inflammatory process by release of cytokines, including IL-4, IL-5, and IL-13. It is these factors that serve to drive inflammation (eg, recruitment of eosinophils) and also regulate IgE production.[32]

Eosinophils are a characteristic feature of allergic inflammation.[32] The biology of eosinophils is well designed to cause airway inflammation, enhancement of airway hyperresponsiveness, and airflow obstruction. Eosinophils are recruited to the airway in asthmatic subjects by families of cytokines, and chemokines (eg, IL-5, RANTES, and eotaxin) undergo cell activation through processes not fully identified and release highly inflammatory granule-associated substances, the actions of which injure the airway and cause persistent inflammation. Eosinophils

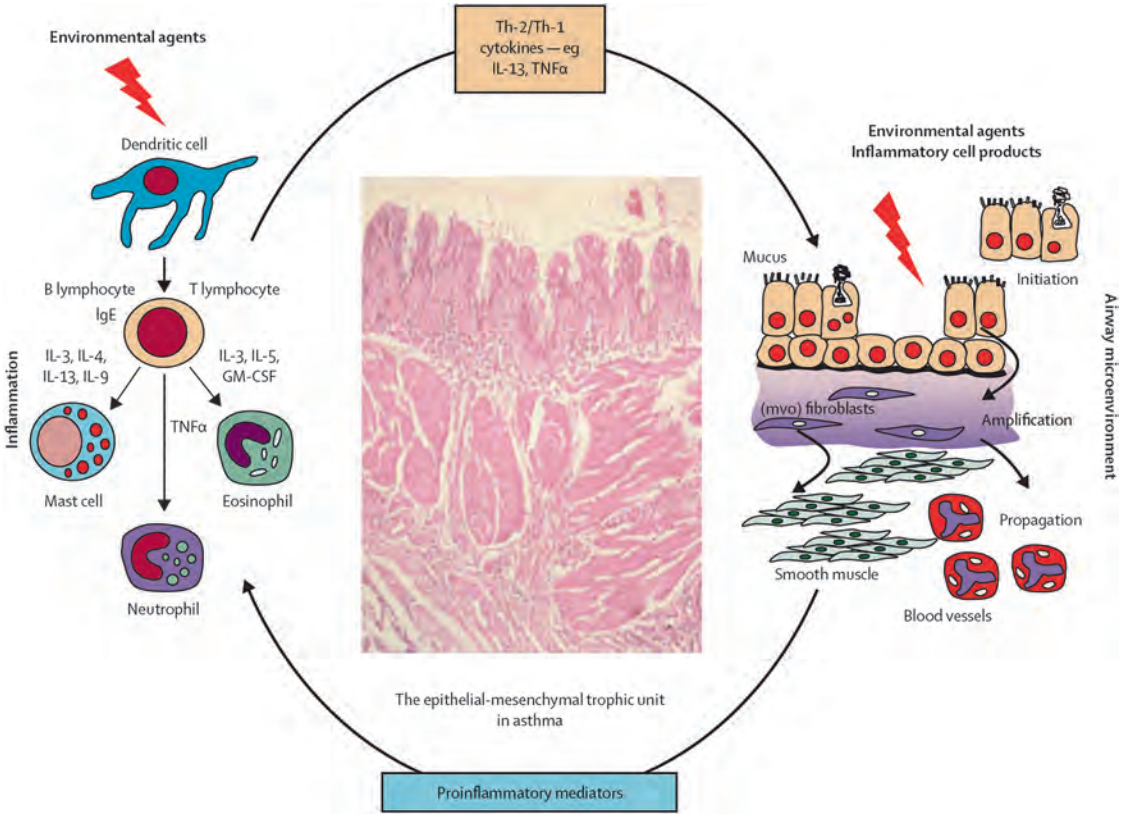

FIG 1. Inflammatory and remodeling responses in asthma with activation of the epithelial mesenchymal trophic unit. The epithelial mesenchymal trophic unit has been defined as bidirectional interaction between the epithelium and underlying mesenchyme involving the release of selective growth factors and cytokines. Epithelial damage alters the set point for communication between bronchial epithelium and underlying mesenchymal cells, leading to myofibroblast activation, an increase in mesenchymal volume, and induction of structural changes throughout airway wall. Used with permission from the *Lancet*.[33]

are also a rich source for leukotrienes, products of oxidative metabolism, and inflammatory cytokines and growth factors.[36] Although the eosinophil is a prominent feature of airway pathology in asthmatic subjects, its precise contribution to airway pathophysiology is undergoing re-evaluation.

The pattern of airway injury in patients with chronic asthma tends to be more variable, with a shift in the histologic picture toward resident cells of the airway as the more likely cause of persistent disease. In some patients there will be a progressive decrease in lung function and the development of chronic irreversible changes in lung function with their asthma. Although these changes likely have their origins at the onset of asthma, many questions remain as to who is at risk for airway remodeling, when this process begins, and what factors regulate the transition from acute to chronic inflammation. The recognition of progressive loss of lung function in asthmatic subjects has led to a renewed interest in the role of resident airway cells in persistent inflammation.

The airway epithelium is both a target and contributor of persistent inflammatory airway changes in asthmatic subjects.[37] Histologic evaluation of airways in asthmatic subjects, particularly those with more severe disease, reveals injury to epithelium and often a loss of these cells. Epithelial cells are also a rich source of inflammatory mediators and growth factors. In addition, airway smooth muscle often shows hypertrophic and hyperplastic changes in subjects with persistent severe asthma. Moreover, the airway smooth muscle can be a source of both inflammatory cytokines and growth factors.[38]

There are other airway cells involved in asthma histopathology, including mucous glands and blood vessels. In subjects with asthma, mucous glands hypertrophy occurs. Activation of these cells leads to the release of mucus to occlude airways and, in severe exacerbations, to become the principal cause for resistance to treatment. Many factors generated in asthma (ie, vascular endothelial growth factor) can act on airway vessels to cause proliferation and, as a process, narrow the airways.

Understanding that heterogeneity exists in the pattern of airway inflammation and the likely molecular factors regulating these processes explains why current therapy is not effective in all subjects with asthma. As the phenotypic features of asthma unfold and with them a recognition of the associated cellular and molecular events, a more specific approach to treatment will follow accompanied by improved control of disease.

Risk factors

Risk factors in relationship to asthma have been evaluated in the context of disease inception (eg, viral infections[1,2,39]), environmental exposures (eg, aeroallergens,[40] pollution,[41-43] and tobacco smoke)[44-47] and lifestyle (eg, living on a farm,[48] diet,[49] and

antibiotic use[50]), comorbid conditions (eg, atopic dermatitis[51] and obesity[52]), and occupational exposures,[3] among others, as well as disease severity (as defined by the risk domain, which is discussed subsequently; hospitalizations,[53,54] frequency and severity of exacerbations,[55] and loss of lung function[8,56]). Genetic factors also contribute significantly to disease expression and severity. Asthma is genetically classified as a complex disorder; as such, it does not follow simple Mendelian inheritance characteristics. Hundreds of genetic association studies on asthma-related phenotypes have been conducted in different populations; these have been recently reviewed.[57] Although the importance of gene-environment interactions in the expression of disease has recently been highlighted,[58] the complexities involved in analyzing these relationships from a functional perspective have proved challenging.[59] Recent pharmacogenetic evaluations in relationship to chronic β-agonist use[60] and corticosteroid efficacy have provided new insights into the variability of response in asthmatic patients.

Exacerbating factors

Allergens. Allergen exposure is important in host allergic sensitization and as a common precipitant of asthmatic symptoms in both children and adults. The formation of antigen-specific IgE antibody to aeroallergens (eg, mites, trees, grasses, and animal dander)—the development of allergic sensitization but not necessarily of allergic disease—does not usually occur until 2 to 3 years of life. Thus aeroallergen-induced asthma is uncommon during the first year of life, begins to increase in prevalence during later childhood and adolescence, and peaks in the second decade of life. Once established in genetically predisposed individuals, IgE-mediated reactions are a major contributor both to acute asthmatic symptoms and chronic airway inflammation. Chronic low-level exposure to indoor allergens, dust mite and cockroach in particular, might play a major role in both asthma inception and subsequent provocation of symptoms.[61] Although a wide variety of inhaled allergens can provoke asthma symptoms, sensitization to house dust mite,[62] cockroach,[63] *Alternaria* species,[64] and possibly cat[40] are important in the pathogenesis of asthma. Dog, but not cat, ownership during infancy has been shown to reduce the subsequent development of allergic sensitization and atopic dermatitis[65]; numbers of pets and not the type of furred pet might also reduce future risk.[66] These diverse findings indicate that these relationships are indeed complex and might involve gene-environment interactions. Pollen immunotherapy in school-aged children with only allergic rhinitis at the start of treatment has been demonstrated to reduce significantly the subsequent risk of the development of airway hyperresponsiveness and asthma.[67]

Infections. Respiratory tract infections caused by viruses,[1,68,69] *Chlamydia* species,[70] and *Mycoplasma* species[70] have been implicated in the pathogenesis of asthma. Of these respiratory pathogens, viruses have been demonstrated to be epidemiologically associated with asthma in at least 3 ways.

First, during infancy, certain viruses have been implicated as potentially being responsible for the inception of the asthmatic phenotype. The viruses most convincingly demonstrated in this regard have been rhinovirus and respiratory syncytial virus (RSV).[1,2] The propensity to respond to these infections differently in persons destined to have asthma might be due to aberrations in innate immune responses, epithelial cell barrier alterations that enhance viral replication, and potentially increased virulence of pathogenic viral strains. However, because nearly every child has been infected at least once with this virus by 2 years of age, additional genetic, environmental, or developmental factors must contribute to the propensity of RSV to be epidemiologically linked with childhood asthma.

Second, in patients with established asthma, particularly children, viral upper respiratory tract infections play a significant role in producing acute exacerbations of airway obstruction that might result in frequent outpatient visits or hospitalizations.[1,71] Rhinovirus, the common cold virus, is the most frequent cause of exacerbations, but other viruses, including parainfluenza, RSV, influenza, and coronavirus, also have been implicated, albeit to a lesser extent. The increased tendency for viral infections to produce lower airway symptoms in asthmatic subjects might be related, at least in part, to interactions among allergic sensitization, allergen exposure, and viral infections acting as cofactors in the induction of acute episodes of airflow obstruction.[72,73] Abnormalities in the innate immune response that would prevent viral replication in airway epithelial cells from asthmatic subjects have recently been demonstrated.[68]

Third, and paradoxically, infections have been considered to have the potential of actually preventing the development of allergic respiratory tract diseases, including asthma. Interest in this area increased after the advancement of the hygiene hypothesis,[74] which proposed that increasing family size coincident with an increased number of infections might protect against these developments. Based on a progressively broader interpretation of this initial hypothesis,[75] a number of other epidemiologic (eg, living on a farm[76] and early pathologic bacterial colonization of the airway[77]) and biologic (eg, probiotics[78]) factors have been evaluated regarding their ability to influence the development of allergic sensitization, asthma, or both.

For infections with other microbial agents, recent attention has focused on *Chlamydia* and *Mycoplasma* species as potential contributors to both exacerbations and the severity of chronic asthma in terms of loss of lung function or medication requirements.[70] Finally, infections involving the upper airways (ie, sinusitis) have been considered to contribute to asthma control instability, evoking the concept of a unified airway in relationship to inflammatory responses and alterations in airway physiology.

Exercise. Exercise is one of the more common precipitants of airway obstruction in asthmatic subjects.[79] The symptoms of exercise-induced bronchospasm (EIB) can include any or all of the following: wheezing, coughing, and shortness of breath and, in children, chest pain or discomfort. The symptoms are most intense for 5 to 10 minutes and usually resolve within 15 to 30 minutes after exercise cessation. Under most circumstances, the degree of bronchoconstriction is rarely severe enough to be life-threatening, and such a situation almost invariably reflects advanced untreated disease, confounding triggering factors (ie, concomitant allergen or irritant exposure), or both. Objective documentation of airflow obstruction after an exercise challenge test ($\geq$15% decrease in FEV_1; $\geq$10% if symptoms accompany the decrease in FEV_1)[79] or a convincing history with an appropriate response to prophylactic or rescue medication is required to make the diagnosis of EIB. Exercise challenge testing, particularly in elite athletes,[80] must be of sufficient intensity and duration to be able to accurately diagnose the condition, keeping in mind that such confounding problems as vocal cord dysfunction might need to be considered in the differential diagnosis.[81] The pathophysiology of EIB can involve exaggerated responses to heat

and water loss and the release of inflammatory mediators as a consequence of these thermodynamic alterations.[82]

Nonsteroidal anti-inflammatory drugs. Approximately 5% to 10% of adult asthmatic patients will have an acute worsening of symptoms to nonsteroidal anti-inflammatory drugs (NSAIDs).[83] The aspirin triad, asthma, nasal polyps, and aspirin sensitivity, is usually found in adult asthmatic patients. The response to aspirin or other NSAIDs begins within an hour of aspirin ingestion and is associated with profound rhinorrhea, eye lacrimation, and, potentially, severe bronchospasm. Patients sensitive to aspirin usually are reactive to all other NSAIDs, and variations in the frequency and severity of adverse responses appear to depend on the potency of each drug within this class of compounds to inhibit the activity of the COX-1 enzyme.[83]

The sensitivity to NSAIDs is not IgE mediated but involves the modulation of eicosanoid production. It has been suggested that NSAIDs act by reducing the formation of prostaglandins, which help maintain normal airway function, while increasing the formation of asthma-provoking eicosanoids, including hydroxyeicosatetraenoic acids and large quantities of cysteinyl leukotrienes.[83] In addition, there is evidence that mast cell activation occurs, and its mediators can be detected in nasal secretions during an episode of aspirin-induced asthma.[84] This syndrome should be of concern in any asthmatic subject with nasal polyposis, chronic sinusitis, and eosinophilia, although the polyposis and sinusitis might precede the onset of recognized NSAID sensitivity by years.

Aspirin desensitization is available for the aspirin-sensitive patient who might need anti-inflammatory treatment or for use in patients with ischemic heart disease. In patients with aspirin-induced asthma, desensitization with aspirin has proved beneficial in improved asthma control, as well as improved sense of smell, reduced purulent sinus infections, and need for further polyp surgery.[85,86]

Gastroesophageal reflux. The true incidence of gastroesophageal reflux disease (GERD) in asthmatic subjects and as a causative factor in disease severity has yet to be established. However, it has been estimated that as many as 45% to 65% of adults and children with asthma have GERD. The mechanisms by which GERD affects asthma are also not established but might include microaspiration or irritation of the esophagus with reflux bronchospasm. Although often asymptomatic in its presentation, many patients have nighttime exacerbations or difficult-to-control symptoms. Confirmation of the importance of GERD to asthma often requires endoscopy and 24-hour monitoring of intraesophageal pH levels with concomitant measures of peak expiratory flow rates.

A number of clinical trials have begun to evaluate the effect of suppressing acid reflux on asthma symptoms. A systematic review of 12 small trials of proton-pump inhibitors used in asthma showed an improvement in asthma-related outcomes, but many studies had design flaws and variability in their outcomes.[87] In one study with patients experiencing nocturnal symptoms and symptomatic gastroesophageal reflux, comparisons were made between placebo and 40 mg of esomeprazole twice daily.[88] Improvements in peak flow were noted but not in FEV_1, rescue inhaler use, or nocturnal awakenings. Finally, the American Lung Association–Asthma Clinical Research Center evaluated 40 mg of esomeprazole twice daily versus placebo in subjects who were asymptomatic for acid reflux disease but had documented acid reflux in 40% of the subjects. Proton-pump inhibitors had no significant effect on a variety of asthma outcomes.[89] These studies suggest that treatment of acid reflux is beneficial, but improvement in symptoms from this condition had no effect on asthma outcomes.

Psychosocial factors. The role of psychosocial factors, or "stress," has undergone an important re-evaluation both in terms of a disease risk factor and a concomitant component of severity. Evidence has shown that parental stress is a risk factor for asthma expression in some children.[90] The mechanisms by which this occurs have not been defined but might include the promotion of allergic inflammation. For example, Liu et al[91] found stress from final examinations to enhance eosinophil recruitment to the airway after an antigen inhalation challenge. Chen et al[92] evaluated the influence of socioeconomic status, which they related to stress, on cytokine generation. With peripheral blood cells from asthmatic subjects but not healthy control subjects, lower socioeconomic status was associated with greater generation of the proinflammatory cytokines IL-5 and IL-13. These data are a further indication that stress can, in asthmatic subjects, promote an inflammatory profile. Recent work has also demonstrated dose-response–type relationships between panic and asthma and bidirectional longitudinal associations between the 2 conditions.[93]

DISEASE PROGRESSION, PREVENTION, AND TREATMENT

Although a number of research groups are investigating strategies aimed at asthma prevention,[94,95] this goal has not yet been achieved. Therefore therapy at present is directed primarily at achieving optimal control while attempting to minimize both short- and long-term side effects from any therapeutic intervention. Asthma control is defined by an understanding of the patient's asthma severity, which can be viewed in 2 domains: impairment and risk. Impairment is an evaluation of the concurrent degree of control in achieving the following: minimal (or none) chronic symptoms, including nocturnal awakenings caused by asthma; minimal (or none) need for acute rescue therapy, such as inhaled β_2-agonists; establishment of a normal lifestyle with no limitations on activities, including exercise; and normalization of pulmonary function. The risk domain includes criteria that deal with future events that the treatment program should either prevent or reduce to the greatest extent possible: reduction (or elimination of) in the frequency and severity of asthma exacerbations; minimal or no loss of lung function over time (considered to be a potential consequence of airway remodeling); and minimal or no adverse effects from medications.

The initial selection of pharmacologic treatment is determined based on the age of the patient and the severity of his or her asthma at the time of evaluation. Because asthma is a variable but chronic disease (or syndrome), specific treatment will need to be adjusted both acutely, or during exacerbations, and chronically in the context of eliminating or reducing both impairment and risk because they dynamically fluctuate over time to achieve acceptable control. a stepwise approach has been adapted for treatment to accomplish these goals (http://www.nhlbi.nih.gov/guidelines/asthma/asthgdln.htm).[4] The basis of the stepwise approach is to increase the number, frequency, and dose of medications with increasing asthma severity until the patient's disease has been put under "control" (ie, achieving optimal control for that patient). Once control has been established, step-down therapy can be

attempted to minimize medication burden, when possible. Recently, the concept of response to therapy has also received increasing attention. Responsiveness is the ease with which asthma control is achieved by therapy. Responsiveness to an asthma treatment is highly variable, and it is likely that both genetic and phenotypic characteristics contribute to this intrapatient and interpatient variability in response over time.[96,97]

In the last few years, a number of published clinical trials with new therapeutic agents or novel treatment strategies are noteworthy based on their potential effect in initiating or adjusting medication based on this stepwise severity scheme. The first set of trials pertains to the treatment of preschool wheezing children. One trial[98] evaluated continuous ICS treatment (2 years receiving therapy with an ICS and the third year receiving as-needed medication, which served as the observation year) in children who had a positive asthma predictive index (API). Children with positive APIs in the first 3 years of life have about a 65% chance of having clinically diagnosed asthma by age 6 years. During the 2 years of treatment with a low-dose ICS (fluticasone, 88 μg twice daily) compared with matching placebo, treated children had significantly greater numbers of episode-free days and reduced exacerbations requiring oral steroid treatment. However, after discontinuation of the ICS treatment at the beginning of the observation period, episode-free days were no different than in the placebo group within about 3 months. Reduced airway resistance in the ICS group at the end of the treatment period was no longer evident at the end of the observation period. Thus early recognition and treatment of high-risk children can reduce symptom burden while receiving therapy but does not appear to alter the natural history of asthma.[98] Similar negative results were seen when intermittent ICS therapy was prescribed.[99] Intermittent therapy with either an ICS or montelukast at the onset of respiratory tract symptoms was able to reduce symptom burden during these illnesses; however, these beneficial effects were only seen in children with positive APIs.[100] In a third study in preschool-aged children with moderate-to-severe, presumed virus-induced wheezing, pre-emptive treatment with high-dose fluticasone (750 μg twice daily at the start of upper respiratory tract symptoms) compared with placebo reduced the use of rescue oral corticosteroids. However, treatment with fluticasone was associated with a smaller gain in height and weight.[101] Finally, in preschool children presenting to a hospital with mild-to-moderate wheezing associated with a presumed viral infection, oral prednisolone was not superior to placebo in reducing the duration of the hospital stay.[102] These disparate results in this age group might relate to host (eg, presence or absence of atopy[103]), viral (cause/pathogenicity of viral infection, such as rhinovirus vs RSV[104]), or both factors that confer differential responses to these types of interventions. More studies are obviously needed before precise recommendations can be made in this age group.

The second set of trials pertains to the use of long-acting β-agonists (LABAs) in combination with ICSs for the treatment of persistent asthma. Although a number of clinical trials have demonstrated both safety and efficacy in terms of asthma control in both the impairment and risk domains,[105] concern has been raised about the potential for adverse outcomes in a small number of patients with the use of LABAs.[106] Recent continued review of the available data has re-emphasized these potential safety issues in both children and adults.[107] Unfortunately, the possible mechanisms underlying these rare events are unknown. Moreover, the numbers of patients needed to be prospectively evaluated to ascertain the precise risks involved might be too large to realistically enroll.[108] Overall, however, the benefits of combination therapy appear to outweigh the risks in the majority of patients. Monotherapy with LABAs in asthmatic subjects should not be prescribed.

SUMMARY

Asthma is a complex genetic disorder that is characterized by airway inflammation and reversible airflow obstruction. It is further distinguished by multiple phenotypes that might differ based on age of onset, triggering factors, and patterns of severity both during acute exacerbations and on a more chronic basis, as reflected by variably reversible loss of lung function. As a result of this clinical heterogeneity, treatment approaches need to be individualized and modified to obtain and maintain adequate symptom and disease control over time. Although current therapy is targeted at the development of secondary and tertiary prevention strategies, ongoing research is evaluating the prospects of primary prevention as well.

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Rhinitis and sinusitis

Mark S. Dykewicz, MD,[a] and Daniel L. Hamilos, MD[b] *Winston-Salem, NC, and Boston, Mass*

Rhinitis and sinusitis are among the most common medical conditions and are frequently associated. In Western societies an estimated 10% to 25% of the population have allergic rhinitis, with 30 to 60 million persons being affected annually in the United States. It is estimated that sinusitis affects 31 million patients annually in the United States. Both rhinitis and sinusitis can significantly decrease quality of life, aggravate comorbid conditions, and require significant direct medical expenditures. Both conditions also create even greater indirect costs to society by causing lost work and school days and reduced workplace productivity and school learning. Management of allergic rhinitis involves avoidance, many pharmacologic options, and, in appropriately selected patients, allergen immunotherapy. Various types of nonallergic rhinitis are treated with avoidance measures and a more limited repertoire of medications. For purposes of this review, *sinusitis* and *rhinosinusitis* are synonymous terms. An acute upper respiratory illness of less than approximately 7 days' duration is most commonly caused by viral illness (viral rhinosinusitis), whereas acute bacterial sinusitis becomes more likely beyond 7 to 10 days. Although the mainstay of management of acute bacterial sinusitis is antibiotics, treatment of chronic sinusitis is less straightforward because only some chronic sinusitis cases have an infectious basis. Chronic rhinosinusitis (CRS) has been subdivided into 3 types, namely CRS without nasal polyps, CRS with nasal polyps, and allergic fungal rhinosinusitis. Depending on the type of CRS present, a variety of medical and surgical approaches might be required. (J Allergy Clin Immunol 2010;125:S103-15.)

Key words: *Rhinitis, sinusitis, rhinosinusitis, allergic, fungal sinusitis, nasal polyposis*

Rhinitis and sinusitis are among the most common medical conditions and are frequently associated.[1-4] An estimated 10% to 25% of the population in Western societies has allergic rhinitis.[1,2]

Abbreviations used

ABRS:	Acute bacterial rhinosinusitis
AERD:	Aspirin-exacerbated respiratory disease
AFRS:	Allergic fungal rhinosinusitis
AR:	Allergic rhinitis
CRS:	Chronic rhinosinusitis
CRScNP:	Chronic rhinosinusitis with nasal polyposis
CRSsNP:	Chronic rhinosinusitis without nasal polyposis
CT:	Computed tomography
FDA:	US Food and Drug Administration
FESS:	Functional endoscopic sinus surgery
INS:	Intranasal corticosteroids
LTRA:	Leukotriene receptor antagonist
NARES:	Nonallergic rhinitis with eosinophilia syndrome
PAR:	Perennial allergic rhinitis
PRN:	As required
SAR:	Seasonal allergic rhinitis
URI:	Upper respiratory tract infection

Sinusitis affects an estimated 31 million persons annually in the United States.[3] Both rhinitis and sinusitis can significantly decrease quality of life, aggravate comorbid conditions, and require significant direct medical expenditures. Both conditions also create even greater indirect costs to society by causing lost work and school days, as well as reduced workplace productivity and school learning.

For the purposes of this review, *sinusitis* and *rhinosinusitis* are synonymous terms.

RHINITIS

Background

Although semantically, the term rhinitis implies inflammation of the nasal mucous membranes, inflammatory cell infiltrates are not characteristic of all disorders considered to be rhinitis. As a clinical term, rhinitis refers to a heterogeneous group of nasal disorders characterized by 1 or more of the following symptoms: sneezing, nasal itching, rhinorrhea, and nasal congestion.[1] Rhinitis can be caused by allergic, nonallergic, infectious, hormonal, occupational, and other factors.[1,2] Allergic rhinitis is the most common type of chronic rhinitis, but 30% to 50% of patients with rhinitis have nonallergic triggers. Preliminary data suggest that 44% to 87% of patients with rhinitis might have mixed rhinitis, a combination of allergic and nonallergic rhinitis.[1,2,5] Worldwide, the prevalence of allergic rhinitis continues to increase. Studies suggest that seasonal allergic rhinitis (hay fever) is found in approximately 10% to 20% of the general population,[1,2] with an even greater prevalence in children. Overall, allergic rhinitis affects 30 to 60 million subjects in the United States annually.[1,6] Severe allergic rhinitis has been associated with diminished quality of life, disordered sleep (in as many as 76% of patients), obstructive sleep apnea, and impairment in work performance.[1,2] In addition, rhinitis can contribute to sinusitis (see the section below on Sinusitis, comorbidities, and allergic rhinitis) and is frequently associated with asthma.

From [a]Allergy and Immunology Unit, Section of Pulmonary, Critical Care Allergy and Immunologic Diseases, Department of Internal Medicine, Center for Human Genomics and Personalized Medicine Research, Wake Forest University School of Medicine, Winston-Salem, and [b]the Division of Rheumatology, Allergy & Immunology, Massachusetts General Hospital/Harvard Medical School, Boston.

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Reprint requests: Mark S. Dykewicz, MD, Center for Human Genomics and Personalized Medicine Research, Wake Forest University School of Medicine, Medical Center Blvd, Winston-Salem, NC 27157. E-mail: dykewicz@wfubmc.edu.

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Pathogenesis

Nasal anatomy and physiology. The nasal cavity (Fig 1) is divided by the nasal septum, which is composed of bone more proximally and cartilage more distally. The inferior, middle, and superior turbinates in the nasal cavity promote air filtration, humidification, and temperature regulation. The nasal cavity and turbinates are lined with mucosa comprised of pseudostratified columnar ciliated epithelium that overlies a basement membrane and the submucosa (lamina propria). The submucosa consists of serous and seromucous nasal glands, nerves, extensive vasculature, and cellular elements. Overlying the nasal epithelium is a thin layer of mucus that dynamically moves by means of ciliary action to the posterior nasopharynx. Infections (viral or bacterial) and allergic inflammation impair mucociliary clearance. Because nasal tissues are highly vascular, vascular changes can lead to significant nasal obstruction. Vasoconstriction and consequent decreases in nasal airway resistance result from sympathetic nerve stimulation. Parasympathetic nerve stimulation promotes secretion from nasal airway glands and nasal congestion. The nasal mucosa also contains nerves of the nonadrenergic noncholinergic system. Neuropeptides from the latter nerves (substance P, neurokinin A and K, and calcitonin gene–related peptide) are thought to play some role in vasodilatation, mucus secretion, plasma extravasation, neurogenic inflammation, and mast cell nerve interactions, but the relative clinical importance of neuropeptides needs further definition.[7]

Allergic rhinitis

Pathophysiology. Common allergens causing allergic rhinitis include proteins and glycoproteins in airborne dust mite fecal particles, cockroach residues, animal danders, molds, and pollens. On inhalation, allergen particles are deposited in nasal mucus, with subsequent elution of allergenic proteins and diffusion into nasal tissues. In addition, small-molecular-weight chemicals in occupational agents or drugs can act as haptens that react with self-proteins in the airway to form complete allergens. Evidence extrapolated from asthma studies suggests that once in nasal tissues, common aeroallergens not only undergo antigen processing to elicit allergen-specific allergic responses but also promote development of allergic airway disease through their inherent properties. For example, protease activities of several common aeroallergens can facilitate allergen access to antigen-presenting cells by cleaving tight junctions in the airway epithelium and activation of protease-activated receptors on epithelial cells.[8] Activated epithelial cells then produce cytokines, chemokines, and thymic stromal lymphopoietin, which interact with interepithelial and subepithelial dendritic cells to skew T-cell development and adaptive allergic sensitization. The house dust mite allergen Der p 2 mimics MD-2, the LPS-binding component of the Toll-like receptor 4 signaling complex,[2] and facilitates Toll-like receptor 4 signaling and airway T_H2-type inflammation.[9]

In the nose allergens are processed by antigen- presenting cells (dendritic cells expressing CD1a and CD11c and macrophages) in the nasal epithelial mucosa, with subsequent presentation of allergenic peptides by MHC class II molecules to T-cell receptors on resting $CD4^+$ T lymphocytes in regional lymph nodes. With costimulatory signals, allergen-stimulated T cells proliferate into T_H2-biased cells that release IL-3, IL-4, IL-5, IL-13, and other cytokines. These cytokines then lead to a cascade of events that promote B-cell isotype switching with subsequent local and systemic production of allergen-specific IgE antibody production by plasma cells, eosinophilic infiltration into the nasal epithelium and mucosa, and mast cell proliferation and infiltration of airway mucosa.

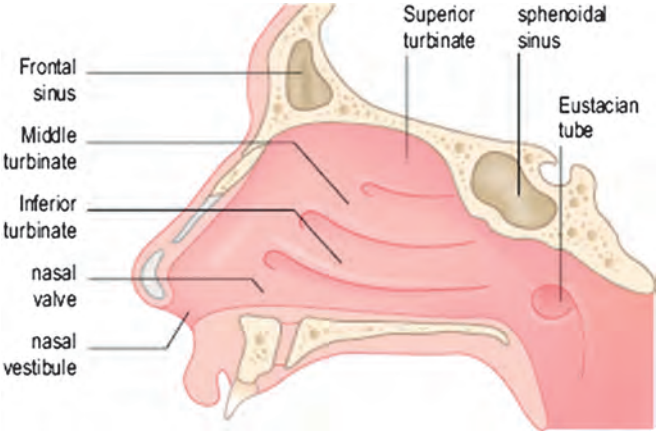

FIG 1. Nasal anatomy. Reprinted with permission from Dykewicz MS. Rhinitis and sinusitis. In: Rich RR, Fleischer TA, Shearer WT, Schroeder HW Jr, Frew AJ, Weyand CM, editors. *Clinical Immunology*. 3rd ed. London: Mosby Elsevier; 2008. p. 626-39.

Early/immediate allergic response. Within minutes of inhalation of allergen in sensitized subjects, deposited allergens are recognized by IgE antibody bound to mast cells and basophils, causing degranulation and release of preformed mediators, such as histamine and tryptase, and the rapid *de novo* generation of mediators, including cysteinyl leukotrienes (leukotrienes C_4, D_4, and E_4) and prostaglandin D_2. Mediators cause plasma leakage from blood vessels and dilation of arteriovenous arteriole venule anastomoses, with consequent edema, pooling of blood in the cavernous sinusoids (the principal cause of the congestion of allergic rhinitis), and occlusion of the nasal passages. Mediators also stimulate active secretion of mucus from glandular and goblet cells. Histamine elicits itching, rhinorrhea, and sneezing, whereas other mediators, such as leukotrienes and prostaglandin D_2, likely have more important roles in the development of nasal congestion. Stimulation of sensory nerves results in the perception of nasal congestion and itching and can provoke systemic reflexes, such as sneezing paroxysms.[1,10]

Late-phase response. Mediators and cytokines released during the early phase set off a cascade of events over the ensuing 4 to 8 hours that lead to an inflammatory response called the late response. Although clinical symptoms during the late phase might be clinically similar to those of the immediate reaction, nasal congestion is more prominent. The cysteinyl leukotrienes also play an active role in recruitment of inflammatory cells. Mediators and cytokines released during the early response act on postcapillary endothelial cells to promote expression of adhesion molecules, such as intercellular adhesion molecule 1, E-selectin, and vascular cell adhesion molecule 1, that promote adherence of circulating leukocytes, such as eosinophils, to endothelial cells. Factors with chemoattractant properties, such as IL-5 for eosinophils, promote the infiltration of the superficial lamina propria of the mucosa with many eosinophils, some neutrophils and basophils, and eventually $CD4^+$ (T_H2) lymphocytes and macrophages.[1] These cells become activated and release more mediators, which in turn activate many of the proinflammatory reactions seen in the immediate response.

Priming effect. The amount of allergen necessary to elicit an immediate response becomes less when allergen challenges are given repeatedly, a phenomenon called the priming effect.[1,11] During ongoing, prolonged allergen exposure and repeated late-phase/inflammatory responses, the nasal mucosa becomes progressively more inflamed and responsive to allergen. Clinically, the priming effect can explain why patients might have increasing symptoms despite decreasing aeroallergen levels as a season progresses and also provides the rationale for initiating effective anti-inflammatory rhinitis therapies before a pollen season or before other chronic or repetitive aeroallergen exposures. In addition, the priming effect from allergen is also associated with mucosal hyperresponsiveness to nonantigenic triggers, such as strong odors and cigarette smoke.

Associated nonnasal symptoms. Allergic rhinitis is often accompanied by allergic conjunctivitis (a complex sometimes referred to as allergic rhinoconjunctivitis) that results in conjunctival injection and chemosis and symptoms of itchy eyes and tearing.[1] The prevalence and severity of conjunctival symptoms associated with allergic rhinitis vary with several factors, but one study found allergic conjunctivitis symptoms in more than 75% of patients with seasonal allergic rhinitis.[12] Sensitivity to pollens is more frequently associated with ocular symptoms than is sensitivity to house dust mites. Itching of the ears and throat can also be associated with allergic rhinitis.

Association with asthma. Allergic asthma and rhinitis are comorbid conditions that are associated pathophysiologically and epidemiologically.[1,2] Both are airway diseases in which IgE antibody sensitization to aeroallergens is a prominent feature. There is some evidence that systemic trafficking of inflammatory cells from local inflammation in one portion of the respiratory tract can induce inflammatory changes in the other, with one example being that segmental bronchial allergen challenge in patients with allergic rhinitis has been shown to result in both bronchial and nasal inflammatory responses.[13] Treatment with intranasal corticosteroids in patients with allergic asthma and rhinitis has been shown to prevent the seasonal increase in bronchial hyperreactivity and to reduce existing bronchial hyperreactivity.[1,14,15] More than 80% of persons with allergic asthma have allergic rhinitis, and allergic rhinitis is a clear risk factor for the eventual development of asthma.[1,2] Guidelines recommend that patients with persistent allergic rhinitis should be evaluated for asthma and patients with asthma should be evaluated for rhinitis.[1,2]

Differential diagnosis, including forms of nonallergic rhinitis. Some of the classic symptoms of allergic rhinitis (rhinorrhea, nasal congestion, sneezing, and nasal itching) overlap with symptoms associated with forms of nonallergic rhinitis (Table I) and various anatomic abnormalities of the upper airway (Table II), sometimes making it difficult to distinguish between these disorders on the basis of history alone.

Nonallergic rhinitis without eosinophilia. Sometimes termed *idiopathic rhinitis*,[2] this manifests as chronic nasal symptoms not caused by allergic or infectious processes. Symptoms are nasal obstruction, increased secretions, or both, with sneezing and pruritus being less common. This clinical presentation is likely caused by a heterogeneous group of disorders with a pathogenesis that is incompletely understood. *Vasomotor rhinitis* is a term that is sometimes used synonymously with the term *nonallergic rhinitis without eosinophilia* but sometimes can more specifically connote nasal symptoms that occur in response to environmental conditions, such as changes in temperature or relative humidity, odors (eg, perfumes or cleaning materials), passive tobacco smoke, alcohol, sexual arousal, and emotional factors. Such hyperreactivity to nonallergic triggers is not mediated by increased neural efferent traffic to the blood vessels supplying the nasal mucosa and can also occur in allergic rhinitis, when the term *mixed rhinitis* is applied.[1,2]

TABLE I. Types of rhinitis

I. Allergic rhinitis
II. Nonallergic rhinitis
A. Vasomotor rhinitis
1. Irritant triggered (eg, chlorine)
2. Cold air
3. Exercise (eg, running)
4. Undetermined or poorly defined triggers
B. Gustatory rhinitis
C. Infectious
D. NARES
III. Occupational rhinitis
A. Caused by protein and chemical allergens; IgE mediated
B. Caused by chemical respiratory sensitizers; immune mechanism uncertain
C. Work-aggravated rhinitis
IV. Other rhinitis syndromes
A. Hormonally induced
1. Pregnancy rhinitis
2. Menstrual cycle related
B. Drug induced
1. Rhinitis medicamentosa
2. Oral contraceptives
3. Antihypertensive and cardiovascular agents
4. Aspirin/NSAIDs
5. Other drugs
C. Atrophic rhinitis
D. Rhinitis associated with inflammatory-immunologic disorders
1. Granulomatous infections
2. Wegener granulomatosis
3. Sarcoidosis
4. Midline granuloma
5. Churg-Strauss syndrome
6. Relapsing polychondritis
7. Amyloidosis

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NSAIDs, Nonsteroidal anti-inflammatory drugs.

Nonallergic rhinitis with eosinophilia syndrome. Nonallergic rhinitis with eosinophilia syndrome (NARES) is characterized by perennial nasal symptoms (particularly nasal congestion), sneezing paroxysms, profuse watery rhinorrhea, nasal pruritus, and occasional loss of smell.[1,2] Nasal smears demonstrate eosinophils (inconsistently defined as >5% to >20%),[16,17] as in allergic rhinitis, but patients lack evidence of allergic disease based on skin testing or serum levels of IgE to environmental allergens. However, similar to histologic findings in patients with allergic rhinitis, mast cells with bound IgE and increased tryptase levels have been found in nasal mucosal biopsy specimens of patients with NARES. Patients are typically middle-aged adults. The prevalence of NARES in the general population is uncertain, but NARES occurs extremely infrequently in childhood and probably accounts for less than 2% of children with nasal eosinophilia.[18] It has been proposed that the syndrome might be an early stage of nasal polyposis and aspirin

TABLE II. Differential diagnosis of rhinitis: Conditions that might mimic symptoms of rhinitis

A. Nasal polyps
B. Structural/mechanical factors
1. Deviated septum/septal wall anomalies
2. Adenoidal hypertrophy
3. Trauma
4. Foreign bodies
5. Nasal tumors
a. Benign
b. Malignant
6. Choanal atresia
7. Cleft palate
8. Pharyngonasal reflux
9. Acromegaly (excess growth hormone)
C. Cerebrospinal fluid rhinorrhea
D. Ciliary dyskinesia syndrome

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sensitivity.[19] Patients with NARES are at risk for obstructive sleep apnea.[20]

Hormonal rhinitis and rhinitis of pregnancy. Rhinitis can be caused by hormonal changes of pregnancy or puberty, the use of oral contraceptives or conjugated estrogens, or thyroid disorders. In *pregnancy rhinitis de novo* nasal congestion develops during pregnancy proposed to occur from hormone-induced nasal vascular pooling resulting from vasodilation and increased blood volume. Symptoms usually disappear within 2 weeks after delivery. However, pre-existing rhinitis is a more common cause of nasal symptoms in pregnant women, with approximately one third of woman with allergic rhinitis having worsened symptoms during pregnancy.[21]

Drug-induced rhinitis. Rhinitis can be caused by either oral or topical medications. Causal oral medications include angiotensin-converting enzyme inhibitors (which can cause nasal symptoms in the absence of the more common adverse effect of cough), β-blockers, various antihypertensive agents, aspirin, other nonsteroidal anti-inflammatory drugs, and oral contraceptives.[1,2] Use of topical α-adrenergic decongestant sprays for more than 5 to 7 days can induce rebound nasal congestion on withdrawal and reduced mucociliary clearance because of loss of ciliated epithelial cells (rhinitis medicamentosa).[22] Repeated use of intranasal cocaine and methamphetamine can also result in rebound congestion and, on occasion, septal erosion and perforation.

Food-induced rhinitis. Ingested food allergens rarely cause isolated rhinitis on an IgE-mediated basis without involvement of other organ systems.[1,2] Ethanol in beer, wine, and other alcoholic drinks can produce symptoms that have been proposed to occur because of pharmacologic nasal vasodilation. Gustatory rhinitis is a cholinergically mediated syndrome of watery rhinorrhea occurring immediately after ingestion of foods, particularly hot and spicy foods.[23] It can occur as a distinct entity or accompany other types of rhinitis.

Atrophic rhinitis. Primary atrophic rhinitis is a chronic condition characterized by progressive atrophy of the nasal mucosa, resorption of underlying bone and turbinates, nasal dryness, and foul-smelling nasal crusts associated with a constant bad smell (ozena).[24,25] Often associated with sinusitis, it occurs more commonly in young to middle-aged adults and is more prevalent in developing countries with warm climates. The nasal cavities appear abnormally wide on examination, and squamous metaplasia, atrophy of glandular cells, and loss of pseudostratified epithelium are found in nasal biopsy specimens. The dryness and reduction of nasal mucosal tissue with the resultant decreased resistance to airflow is, paradoxically, perceived by patients as severe nasal congestion. An infectious basis has been proposed. Secondary atrophic rhinitis can be less severe and progressive than primary atrophic rhinitis and develops as a direct result of other primary conditions, such as chronic granulomatous nasal infections, chronic sinusitis, excessive nasal surgery, trauma, and irradiation.

Infectious rhinosinusitis. Acute viral upper respiratory tract infection (URI) presents with nasal symptoms and constitutional symptoms (fever, myalgias, and malaise). Pruritus is typically absent, and symptoms resolve within 7 to 10 days. Acute and chronic bacterial sinusitis can be difficult to distinguish from rhinitis on the basis of history (see the section on infectious rhinosinusitis).

Differential considerations other than rhinosinusitis

For more information on differential considerations other than rhinosinusitis, see Table II. Anatomic abnormalities usually present with prominent obstructive symptoms with less prominent symptoms of rhinorrhea. Septal deviation can cause symptoms of unilateral or bilateral congestion or recurrent sinusitis, although more often it is asymptomatic. Septal deviations can often be diagnosed by seeing the external deviation of the nose or by looking anteriorly with a nasal speculum. Diagnosis might require fiberoptic rhinopharyngoscopy or computed tomographic (CT) scanning. Nasal polyps are benign inflammatory growths that arise from the inflamed mucosa lining the paranasal sinuses. They can cause invariant unilateral or bilateral nasal obstruction and loss of smell or rhinorrhea (see the section on CRS with nasal polyposis [CRScNP]). Polyps are infrequent in children, except for those with cystic fibrosis, in whom polyps with neutrophilic infiltrates are characteristic,[26] in contrast to eosinophilic infiltrates typical of most nasal polyps. Unilateral nasal polyps should raise consideration of a possible neoplasm.

Other differential considerations for nasal symptoms include nasal tumors that can be benign or malignant. The most common presentation of tumors is obstruction. Juvenile angiofibromas often present with bleeding in adolescent males. Nasal carcinoma can present with unilateral epistaxis and nasal pain. Young children might place intranasal foreign bodies in their noses (eg, small parts of toys), leading to foul-smelling, purulent discharge and unilateral nasal obstruction that predisposes to sinusitis. Adenoidal hypertrophy in young children causes bilateral nasal obstruction and is often associated with nocturnal mouth breathing and snoring. Wegener granulomatosis can present with nasal and sinus complaints, including purulent rhinorrhea and occasionally septal erosions and perforations. Sjögren syndrome can cause nasal dryness, congestion, and crusting. Sarcoidosis can present with nasal congestion.

Diagnosis. Full evaluation of a patient with rhinitis should include assessment of specific symptoms bothersome to the patient (eg, nasal congestion, pruritus, rhinorrhea, and sneezing), the pattern of symptoms (eg, infrequent/intermittent, seasonal, and perennial) that might affect therapeutic choices, identification of precipitating factors that might be avoided, previous response to medications, coexisting conditions, and a detailed environmental history, including home and occupational exposures.[1,2] Nasal itching is more suggestive of allergic rhinitis than nonallergic rhinitis. Because allergic rhinitis is frequently associated with

allergic conjunctivitis, the presence of eye pruritus and lacrimation is a helpful indication that a patient's rhinitis has an allergic basis. Pollens are generally associated with seasonal allergic rhinitis. In most regions of the United States, trees pollinate in the spring, grasses in the late spring and early summer, and weeds in the late summer and fall. However, in some regions (eg, portions of California) pollens can cause perennial symptoms. Perennial allergens, such as house dust mites, cockroaches, and animals, cause symptoms that vary little between seasons, making it difficult to accurately distinguish between allergic and nonallergic rhinitis on the basis of history alone. Family history is an important clue in making the diagnosis of allergic rhinitis in children. A handheld otoscope or headlamp with nasal speculum permits viewing of the anterior third of the nasal airway, including the anterior tip of the inferior turbinates (and occasionally the anterior tip of the middle turbinates) and portions of the nasal septum. Treatment with a topical decongestant improves visualization of the nasal cavity. However, some nasal polyps, septal deviation, and masses can be missed because of the inability to visualize the posterior and superior nasal airways. Typically, patients with allergic rhinitis have clear discharge, swollen turbinates, and bluish or pale mucosa. Pale or erythematous mucosa can be seen in various types of nonallergic rhinitis. Both allergic and nonallergic rhinitis can cause *allergic shiners*, infraorbital darkening thought to be caused by chronic venous pooling, or an *allergic salute* in children who rub their noses upward because of nasal discomfort, sometimes producing a persistent horizontal crease across the nose. In association with rhinitis, physical findings of bilateral conjunctivitis (mild injection with nonpurulent discharge) are suggestive of allergy. Patients with nasal disease require appropriate examination for associated diseases, such as sinusitis and otitis media.

Determination of specific IgE antibodies to known allergens by means of skin testing or *in vitro* tests is indicated to provide evidence of an allergic basis for the patient's symptoms, to confirm or exclude suspected causes of the patient's symptoms, or to assess the sensitivity to a specific allergen for avoidance measures, allergen immunotherapy, or both.[1,2] Skin testing is preferred for its simplicity, ease, and rapidity of performance; low cost; and high sensitivity.[1] In patients with perennial rhinitis, history is usually insufficient for distinguishing allergic from nonallergic rhinitis, and testing is of added importance. Neither total serum IgE levels nor total circulating eosinophil counts are routinely indicated in the diagnosis of rhinitis because they are neither sensitive nor specific for allergic rhinitis.[1]

Nasal cytology might aid in differentiating allergic rhinitis and NARES from other forms of rhinitis, such as vasomotor or infectious rhinitis, if the correct procedure is followed and the appropriate stains are used. However, there is lack of expert consensus about whether nasal cytology should be routinely used in the diagnosis of rhinitis.[1] In selected cases special techniques, such as fiberoptic nasal endoscopy, inspiratory peak flow measurements, acoustic rhinometry, or rhinomanometry, to assess airway function might be useful in evaluating patients presenting with rhinitis symptoms.

Treatment. *Avoidance measures.* Avoidance of inciting factors, such as allergens (house dust mites, molds, pets, pollens, and cockroaches), irritants, and medications, can effectively reduce symptoms of rhinitis. In particular, patients allergic to house dust mites should use allergen-impermeable encasings on the bed and all pillows. Pollen exposure can be reduced by keeping windows closed, using an air conditioner, and limiting the amount of time spent outdoors.

Medications. Selection of medications should be individualized based on multiple considerations, including patient preference (eg, intranasal vs oral), individual response (which can differ from average responses in the general population), and cost.[1] Some medications are more effective for treating certain types of rhinitis (eg, allergic vs nonallergic), more severe symptoms, or particular rhinitis symptoms that are more bothersome to a patient (eg, nasal congestion).[1,2] Medications also differ in onset of action, with those having more rapid symptom relief better suited to treating episodic rhinitis (defined by the Joint Task Force as allergic nasal symptoms elicited by sporadic exposures to inhalant aeroallergens that are not usually encountered in the patient's indoor or outdoor environment)[1] or intermittent symptoms (defined by Allergic Rhinitis and Its Impact on Asthma guidelines as present <4 days per week or <4 weeks duration).[2]Table III reviews principal medication options for rhinitis (both monotherapy and combination regimens), listing therapeutic considerations for treatment of allergic rhinitis and then for nonallergic rhinitis.

Allergen immunotherapy/allergy vaccination. Subcutaneous allergen immunotherapy can be highly effective in controlling symptoms of allergic rhinitis and favorably modifies the long-term course of the disease.[27] Sublingual immunotherapy with single allergens, although part of clinical practice for the treatment of rhinitis in Europe, is undergoing clinical trials in the United States and is not approved by the US Food and Drug Administration (FDA) at the time of this manuscript's submission. Patients with allergic rhinitis should be considered candidates for immunotherapy on the basis of the severity of their symptoms, failure or unacceptability of other treatment modalities, presence of comorbid conditions, and possibly as a means of preventing worsening of the condition or the development of comorbid conditions (eg, asthma and sinusitis).[1,27] Approximately 80% of patients will experience symptomatic improvement after 1 to 2 years of subcutaneous immunotherapy, and guidelines recommend that treatment be continued for a total of 4 to 5 years.[27] In many patients the beneficial effects persist for years after injections are stopped. Allergen immunotherapy for allergic rhinitis can reduce the development of asthma in children and possibly in adults.[1,27,28]

Considerations in select populations. *Children.* Because some, although not all, nasal corticosteroid preparations have been reported to reduce linear growth (at least temporarily),[29-31] growth should be monitored in children receiving these agents.

Elderly. Allergy is an uncommon cause of perennial rhinitis in individuals older than 65 years. More commonly, rhinitis in the elderly is due to cholinergic hyperreactivity (associated with profuse watery rhinorrhea, which might be aggravated after eating [ie, gustatory rhinitis]), α-adrenergic hyperactivity (congestion associated with antihypertensive drug therapy), or sinusitis.[1] Because the elderly might have increased susceptibility to the adverse central nervous system and anticholinergic effects of antihistamines, nonsedating agents are recommended if antihistamines are used for allergic rhinitis. Oral decongestants should be used with caution in this patient subset because of their effects on the central nervous system, heart, and bladder function.

Pregnancy. The time for greatest concern about potential congenital malformation caused by medication use is the first trimester, when organogenesis occurs.[1,32,33] When selecting medications for treating rhinitis in pregnancy, the clinician might

TABLE III. Principal medication options for rhinitis (listed in alphabetical order)

For AR, both seasonal and perennial

	Therapeutic considerations
Monotherapy	
Oral agents	
Antihistamines, oral (H1 receptor antagonists)	● Continuous use is most effective for SAR and PAR but appropriate for PRN use in episodic or intermittent AR because of relatively rapid onset of action. ● Less effective for nasal congestion than for other nasal symptoms ● Less effective for AR than INSs, with similar effectiveness to INSs for associated ocular symptoms ● Because they are generally ineffective for non-AR, other choices are typically better for mixed rhinitis. ● To avoid sedation (often subjectively unperceived), performance impairment, or anticholinergic effects of first-generation antihistamines, second-generation agents are generally preferred. ● Of these, fexofenadine, loratadine, and desloratadine without sedation at recommended doses
Corticosteroids, oral	● A short course (5-7 days) might be appropriate for very severe nasal symptoms. ● Preferred to single or recurrent administration of intramuscular corticosteroids
Decongestants, oral	● Pseudoephedrine reduces nasal congestion. ● Side effects include insomnia, irritability, palpitations, and hypertension.
Leukotriene receptor antagonists (LTRAs)	● Montelukast is approved for SAR and PAR. ● The efficacies of LTRAs and oral antihistamines are similar (with loratadine as the usual comparator). ● Because approved for both rhinitis and asthma, can be considered when both conditions are present. ● Side effects are minimal.
Intranasal agents	
Intranasal antihistamines	● Effectiveness for AR is equal or superior to that of oral second-generation antihistamines with a clinically significant effect on nasal congestion. ● Generally less effective than INSs for nasal symptoms ● Clinically significant rapid onset of action (within several hours or less), making them appropriate for PRN use in patients with episodic AR ● Because azelastine nasal spray is approved for vasomotor rhinitis, appropriate choice for mixed rhinitis ● Side effects with intranasal azelastine are bitter taste and somnolence.
Intranasal anticholinergic (ipratropium)	● Reduces rhinorrhea but not other symptoms of AR. ● Appropriate for episodic AR because of rapid onset of action ● Side effects are minimal, but nasal dryness can occur.
Intranasal corticosteroids (INSs)	● Most effective monotherapy for AR ● Effective for all symptoms of SAR and PAR, including nasal congestion ● The usual onset of action is less rapid than that of oral or intranasal antihistamines, usually occurs within 12 hours, and can start as early as 3-4 hours in some patients. ● Might be considered for episodic AR ● PRN use (eg, >50% days use) is effective for SAR. ● More effective than the combination of an oral antihistamine and LTRA for SAR and PAR ● Similar effectiveness to oral antihistamines for associated ocular symptoms of AR ● Appropriate choice for mixed rhinitis because agents in this class are also effective for some cases of non-AR ● Without significant systemic side effects in adults ● Growth suppression in children with PAR has not been demonstrated when used at recommended doses. ● Local side effects are minimal, but nasal bleeding can occur, as well as rare nasal septal perforation.
Intranasal cromolyn	● Used for maintenance treatment of AR; onset of action within 4-7 days; full benefit can take weeks. ● For episodic rhinitis, administration just before allergen exposure protects for 4-8 hours against allergic response. ● Less effective than nasal corticosteroids, and there are inadequate data for comparison with leukotriene antagonists and antihistamines. ● Minimal side effects
Intranasal decongestants	● Useful for the short-term and possibly for episodic therapy of nasal congestion but inappropriate for daily use because of risk for rhinitis medicamentosa

(Continued)

TABLE III. *(Continued)*

	Therapeutic considerations
Combination therapy	
Antihistamine, oral with decongestant, oral	● Provides more effective relief of nasal congestion than antihistamines alone
Antihistamine, oral with LTRA, oral	● Might be more effective than monotherapy with an antihistamine or LTRA ● Combination is less effective than INSs. ● Alternative if patients are unresponsive to or not compliant with INSs
Antihistamine, oral with intranasal antihistamine	● Combination can be considered, although controlled studies of additive benefit are lacking.
Antihistamine, oral with INS	● Combination can be considered, although supporting studies are limited, and many studies are unsupportive of the additive benefit of adding an antihistamine to an intranasal steroid.
Intranasal anticholinergic with INS	● Concomitant ipratropium bromide nasal spray with INS is more effective for rhinorrhea than administration of either drug alone.
Intranasal antihistamine with INS	● Combination can be considered based on limited data indicating additive benefit. ● There are inadequate data about the optimal interval between administration of the 2 sprays. ● For mixed rhinitis, there is a possible added benefit to combination of intranasal antihistamine with INS.
LTRA, oral with INS	● Provides subjective additive relief in limited studies; data are inadequate.

For nonallergic (idiopathic) rhinitis

	Therapeutic considerations (for side effects, see AR table)
Monotherapy	
Oral agents	
Antihistamines, oral (H1 receptor antagonists)	● Generally ineffective for non-AR
Decongestants, oral	● Pseudoephedrine reduces nasal congestion.
Intranasal agents	
Intranasal antihistamines	● Effective for vasomotor rhinitis
Intranasal anticholinergic (ipratropium)	● Effective only for rhinorrhea of non-AR syndromes ● Special role for preventing rhinorrhea of gustatory rhinitis
INSs	● Effective for some forms of non-AR, including vasomotor rhinitis and NARES
Combination therapy	● There are inadequate data to provide firm recommendations in non-AR.

Adapted from Wallace et al.[1]
AR, Allergic rhinitis; *INS*, intranasal corticosteroids; *LTRA*, leukotriene receptor antagonist; *PAR*, perennial allergic rhinitis; *PRN*, as required; *SAR*, seasonal allergic rhinitis.

consider the FDA risk categories (category B being more favorable than category C) that are based largely on animal data and limited human studies.[1] However, it is also suggested that a clinician consider human cohort and case-control studies, as well as birth registry data.[1] Nasal cromolyn has the most reassuring safety profile in pregnancy. Cetirizine, chlorpheniramine, loratadine, and tripelennamine have been rated FDA pregnancy category B, whereas many other antihistamines have a category C rating. Intranasal budesonide has a category B rating, whereas other nasal corticosteroids are rated category C. Oral decongestants are best avoided in the first trimester because of the risk of gastroschisis in the newborn.[34] Allergen immunotherapy should not be started or advanced in dose during pregnancy but might be continued at a stable dose.

SINUSITIS (RHINOSINUSITIS)

Sinus anatomy and physiology

Normal sinus function requires (1) patency of each sinus ostia, (2) normal mucociliary function, and (3) normal systemic and local immune function. Epithelial cilia in the sinuses normally beat mucus in an ordered fashion toward the ostia that communicate with the nasal cavity. The maxillary, anterior ethmoid, and frontal sinuses drain through a comparatively narrow drainage pathway, the ostiomeatal unit (complex), which communicates into the middle meatus, a space between the inferior and middle turbinates (Fig 2). In 50% of cases, the frontal sinus drains just anterior to this region. The posterior ethmoid and sphenoid sinuses drain through the sphenoethmoidal recess. Sinus ostial obstruction is common in patients with acute rhinosinusitis and CRS. Mucociliary function is grossly abnormal in diseases, such as cystic fibrosis, or in ciliary dysmotility syndrome (Kartagener syndrome). Mucociliary function might be impaired by cigarette smoke, environmental pollutants, or viral URIs.[35] Systemic immune function is impaired by hypogammaglobulinemia, severe T-cell dysfunction, or immune suppression. It has been suggested but not proved that defects in local innate immune function might predispose to sinus infections. Local innate function involves (1) pathogen recognition and signaling through epithelial Toll-like and other innate receptors and (2) secretion of collectins and antimicrobial peptides.[36] Defects in either pathway remain largely unstudied in sinusitis.

Rationale for rhinosinusitis rather than sinusitis

The symptoms of rhinitis and sinusitis overlap, and sinusitis rarely occurs in the absence of rhinitis.[4] Second, there is an important interrelationship between the middle turbinate and the ethmoid sinus such that cyclic variations in nasal turbinate swelling occurring during the normal nasal cycle can cause mucosal thickening in the ethmoidal infundibulum. This thickening might be interpreted as ethmoid sinusitis.[37] The ethmoid infundibulum and the nose represent contiguous structures sharing vascular, neuronal, and interconnecting anatomic pathways. For these reasons, some expert panels have adopted the term

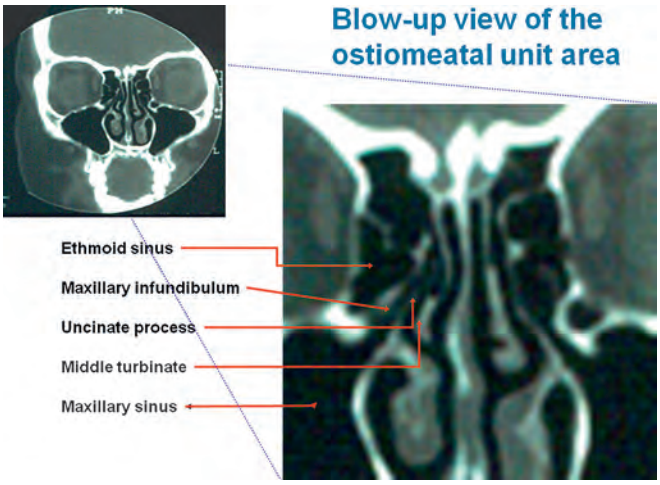

FIG 2. The ostiomeatal unit is well visualized on this blow-up of a normal sinus CT scan. The major structures illustrated include the maxillary infundibulum, the ethmoid infundibulum, the uncinate process, and the middle turbinate. The ostiomeatal unit is a 3-dimensional structure made up of these individual components.

rhinosinusitis rather than sinusitis, emphasizing that sinusitis typically involves the nasal passages and the paranasal sinuses.[4,38] For the purposes of this review, sinusitis and rhinosinusitis are synonymous terms.

Infectious rhinosinusitis

Viral rhinosinusitis is defined as acute rhinosinusitis caused by viral infection. Viral rhinosinusitis is often difficult to distinguish from acute bacterial rhinosinusitis (ABRS) and can be accompanied by inflammatory changes in the sinuses.[39] ABRS, by definition, is caused by a bacterial pathogen. CRS is an inflammatory condition in which infection plays an important role. Each of these entities is discussed further below.

The transition from viral rhinosinusitis to ABRS

The principal inciting event for ABRS is viral rhinosinusitis (also known as a viral URI). The transition from viral rhinosinusitis to ABRS is variable and only occurs in 0.5% to 2% of cases.[39] Viral rhinosinusitis is typically accompanied by clear rather than thick or colored secretions. Symptoms can persist up to 14 days or longer. An acute upper respiratory illness of less than approximately 7 days' duration is most commonly caused by viral illness (viral rhinosinusitis), whereas acute bacterial sinusitis becomes more likely beyond 7 to 10 days.[3,4] Transition from viral URI to ABRS can occur at any time during the viral URI.[40]

Acute and chronic rhinosinusitis: Definitions and symptoms

Rhinosinusitis is defined as inflammation of the nose and paranasal sinuses. Acute rhinosinusitis is usually infectious, whereas CRS is less clearly infectious and often more inflammatory.[4] However, infection still plays an important role in CRS. *Acute rhinosinusitis* is defined as up to 4 weeks of purulent (not clear) nasal drainage (anterior, posterior, or both) accompanied by nasal obstruction, facial pain-pressure-fullness, or both. *Subacute rhinosinusitis* is defined in some expert reports[3] as rhinosinusitis of between 4 and 8 weeks' duration. CRS is defined as an inflammatory condition involving the paranasal sinuses and nasal passages with a minimum duration of either 8 or 12 weeks despite attempts at medical management.[3,4]

The 4 major symptoms of CRS are (1) anterior, posterior, or both mucopurulent drainage; (2) nasal obstruction or blockage; (3) facial pain, pressure, and/or fullness; and (4) decreased sense of smell. Two or more symptoms must be present to make the diagnosis.[4,41] In addition, objective documentation of mucosal inflammation is required.

The symptoms of CRS do not reliably correlate with specific objective findings nor do they accurately differentiate CRS subtypes (see below).

Facial pain, pressure, and/or headache are commonly reported symptoms (83% in one series).[42] The pain is usually described as a dull pain or pressure in the upper cheeks, between the eyes, or in the forehead. Sharp localizing pain is less common. Anterior, posterior, or both nasal drainage of CRS is usually opaque white or light yellow. Thick yellow, green, or brown mucus can occur, although this is more characteristic of recurrent acute rhinosinusitis or AFRS. Nasal congestion can be described as nasal blockage or stuffiness or less commonly as nasal fullness. Disturbance in sense of smell can be partial (hyposmia) or complete (anosmia) and is usually associated with mucosal thickening or opacification in the anterior ethmoid sinuses. Rarely, hyposmia/anosmia is caused by olfactory neuronal degeneration or other diseases. Patients with anosmia often report ageusia, a reduced ability to taste foods.

There is a poor correlation between the symptoms of CRS and objective findings on imaging of the paranasal sinuses.[43,44]

Differential considerations other than rhinitis

Facial pain can be caused by nonrhinogenic conditions, including migraine headaches, tension headaches, cluster headaches, and other poorly understood facial pain syndromes.[45,46] Facial pain, pressure, or both are not reliable for predicting the presence of objective findings of rhinosinusitis.[46] Focal and sharp facial pain might be a symptom of CRS but is often not associated with radiographic evidence of sinus disease. Pain in the upper teeth, which is suggestive of nerve irritation in adjacent tooth roots, can be a symptom of maxillary sinus infection.

The differential diagnosis of nasal congestion includes allergic rhinitis, chronic nonallergic (idiopathic) rhinitis, rhinitis associated with medication use, secondary atrophic rhinitis (ie, empty nose syndrome), and cerebrospinal fluid rhinorrhea. Unilateral nasal congestion/blockage raises the question of a local anatomic problem, such as an antral choanal cyst, or tumor, such as an inverted papilloma.[47]

Subtypes of CRS

CRS can be divided into 3 clinical subtypes with distinctive but overlapping clinical features (Table IV).

1. CRS without nasal polyposis (CRSsNP) accounts for approximately 60% of CRS cases. It is a heterogeneous condition in which allergic factors, structural abnormalities, and viral and bacterial infection variably contribute to the disease. Facial pain, pressure, and/or fullness are more common in CRSsNP than in CRScNP (see below). Bacterial organisms isolated from diseased sinus cavities

can include: *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Staphylococcus aureus*, coagulase-negative staphylococci, and, less commonly, gram-negative enteric bacteria. The importance of anaerobic bacteria in causing CRS is controversial.[4] Sinus ostial blockage is the inciting event, in most cases leading to obstruction of sinus drainage and bacterial infection. As the condition becomes chronic, a chronic inflammatory infiltrate containing neutrophils, mononuclear cells, and some eosinophils is seen. Glandular hyperplasia and submucosal fibrosis are typically present histologically in patients with CRSsNP but absent in patients with CRScNP.[48,49]

2. CRScNP accounts for 20% to 33% of CRS cases. The symptoms are similar to those of CRSsNP, although hyposmia/anosmia is more common in patients with CRScNP.[50] Nasal polyps are typically bilateral in the middle meatus unless they have been previously removed. Unilateral polyps are relatively uncommon and should prompt consideration of other diagnoses, including inverted papilloma or other nasal tumors. CRScNP is more likely than CRSsNP to be associated with asthma and aspirin-exacerbated respiratory disease (AERD). The initial trigger for nasal polyp development is unknown. Polyp tissue typically contains a predominance of eosinophils, high levels of histamine, and high levels of the T_H2 cytokines IL-5 and IL-13.[51,52]
3. AFRS is defined as CRS accompanied by (1) the presence of allergic mucin (thick inspissated mucus that ranges in color from light tan to brown to dark green and that contains degranulated eosinophils), (2) fungal hyphae in the mucin, and (3) evidence of IgE-mediated fungal allergy.[4] Allergic mucin is thick inspissated mucus that ranges in color from light tan to brown to dark green and that contains degranulated eosinophils. Sinus surgery is usually required to remove allergic mucin and establish the diagnosis of AFRS. Fungal hyphae are found within the allergic mucin, suggesting fungal colonization. The fungi are strictly noninvasive. Patients with AFRS usually have nasal polyps and are immunocompetent. Symptoms are similar to those of other forms of CRS. Fever is uncommon. Occasionally, AFRS presents dramatically with complete nasal obstruction, gross facial dysmorphia, and/or visual changes.[4] The pathophysiology of AFRS is most consistent with chronic, intense allergic inflammation directed against colonizing fungi. Histologically, allergic mucin demonstrates intense eosinophilic degranulation, mucostasis, and inspissations.[53]

Distinct pathologic features of rhinosinusitis

Allergic mucin. Allergic mucin is a classic feature of AFRS.[54,55] However, allergic mucin is occasionally found in the absence of colonizing fungi in some cases of CRSsNP or CRScNP.

Hyperdensities on sinus CT scanning. Opacified sinus cavities might contain inspissated mucus that produces an inhomogeneous hyperdense pattern on sinus CT scanning. Hyperdensities suggest the presence of allergic mucin. They are a classic feature of AFRS (in which case the allergic mucin also contains fungal hyphae), but they can be seen in both CRSsNP and CRScNP.

TABLE IV. Definitions of rhinosinusitis based on disease classification

Recurrent acute rhinosinusitis
A. Recurrent acute rhinosinusitis >3 times per year
B. Requires >2 of the following symptoms:
• Anterior or posterior mucopurulent drainage
• Nasal congestion
• Facial pain/pressure
• Decreased sense of smell
C. Normal between episodes
Chronic rhinosinusitis with nasal polyps
A. Symptoms present for >12 weeks
B. Requires >2 of the following symptoms
• Anterior or posterior mucopurulent drainage
• Nasal congestion
• Facial pain/pressure
• Decreased sense of smell
C. Objective documentation
• Rhinoscopic examination OR
• Radiograph (sinus CT scan preferred)
D. Bilateral nasal polyps in middle meatus
Chronic rhinosinusitis without nasal polyps
A. Symptoms present for >12 weeks
B. Requires >2 of the following symptoms:
• Anterior or posterior mucopurulent drainage
• Nasal congestion
• Facial pain/pressure
• Decreased sense of smell
C. Objective documentation
• Rhinoscopic examination OR
• Radiography (sinus CT preferred)
AFRS
A. Symptoms present for >12 weeks
B. Requires >2 of the following symptoms
• Anterior or posterior mucopurulent drainage
• Nasal congestion
• Facial pain/pressure
• Decreased sense of smell
C. Objective documentation
• Rhinoscopic examination OR
• Radiography (sinus CT scan preferred)
D. AFRS criteria
• Positive fungal stain or culture of allergic mucin AND
• IgE-mediated fungal allergy

A potential role for colonizing fungi in CRS. Patients with CRS (including those with CRSsNP and CRScNP) have been found to have immune hypersensitivity to fungi, such as *Alternaria* species, that commonly colonize sinus mucus.[56,57] Although most patients do not produce a classic IgE-mediated response against these fungi, eosinophilic inflammation caused by a T_H2-type sensitization is present. The T-cell cytokines involved include IL-5 and IL-13. The eosinophilic inflammation is most intense in the mucus, where the eosinophils physically associate with fungal hyphae.[58]

Role of bacterial infection. CRS is a complex inflammatory disorder rather than a simple infectious process. Bacterial infections can complicate all forms of CRS. Bacteria can be involved in the pathogenesis of CRS, in the following ways:

- ABRS might fail to resolve, leading to a chronic infection in 1 or more sinuses.
- Bacterial colonization with enterotoxin-producing *S aureus* is found with increased prevalence in patients with

CRScNP and is associated with local production of enterotoxin-specific IgE antibodies.[59,60] These antibodies can be measured in sinus tissues, although levels in the blood might be undetectable. The enterotoxins act as superantigens and locally activate T lymphocytes.[59] In contrast, patients with CRSsNP do not have an increased prevalence of enterotoxin-specific IgE antibodies.

- Bacteria can form biofilm on the sinus epithelium. Sequestration of bacteria within biofilms allows the bacteria to resist antibiotic treatment and persist as a low-grade infection within the sinus mucosa.[61,62]
- Drug-resistant infection can occur with gram-negative bacteria or methicillin-resistant *S aureus*.[41]
- Acute bacterial infection can lead to osteitis of the underlying bone, although actual invasion of the bone has not been conclusively demonstrated.[63]

Physical findings

The diagnosis of ABRS requires the presence of purulent nasal discharge (secretions that are cloudy or colored) and nasal obstruction (congestion, blockage, or stuffiness), facial pain-pressure-fullness, or both.[40] Using a positive sinus radiograph as a gold standard for confirmation of disease, this symptom definition only allows for correct diagnosis in approximately 40% to 50% of cases.[64] Nonetheless, ABRS remains a clinical diagnosis.

The definitive diagnosis of CRS requires objective confirmation of disease either with nasal endoscopy or sinus CT scanning. Nasal endoscopy might reveal discolored mucus or edema in the middle meatus or sphenoethmoidal recess or similar findings in the sinus cavities of patients who have undergone previous surgery. Typical findings on sinus CT include sinus ostial narrowing or obstruction, sinus mucosal thickening or opacification, and, less commonly, air-fluid levels in the sinuses.

Diagnostic testing

Sinus imaging with plain radiography or sinus CT scanning is not recommended in patients with uncomplicated ABRS unless symptoms or signs suggesting extrasinus involvement are present.[40] Sinus CT scanning is the imaging study of choice for evaluation of CRS.[4,40] Coronal images are commonly obtained, although multiplanar images are available in many institutions. Nasal endoscopy is sufficient to establish the diagnosis of CRS but is insufficient to establish the extent of sinus involvement unless extensive prior sinus surgery has been performed.

Because CRS is associated with allergic rhinitis in 60% of adults and 36% to 60% of children, patients with CRS should be evaluated for allergy so that environmental control measures or other interventions appropriate for allergic disease can be implemented.[3]

Initial treatment of ABRS

An initial period of watchful waiting without initiation of antibiotics can be considered in adults with uncomplicated ABRS who have mild illness (mild pain and temperature <38.3 °C) and assurance of follow-up.[40] Spontaneous resolution has been reported in 62% to 69% of patients in placebo-controlled clinical trials.[40] Patients with more severe symptoms should be treated with an antibiotic. The most common bacteria isolated from the maxillary sinuses of patients with ABRS include *S pneumoniae*, *H influenzae*, and *M catarrhalis*, the latter being more common in children.[40] If a decision is made to treat with an antibiotic, amoxicillin is considered first-line therapy for most adults. For patients with penicillin allergy, trimethoprim-sulfamethoxazole or macrolide antibiotics are cost-effective alternatives. Several additional antibiotics, including cephalosporins and fluoroquinolones, are FDA approved for treatment of ABRS.

Intranasal decongestants might relieve nasal congestion but should be limited to 3 days to avoid rebound nasal congestion.[40] Intranasal corticosteroid sprays have been studied but are not approved as adjunctive therapy.

When initial therapy of ABRS fails

If ABRS does not improve after several days of antibiotics, prescription of an alternative antibiotic for several additional weeks should be considered.[3] If there is still no response, a sinus CT scan is indicated to confirm the presence of sinusitis and determine whether anatomic abnormalities might be predisposing to sinusitis. Underlying medical conditions should also be considered, including immune deficiency, gastroesophageal reflux disease, or defects in mucociliary clearance (see the section on chronic rhinosinusitis comorbidities). Specialist evaluation is appropriate when sinusitis is refractory to treatment or is recurrent.

Findings that suggest need for immediate referral

The following symptoms and signs are suggestive of other conditions that require immediate evaluation: double or reduced vision, proptosis, dramatic periorbital edema, ophthalmoplegia, other focal neurologic signs, severe headache, and meningeal signs.[65] Extrasinus extension of sinus disease is the most ominous complication of acute rhinosinusitis or CRS. Complications of acute sinusitis include orbital cellulitis, cavernous vein thrombosis, brain abscess, meningitis, localized osteomyelitis, and oral-antral fistula. Complications of chronic sinusitis include localized osteomyelitis, oral-antral fistula, mucocele, and brain abscess.

Treatment of chronic rhinosinusitis

Topical corticosteroid nasal sprays are recommended for all forms of CRS.[66] Antihistamines might be helpful in patients with underlying allergic rhinitis.[66] Antibiotics should be used to treat infection if nasal purulence is present, although antibiotics have not been officially approved for use in CRS. Antifungals, including oral terbinafine and topical amphotericin B, have been studied in patients with CRS.[67-69] Most antifungal trials have failed to show efficacy, and antifungal agents are not recommended.

CRScNP. Patients might benefit from a brief course (10-15 days) of oral corticosteroids to shrink nasal polyps.[70] Topical corticosteroid nasal sprays are recommended.[71] In patients with severe polyposis, sinus surgery with debulking of nasal polyps might be necessary. Topical corticosteroid nasal sprays are recommended to prevent recurrence of nasal polyps, although they are not always effective. Antileukotriene agents (eg, zafirlukast, montelukast, and zileuton) have received limited study and are not FDA approved for the treatment of nasal polyps. Patients with nasal polyps who have AERD might benefit from aspirin desensitization and daily aspirin therapy, provided they have no contraindications to aspirin therapy.[66]

AFRS. Sinus surgery is almost always required to establish the diagnosis of AFRS, remove inspissated mucus, and restore sinus patency. Nearly all patients with AFRS have nasal polyps. After surgery, oral corticosteroids are recommended at 0.5 mg/kg daily, with gradual tapering of the dose to the lowest possible dose necessary to maintain control of sinus symptoms. Topical corticosteroid nasal sprays are also recommended to control inflammation and prevent recurrence of nasal polyps.

Indications for sinus surgery

Functional endoscopic sinus surgery (FESS) is the procedure of choice for surgical management of refractory CRS. FESS is predicated on the observation that CRS "usually starts in the nose and spreads through the ethmoidal prechambers to the frontal and maxillary sinuses, with infections of these latter sinuses thus usually being of secondary nature."[72] The principal goal of FESS is to restore patency to the ostiomeatal unit, the key anatomic area of drainage of the maxillary and anterior ethmoid sinuses (Fig 2). A typical FESS procedure includes (on each side) removal of the uncinate process, creation of a widened maxillary antrostomy, an ethmoidectomy, and (in some cases) a sphenoidotomy. Additional goals of FESS might include correction of septal deformities, removal of severe concha bullosa deformity (enlarged middle turbinate containing an air cell), and restoration of patency to the frontal sinus. Several studies have reported a high success rate for FESS in improving the symptoms of CRS.[73-75]

The classic indications for FESS include (1) persistence of CRS symptoms despite medical therapy, (2) correction of anatomic deformities believed to be contributing to persistence of disease, and (3) debulking of advanced nasal polyposis.

Comorbidities

Allergic rhinitis. IgE-mediated allergy to environmental allergens is found in 60% of patients with CRS (including CRSsNP and CRScNP) compared with 30% to 40% for the general population.[76] Patients with CRS are typically sensitized to perennial rather than seasonal (ie, pollen) allergens.[76] By definition, all patients with AFRS have IgE-mediated allergy to fungi. Fungal spores can germinate in sinus mucus, thereby increasing the allergenic stimulus.

Histopathologic studies of ethmoidal tissue from patients with CRSsNP and nasal polyps from patients with CRScNP have shown that patients with CRS with associated allergies have mucosal T_H2 cell infiltration with production of classic T_H2 cytokines, including IL-4, IL-5, and IL-13.[77,78] This suggests that allergens contribute to chronic allergic sinus inflammation.

Immunodeficiency. Deficient antibody production in response to vaccination or hypogammaglobulinemia is found in approximately 12% of adults with CRSsNP.[50] Immunodeficiency is rare in patients with CRScNP or AFRS. Most patients with deficient antibody production or hypogammaglobulinemia have a pattern of recurrent acute episodes of purulent infection. They might also have a history of concomitant pulmonary infections or recurrent otitis media. Although the nasal and sinus epithelium expresses Toll-like and other innate receptors and produces a variety of antimicrobial proteins, such as lactoferrin, lysozyme, defensins, collectins, and cathelicidins, there are limited data about CRS risk in patients with defects in innate immunity.[79]

Gastroesophageal reflux disease. Sinusitis is considered a possible extraesophageal manifestation of gastroesophageal reflux disease. The mechanism is believed to be due to direct reflux of gastric acid into the pharynx and nasopharynx, causing inflammation of the sinus ostium and leading to sinusitis.[80,81]

Defects in mucociliary clearance. Defects in mucociliary clearance, such as those found in patients with cystic fibrosis and primary ciliary dyskinesia, dramatically increase the risk of CRS.

Viral infections. In a small number of cases, patients appear to have CRS after a period of repeated exposure to viral URIs. This is characteristically seen in patients exposed to health care settings, day care centers, schools, or homes with small children. However, data clearly implicating viral agents in the pathogenesis of CRS are scarce,[82] and the role of viral infection in patients with CRS is controversial.

Systemic diseases. CRS might be the presenting feature of an underlying systemic illness, such as Wegener granulomatosis or Churg-Strauss vasculitis[83,84] or, less commonly, sarcoidosis.[85]

Anatomic abnormalities. Several common anatomic variants can be seen in patients with CRS, including nasal septal deviation, concha bullosa deformity, Haller cells, agar nasi cells, and paradoxical curvature of the middle turbinate. However, these abnormalities are also seen in otherwise healthy subjects and are not clearly epidemiologically linked to an increased risk of sinusitis.[86-89]

Associated conditions

Both asthma and AERD are associated with CRS. Approximately 20% of patients with CRS have concomitant asthma. Conversely, approximately two thirds of asthmatic subjects, including both children and adults, have evidence of chronic sinus mucosal thickening or sinus opacification in cross-sectional studies.[90] The combination of aspirin sensitivity, asthma, and nasal polyposis is referred to as triad asthma, Samter syndrome, or AERD.[91,92]

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Food allergy

Scott H. Sicherer, MD, and Hugh A. Sampson, MD *New York, NY*

Adverse immune responses to foods affect approximately 5% of young children and 3% to 4% of adults in westernized countries and appear to have increased in prevalence. Food-induced allergic reactions are responsible for a variety of symptoms and disorders involving the skin and gastrointestinal and respiratory tracts and can be attributed to IgE-mediated and non–IgE-mediated (cellular) mechanisms. Genetic disposition and environmental factors might abrogate oral tolerance, leading to food allergy. Disease outcomes are influenced by the characteristics of the immune response and of the triggering allergen. Diagnosis is complicated by the observation that detection of food-specific IgE (sensitization) does not necessarily indicate clinical allergy. Therefore diagnosis requires a careful medical history, laboratory studies, and, in many cases, an oral food challenge to confirm a diagnosis. Novel diagnostic methods, including ones that focus on immune responses to specific food proteins or epitopes of specific proteins, are under study. Currently, management of food allergies consists of educating the patient to avoid ingesting the responsible allergen and to initiate therapy (eg, with injected epinephrine for anaphylaxis) in case of an unintended ingestion. Improved therapeutic strategies under study include oral and sublingual immunotherapy, Chinese herbal medicine, anti-IgE antibodies, and modified vaccines. (J Allergy Clin Immunol 2010;125:S116-25.)

Key words: *Food allergy, food hypersensitivity, oral tolerance, gastrointestinal food hypersensitivity, food allergens, anaphylaxis*

Abbreviations used
OFC: Oral food challenge
OIT: Oral immunotherapy
SPT: Skin prick test

The term *food allergy* is used to describe an adverse immune response to foods.[1] Considering allergy to milk, egg, peanut, and seafood in a meta-analysis of 51 studies, self-reported allergy ranged from 3% to 35%, whereas estimates from 6 studies using oral food challenges (OFCs) estimated rates of 1% to 10.8%.[2] In a meta-analysis including 36 population-based studies focusing on allergy to fruits and vegetables (excluding peanut),[3] only 6 included OFCs, and estimates of allergy varied widely from 0.1% to 4.3% for fruits and tree nuts to 0.1% to 1.4% for vegetables and less than 1% for wheat, soy, and sesame. Although an allergy could be triggered by virtually any food, "major allergens" responsible for most significant reactions include milk, egg, peanut, tree nuts, shellfish, fish, wheat, and soy. Allergy to additives and preservatives is generally uncommon.[4]

Food allergy rates vary by age, local diet, and many other factors. Studies in the United Kingdom and North America focusing on peanut indicate that prevalence rates in children have increased, essentially doubling, and exceed 1% in school-aged children.[5] A 2008 Centers for Disease Control and Prevention report indicated an 18% increase in childhood food allergy from 1997 to 2007, with an estimated 3.9% of children currently affected.[6] Extrapolation from US studies indicates approximately 125,000 emergency department visits[7] and 53,700 episodes of anaphylaxis[8] from foods each year. Fatalities are primarily reported from allergic reactions to peanuts and tree nuts, appear to be associated with delayed treatment with epinephrine, and occur more often in teenagers and young adults with asthma and a previously diagnosed food allergy.[9] The determination of accurate food allergy prevalence rates is hampered by the lack of studies applying reliable diagnostic methodologies, such as supervised OFCs, to large unselected populations. Table I presents estimated rates of food allergies in North America based primarily on data from studies conducted there when possible.[2,3,10]

Although prior studies indicated childhood food allergies typically resolved by age 3 years, recent studies, albeit possibly affected by selection bias because of referral patterns, indicated only 11% resolved egg and 19% resolved milk allergy by age 4 years; however, about 80% resolved these allergies by age 16 years.[11,12] Peanut allergy, which is typically considered a persistent allergy, can resolve for about 20% of young children by school age, although recurrence of peanut allergy has also been described primarily in those who tolerated an OFC but did not continue to consume the food.[5] Studies to address the reasons for increased prevalence and persistence of food allergies, focusing primarily on peanut, have included the hygiene hypothesis; changes in the components of the diet, including antioxidants, fats, and nutrients, such as vitamin D; the use of antacids, resulting in exposure to more intact protein; food processing, such as for peanut roasting and emulsification to produce peanut butter compared with fried or boiled peanut; and extensive delay of oral exposure, thus increasing topical (possibly sensitizing) rather than oral (possibly tolerizing) exposure to food allergens.[5,13] Evidence supporting this latter hypothesis is supported by one study showing peanut allergy rates in a school-aged cohort of Israeli Jewish children to be 0.17% compared with those in a cohort of Jewish children in the

From the Elliot and Roslyn Jaffe Food Allergy Institute, Division of Allergy and Immunology, Department of Pediatrics, Mount Sinai School of Medicine.

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Reprint requests: Scott H. Sicherer, MD, Division of Allergy/Immunology, Mount Sinai Hospital, Box 1198, One Gustave L. Levy Place, New York, NY 10029-6574. E-mail: scott.sicherer@mssm.edu.

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United Kingdom, where the rate was about 10-fold higher (1.85%, $P < .001$), in the context of data showing consumption of peanut at ages 8 to 14 months was 7.1 g in Israel compared with 0 g in the United Kingdom ($P < .0001$).[14] A case-control study additionally found that peanut allergy was associated with household peanut consumption rather than maternal or infant peanut consumption.[15] However, randomized controlled trials are needed to confirm the hypothesis that earlier ingestion of peanut is protective.

TABLE I. Estimated food allergy rates in North America

Prevalence	Infant/child	Adult
Milk	2.5%	0.3%
Egg	1.5%	0.2%
Peanut	1%	0.6%
Tree nuts	0.5%	0.6%
Fish	0.1%	0.4%
Shellfish	0.1%	2%
Wheat, soy	0.4%	0.3%
Sesame	0.1%	0.1%
Overall	5%	3% to 4%

PATHOGENESIS

Oral tolerance induction and immune response to food proteins

The gastrointestinal tract encompasses the largest surface area in the human body and is comprised of a single-cell layer of columnar intestinal epithelial cells separating the internal sterile environment from the external world.[16] Its main function is to process ingested food into a form that can be absorbed and used for energy and growth, while at the same time preventing the penetration of harmful pathogens into the body. An intricate "gastrointestinal mucosal barrier" has evolved that consists of physiologic and immunologic components to accomplish this. The physiologic barrier includes a single layer of epithelial cells joined by tight junctions and covered with a thick mucus layer that traps particles, bacteria, and viruses. Trefoil factors are secreted by mucus-secreting cells of the stomach and intestine to help strengthen and promote restoration of the mucosal barrier. In addition, luminal and brush border enzymes, bile salts, and extremes of pH serve to destroy pathogens and render antigens less immunogenic. The immunologic component consists of innate (polymorphonuclear neutrophils, macrophages, natural killer cells, epithelial cells, and Toll-like receptors) and adaptive immune (intraepithelial and lamina propria lymphocytes, Peyer patches, secretory IgA, and cytokines) cells and factors, which also provide an active barrier to foreign antigens. However, the efficiency of this mucosal barrier in infants and young children is not optimal because of the developmental immaturity of various components of the gut barrier and immune system (eg, the activity of various enzymes is suboptimal in the newborn period and the secretory IgA system is not fully mature until 4 years of age).[16] Consequently, this immaturity might play a role in the increased prevalence of both gastrointestinal tract infections and food allergies seen in the first several years of life. Recently, studies in both murine models and human subjects have suggested that alteration of the physiologic barrier function (eg, decreased gastric acidity caused by potent antacids) can lead to increased IgE sensitization in both children and adults.[17] Additionally, altered intestinal permeability leading to increased exposure to intact proteins might promote sensitization and might enhance the severity of food-induced allergic reactions.[18]

Whereas the systemic immune system is typically confronted with relatively small quantities of foreign antigen and mounts a brisk inflammatory response, the mucosal immune system regularly encounters enormous quantities of antigen and must suppress immune reactivity to food and harmless foreign commensal organisms (ie, develop oral tolerance). Antigen-presenting cells, including intestinal epithelial cells and dendritic cells, and regulatory T cells play a central role in the development of oral tolerance.[16,19,20] Several types of regulatory T cells have been identified in conjunction with intestinal immunity: T_H3 cells, a population of $CD4^+$ cells that secrete TGF-β; T_R1 cells, a population of $CD4^+$ cells that secrete IL-10; $CD4^+$ and $CD25^+$ regulatory T cells; $CD8^+$ suppressor T cells; and γδ T cells.[16] In addition, intestinal epithelial cells can process luminal antigen and present it to T cells on an MHC class II complex but lack a "second signal," thus leading to anergy and suggesting their role in tolerance induction to food antigens as nonprofessional antigen-presenting cells. Despite the evolution of this elegant gastrointestinal barrier, about 2% of ingested food antigens are absorbed and transported throughout the body in "immunologically" intact forms, even through the normal mature gut.[21] In a series of experiments performed more than 75 years ago, Walzer and colleagues[22,23] passively sensitized volunteers with sera from patients with food allergy and demonstrated that immunologically intact antigens cross the mucosal barrier and disseminate rapidly throughout the body to activate local mast cells.

Several nonhost factors can influence the development of oral tolerance, such as physical properties of the antigen and the dose and frequency of exposure. Studies in murine models indicated differences in immune responses depending on the dose of antigen ingested: high-dose tolerance involves deletion of effector T cells, and low-dose tolerance is the result of activation of regulatory T cells with suppressor functions.[16]

Ongoing studies indicate that commensal gut flora also likely play a role in oral tolerance induction, as initially suggested by the observation that mice raised in a germ-free environment do not have normal tolerance.[24] In one study mice treated with antibiotics or lacking Toll-like receptor 4–recognizing bacterial LPSs and then exposed to a sensitizing regimen of peanut were more prone to peanut allergy than wild-type control animals.[25] Population-based observational studies relating the presence of atopic dermatitis to stool bacterial patterns and interventional studies administering probiotics suggest a potential for allergy prevention by creating a tolerogenic bacterial milieu, although clinical studies are conflicting.[26]

IgE-mediated hypersensitivity responses are attributed to the generation of T_H2 cells that produce IL-4, IL-5, and IL-13. Murine models demonstrate a role of T_H2 skewing at the time of gut antigen presentation by dendritic cells.[27,28] To explore the relative role of a T_H2- or T_H1-biased immune response in food allergy, Turcanu et al[29] expanded human peanut-specific T cells *in vitro* from the peripheral blood of patients with peanut allergy using peanut antigen and then stimulated the cells with phorbol 12-myristate 13-acetate and ionomycin to maximize cytokine secretion. Expanded T cells from 9 subjects with peanut allergy were found to be T_H2 biased. However, Thottingal et al[30] measured peanut allergen–driven cytokine responses in short-term primary cultures of PBMCs from adults with peanut allergy and peanut-tolerant adults with or without peanut-specific IgE.

Subjects with positive skin test responses had more frequent or intense IL-5 and IL-13 responses than those without, irrespective of whether they had clinically symptomatic peanut allergy. Surprisingly, the 3 groups were not distinguishable based on IFN-γ responses, which were absent, suggesting that a protective T_H1 bias does not explain the distinction in clinical outcomes, whereas a spectrum of T_H2 responses might.

In susceptible hosts oral tolerance might not develop after antigen ingestion, or it might be bypassed altogether by presentation of proteins through alternate routes, such as the respiratory tract or skin. Oral allergy syndrome/pollen-food–related syndrome is an example in which oral tolerance is bypassed because sensitization occurs through the respiratory route.[31] Respiratory sensitization to Bet v 1 in birch pollen might lead to oral pruritus in allergic patients when eating raw apples because of cross-reactivity to a homologous apple protein, Mal d 1. Application of food proteins to the skin of mice has been shown to result in systemic allergic symptoms after oral exposure.[32,33] As described above, there are epidemiologic studies from Israel and the United Kingdom that support the notion that environmental, rather than or perhaps in the absence of, oral exposure to peanut might promote sensitization and allergy.[13,15] The loss of skin barrier provides a portal for sensitization to food allergens in the environment and is increasingly being considered a potential route by which food allergens can evade oral tolerance.[13]

The immunopathophysiology of non–IgE-mediated gastrointestinal food allergy disorders are also being evaluated. In infants with food protein–induced enterocolitis syndrome, detection of TNF-α from PBMCs cultured *in vitro* with food proteins responsible for the reaction has been shown.[34] Chung et al[35] found increased staining for TNF-α and decreased staining for the regulatory cytokine receptor TGF-β1 in duodenal biopsy specimens of affected infants. More work is clearly needed to elucidate the immunologic basis of this disorder, but these studies suggest that a deficit in TGF-β1 response and excessive TNF-α response might be important pathogenic factors.

Healthy subjects without food allergy frequently have low concentrations of food-specific IgG, IgM, and IgA antibodies in their serum. Food protein–specific IgG antibodies tend to increase in the first months after the introduction of a food and then generally decrease, even though the food protein continues to be ingested.[36] Subjects with various inflammatory bowel disorders (eg, celiac disease, inflammatory bowel disease, and food allergy) frequently have high levels of food-specific IgG and IgM antibodies, but there is no evidence that these antibodies are pathogenic.[37]

The role of food proteins

Allergic reactions to egg, milk, peanut, tree nuts, fish, shellfish, wheat, and soy account for most significant food allergies in the United States, although any food can trigger an allergic response.[38] However, relatively few protein families account for the vast majority of allergic reactions.[39] In a study by Jenkins et al[40] comparing animal food allergens and their human homologs (considering protein families, sequence analysis, and evolutionary relationships), they noted that sequence identities to human homologs of greater than 62% typically excluded the protein from being allergenic in human subjects. Major food allergens share a number of common features; they are water-soluble glycoproteins, 10 to 70 kd in size, and relatively stable to heat, acid, and proteases.

However, it is clear that additional aspects, such as food preparation, can affect allergenicity. One theory proposed to explain a higher rate of peanut allergy in westernized countries, where peanut is consumed roasted, compared with lower prevalence rates in China, where peanut is primarily boiled or fried, regards the differential effect of these preparation methods.[5] The high temperature of roasting (180 °C) peanuts leads to a Maillard reaction that appears to increase stability and allergenicity.[41,42] Another theory posits that emulsification (peanut butter) increases allergenicity through an adjuvant effect.[5] Additional characteristics of the manner in which foods are ingested might be relevant. For example, recent studies suggest that 70% to 80% of young children allergic to milk or eggs can tolerate baked (heat-denatured) forms of the protein but not the unbaked form.[43,44] It is suggested that these children make IgE antibodies primarily to conformational epitopes on the food proteins and represent the children who will naturally outgrow their food allergies.

Two recent studies suggest that the carbohydrate moiety of certain glycoproteins might play a significant role in the allergenicity of food proteins. Shreffler et al[45] showed that glycosylated Ara h 1, a major peanut allergen, but not the deglycosylated form, acted as a T_H2 adjuvant by activating dendritic cells to drive the maturation of T_H2 cells. Additionally, Ara h 1 acts as a ligand for DC-SIGN (dendritic cell–specific intercellular adhesion molecule 3–grabbing nonintegrin, an ITAM I [immunoreceptor tyrosine-based activation motif–containing type II member of the C-type lectin family]), which also has been shown to interact with schistosome glycoproteins and induce T_H2 responses.[46] Commins et al[47] identified 24 adults who reported urticaria, angioedema, or anaphylaxis 3 to 6 hours after ingesting beef, lamb, or pork. These patients were all found to have positive skin test results and serum IgE antibodies to galactose-α-1,3-galactose, the carbohydrate moiety of these glycoproteins. This is the first demonstration of IgE antibodies directed at a carbohydrate epitope leading to clinical symptoms.

CLINICAL DISORDERS

In addressing possible food-induced allergic disease, the clinician must consider a variety of adverse reactions to foods that are not food allergies, especially because more than 20% of adults and children alter their diets for perceived adverse reactions/allergies.[2] Adverse reactions that are not classified as food allergies include host-specific metabolic disorders (eg, lactose intolerance, galactosemia, and alcohol intolerance), a response to a pharmacologically active component (eg, caffeine, tyramine in aged cheeses triggering migraine, and histaminic chemicals in spoiled dark-meat fish resulting in scombroid poisoning masquerading as an allergic response), or toxins (eg, food poisoning). Additionally, psychologic (food aversion and anorexia nervosa) or neurologic (eg, auriculotemporal syndrome manifested by a facial flush from tart foods or gustatory rhinitis manifested by rhinorrhea from hot or spicy foods) responses can mimic food allergies.

It is conceptually and diagnostically helpful to categorize food-induced allergic disorders based on immunopathology among those that are and are not mediated by IgE antibodies. Disorders with an acute onset of symptoms after ingestion are typically mediated by IgE antibody. Food-specific IgE antibodies arm tissue mast cells and blood basophils, a state termed *sensitization*.

On re-exposure, the causal food proteins bind to the IgE antibodies specific for them and trigger the release of mediators, such as histamine, that cause the symptoms. Another group of food hypersensitivity disorders are subacute or chronic and are mediated primarily by T cells. A third group of chronic disorders attributed to food allergy are variably associated with detectable IgE antibody (IgE-associated/cell-mediated disorders). Table II lists the features of a spectrum of the most common food-induced allergic disorders categorized by pathophysiology.[4,48] The table does not include disorders such as recalcitrant childhood gastroesophageal reflux, constipation, and irritable bowel syndrome, which are sometimes attributed to food allergy.[49] Detection of IgG antibodies to foods is not considered diagnostic of food allergy.[1,4,37] However, Heiner syndrome, a rare infantile disorder characterized by pulmonary hemosiderosis triggered by milk protein, is associated with increased milk-specific IgG antibodies. Celiac disease and the related skin disorder dermatitis herpetiformis can be considered food allergies because an immune response to gluten in grains, such as wheat, rye, and barley, is responsible, but these disorders are not discussed further here. Dietary (food) protein–induced enteropathy is another malabsorption syndrome, but unlike celiac disease, it is usually caused by cow's milk, is transient, is not associated with malignancy or dermatitis, and, for unclear reasons, has been rarely described in the past decade. Although symptoms of mucous and bloody stools in breast-fed infants have typically been attributed to dietary proctitis/proctocolitis caused by immune responses to maternal ingestants, such as cow's milk, studies have recently emphasized that alternative causes, such as infection or other inflammatory disorders, should be considered.[50,51] Thus empiric maternal dietary interventions should be undertaken with consideration that alternative explanations might exist, and retrials of the avoided allergen can be considered shortly after resolution of symptoms if other signs of allergy are absent. Lastly, contact dermatitis has also been attributed to foods, particularly with occupational exposure.

DIAGNOSIS

The evaluation requires a thorough history and physical examination to consider a broad differential diagnosis, to ascertain possible trigger foods, and to determine a likely general pathophysiologic basis, specifically whether the food-induced allergic disorder is likely IgE mediated, which guides testing. The history should determine the possible causal food or foods, quantity ingested, time course of reaction, ancillary factors (exercise, aspirin, and alcohol), and reaction consistency.[4] The history also focuses on details that might contribute to estimating the prior probability of an allergic reaction to a specific food. For example, reasoning dictates that a food ingested infrequently is more likely responsible for an acute reaction than one previously tolerated; that contamination of a meal by a previously diagnosed allergen should be considered ahead of a less likely explanation, such as development of a new allergy to a previously tolerated food; and that major allergens are inherently more likely to be triggers than other foods. To arrive at a diagnosis, the clinician should consider the epidemiologic aspects of the disease (eg, common triggers and common associations) and the details of the specific history and then consider appropriate testing that can be evaluated in the context of these prior probability estimates.[4]

For IgE-mediated disorders, skin prick tests (SPTs) provide a rapid means to detect sensitization.[4] Negative SPT responses essentially confirm the absence of IgE-mediated allergic reactivity (negative predictive accuracy, >90%). However, a positive test response does not necessarily prove that the food is causal (specificity, <100%). Consideration of the clinical history and disease pathophysiology is required to maximize the utility of test results. For example, a positive SPT response can be considered confirmatory when combined with a recent clear history of a food-induced allergic reaction to the tested food. Additionally, increasing SPT wheal size is correlated with an increasing likelihood of clinical allergy.[4,52] Studies have attempted to define wheal sizes above which allergy is virtually confirmed based on the test result alone[53,54]; however, these studies have been limited to a few foods in infants using specific techniques in only a few populations.[4] In one study of 140 children evaluated for peanut allergy, 64 had positive SPT responses, and 18 reacted during oral peanut challenge.[55] Of 17 children with an SPT wheal of greater than 10 mm, only 8 reacted during the challenge. Thus additional studies are needed to continue to define the diagnostic accuracy of skin test wheal sizes for different foods, ages, disease, and populations; wheal size has not been correlated to severity of outcomes. When evaluating allergy to many fruits and vegetables, commercially prepared extracts are often inadequate because of the lability of the responsible allergen, and therefore the fresh food might be used for testing.

Serum immunoassays to determine food-specific IgE antibodies (the term RAST is now antiquated) provide another modality to evaluate IgE-mediated food allergy.[56] Increasingly higher concentrations of food-specific IgE levels correlate with an increasing likelihood of a clinical reaction but do not generally correlate very well with reaction severity.[57-62] Different predictive values are being generated from emerging studies, which might represent nuances of diet, age, disease, and challenge protocols.[60,61,63] Particular values associated with a high likelihood of clinical allergy (eg, >95%) are often referred to as diagnostic values. Undetectable serum food-specific IgE might be associated with clinical reactions for 10% to 25%.[57,64] Consequently, if there is a suspicion of possible allergic reactivity, a negative SPT response, negative physician-supervised food challenge result, or both are necessary to confirm the absence of clinical allergy. Nomograms are available where prior probabilities can be used along with likelihood ratios (determined from studies evaluating the diagnostic utility of tests) to predict a diagnosis; however, there are few studies providing likelihood ratios, and results vary.[4] A decrease in specific IgE concentration is associated with an increasing chance of allergy resolution.[65] A complete primer of food allergy diagnosis is beyond the scope of this review, but Table III provides additional insights and information that are key to accurate diagnostics.[57-62,66-68]

Although not commercially available, determination of specific IgE-binding epitopes on an allergen might provide increased diagnostic utility.[69] The specific profiles of epitopes bound might reflect distinctions in binding to areas of an allergen that are dependent on protein folding (conformational epitopes) and are a feature of mild/transient allergy versus areas that represent linear binding regions that are stable, reflecting a severe persistent allergy. Additionally, IgE responses to specific proteins in foods might account for particular outcomes.[70] For example, identification of IgE binding to labile birch pollen–related proteins is associated with mild reactions, whereas binding to stable lipid transfer proteins in the same foods is associated with more severe reactions. This observation forms the basis for an approach termed component-resolved diagnostics.

TABLE II. Food-induced allergic disorders (also see text)

Immunopathology	Disorder	Key features	Additional immunopathology	Typical age	Most common causal foods	Natural course
IgE antibody dependent (acute onset)						
	Urticaria/ angioedema	Triggered by ingestion or direct skin contact (contact urticaria); food commonly causes acute (20%) but rarely chronic (2%) urticaria		Children > adults	Primarily major allergens	Depending on food
	Oral allergy syndrome (pollen–food related)	Pruritus, mild edema confined to oral cavity Uncommonly progresses beyond mouth (~7%) or anaphylaxis (1% to 2%) Might increase after pollen season	Sensitization to pollen proteins by the respiratory route results in IgE that binds certain homologous, typically labile food proteins (in certain fruits/ vegetables (eg, apple Mal d 1 and birch bet v 1)	Onset after pollen allergy established (adult > young child)	Raw fruit/vegetables Cooked forms tolerated Examples of relationships: birch (apple, peach, pear, carrot), ragweed (melons)	Might be long-lived and vary with seasons
	Rhinitis, asthma	Symptoms might accompany a food-induced allergic reaction but rarely an isolated or chronic symptom Symptoms might also be triggered by inhalation of aerosolized food protein		Infant/child > adult, except for occupational disease (eg, baker's asthma)	General: major allergens Occupational: wheat, egg, and seafood, for example	Depending on food
	Anaphylaxis	Rapidly progressive, multiple organ system reaction can include cardiovascular collapse	Massive release of mediators, such as histamine, although mast cell tryptase levels not always increased Key role of platelet-activating factor	Any	Any but more commonly peanut, tree nuts, shellfish, fish, milk, and egg	Depending on food
	Food-associated, exercise-induced anaphylaxis	Food triggers anaphylaxis only if ingestion followed temporally by exercise	Exercise is presumed to alter gut absorption, allergen digestion, or both	Onset more commonly later childhood/adult	Wheat, shellfish, and celery are most described	Presumed persistent
IgE antibody associated/cell-mediated (delayed onset/chronic)						
	Atopic dermatitis	Associated with food in ~35% of children with moderate-to-severe rash	Might relate to homing of food-responsive T cells to the skin	Infant > child > adult	Major allergens, particularly egg and milk	Typically resolves

(*Continued*)

TABLE II. (*Continued*)

Immunopathology	Disorder	Key features	Additional immunopathology	Typical age	Most common causal foods	Natural course
	Eosinophilic gastroenteropathies	Symptoms vary on site(s)/degree of eosinophilic inflammation Esophageal: dysphagia and pain Generalized: ascites, weight loss, edema, and obstruction	Mediators that home and activate eosinophils play a role, such as eotaxin and IL-5	Any	Multiple	Likely persistent
Cell-mediated (delayed onset/ chronic)						
	Dietary protein enterocolitis	Primarily affects infants Chronic exposure: emesis, diarrhea, poor growth, and lethargy Re-exposure after restriction: emesis, diarrhea, and hypotension (15%) 2 hours after ingestion	Increased TNF-α response, decreased response to TGF-β	Infancy	Cow's milk, soy, rice and oat	Usually resolves
	Dietary protein proctitis	Mucus-laden, bloody stools in infants	Eosinophilic inflammation	Infancy	Milk (through breast-feeding)	Usually resolves

Increasingly, studies are evaluating the utility of the atopy patch test for disorders in which symptoms are delayed after food ingestion, such as atopic dermatitis,[71] eosinophilic esophagitis,[72] and food protein–induced enterocolitis syndrome.[73] The test is performed by placing foods under Finn chambers in a manner akin to testing for contact allergens. Although the atopy patch test shows promise, there are currently no standardized reagents, methods of application, or interpretations, and the additional diagnostic information in some studies appears marginal.[71,72] Additional future diagnostic modalities might include the basophil activation test.[74] Various tests and procedures (eg, endoscopy/biopsy and breath hydrogen tests) might be required to evaluate possible gastrointestinal allergy.[75] Unproved or disproved tests, such as the pulse test, applied kinesiology (muscle strength tests), cytotoxic tests, electrodermal tests, and IgG testing, should not be used.[76]

The OFC is comprised of a gradual feeding of a possible allergen under medical supervision to determine tolerance or clinical reactivity. Severe reactions could be elicited, and therefore the procedure is undertaken by properly trained personnel with medications and equipment to treat anaphylaxis on hand. Feeding is generally stopped when objective or persistent subjective symptoms are elicited.[62] For chronic disorders in which an ingested food is currently a part of the diet, diagnosis typically includes a period of elimination of the possible trigger food or foods to determine whether symptoms resolve before an OFC. Caution is advised because acute severe reactions are sometimes noted after reintroduction of a potential allergen (eg, positive test result for IgE or suspicion of allergy) after prolonged dietary elimination.[77] Open or single-blind OFCs are often used to screen for reactions. The double-blind, placebo-controlled OFC is the gold standard for the diagnosis of food allergies because bias is minimized.[78] If the blinded challenge result is negative, it must be confirmed by means of an open supervised feeding of a typical serving of the food in its natural form to rule out a false-negative challenge result (approximately 1% to 3%). A number of reviews have outlined the procedures involved for OFCs,[78,79] and a comprehensive clinically oriented guide has been recently published.[80]

MANAGEMENT

The primary therapy for food allergy is to avoid the causal food or foods. Education about avoidance includes careful attention to label reading, care in obtaining foods from restaurants/food establishments, and avoidance of cross-contact of foods with an allergen during meal preparation, such as avoiding shared cutting boards, slicers, and mixers. Food-labeling laws in the United States require simple English terms, such as "milk" instead of "casein," to indicate the presence of specific regulated food allergens, including only milk, egg, wheat, soy, peanut, tree nuts, fish, and crustacean shellfish. Patients and caregivers should be encouraged to obtain medical identification jewelry, taught to recognize symptoms, and instructed on using self-injectable epinephrine and activating emergency services. Comprehensive educational materials are available through organizations such as the Food Allergy & Anaphylaxis Network (Fairfax, Va; 1-800-929-4040 or http://www.foodallergy.org).

Various medications can provide relief for certain aspects of food-induced disorders. Antihistamines might partially relieve symptoms of oral allergy syndrome and IgE-mediated skin symptoms. Anti-inflammatory therapies might be beneficial for allergic eosinophilic esophagitis or gastroenteritis.[81] It is

TABLE III. Pearls and pitfalls regarding the diagnosis of food allergy

<table>
<tr><th>Pearl/observation</th><th>Additional details</th><th>Clinical application</th></tr>
<tr><td>A positive skin test or serum food-specific IgE test result indicates sensitization but not necessarily clinical allergy</td><td>Screening with indiscriminate panels of tests is poorly informative</td><td>The history and epidemiologic considerations should guide test selection
Tolerated foods generally need not be tested
Differential diagnosis should include alternative allergen triggers (environmental aeroallergens) and nonallergic diseases (eg, intolerance)</td></tr>
<tr><td>Dose, manner of preparation, and ancillary (eliciting) factors might alter reaction outcomes</td><td>Alcohol, NSAIDs, and exercise are among eliciting factors that might facilitate a reaction
Heating can alter allergenicity (eg, bakery products with egg/milk might be tolerated when whole forms are not, and cooked fruits might be tolerated when raw fruits are not)
A low dose might be tolerated, whereas larger amounts might not</td><td>The history should focus on amounts triggering a reaction and ancillary factors
The history should explore the types of foods tolerated or not tolerated</td></tr>
<tr><td>IgE binding to homologous proteins among food groups and between foods and pollens might have variable clinical relevance</td><td>Rates of clinical cross reactivity:
<table><tr><th>Allergy to:</th><th>Related food</th><th>Approximate clinical reaction rate</th></tr><tr><td>Peanut</td><td>Most beans</td><td>5%</td></tr><tr><td>A tree nut</td><td>Other tree nut</td><td>35%
Higher for: walnut-pecan, almond-hazel, cashew-pistachio</td></tr><tr><td>A fish</td><td>Other fish</td><td>50%</td></tr><tr><td>Shellfish</td><td>Another shellfish</td><td>75%</td></tr><tr><td>Grain</td><td>Another grain</td><td>20%</td></tr><tr><td>Cow's milk</td><td>Goat/sheep milk
Mare's milk
Beef</td><td>>90%
5%
10%</td></tr></table></td><td>Care should be used in not overtesting
For some categories and foods, avoidance of the entire group might be prudent, especially to avoid cross-contact in preparation, but individualization might be possible</td></tr>
<tr><td>Tests for serum food-specific IgE might not provide comparable results among manufacturers</td><td>In the United States there are 3 major test manufacturers</td><td>Care must be taken in evaluating test results over time when different manufacturers are used</td></tr>
<tr><td>Serum/skin tests might be negative despite clinical reactivity</td><td>Might be due to reagent lacking relevant protein
Might be because reaction is not IgE mediated</td><td>Do not discount a convincing history because of a negative test result
Consider testing with fresh food (prick-prick test)
Be cognizant of non–IgE-mediated allergic reactions</td></tr>
<tr><td>Increasingly high serum food-specific IgE levels or increasingly larger skin test wheal sizes indicate greater chances of clinical allergy</td><td>Correlation of tests with outcomes vary by centers, age, and disease (equivalent results are generally more predictive of allergy in a younger patient)
Results are not strongly reflective of severity</td><td>Tests should not be viewed solely as positive/negative
Results can be followed over time to monitor allergy persistence/resolution
Specific correlative values might not be applicable over all patient groups</td></tr>
<tr><td>At specific high levels of IgE or large skin tests, clinical reactivity is highly likely; however, studies are limited, and variations in diagnostic cutoff values are reported</td><td><table><tr><th>Food</th><th>Mean age 5 y, 50% react</th><th>Mean age 5 y, ~95% react</th><th>Age <2 y, ~95% react</th></tr><tr><td>Egg (kUa/L)</td><td>2</td><td>7</td><td>2</td></tr><tr><td>Milk (kUa/L)</td><td>2</td><td>15</td><td>5</td></tr><tr><td>Peanut (kUa/L)</td><td>2/5</td><td>14</td><td></td></tr></table></td><td>Oral food challenges might be deferred, particularly if there is a clinical history</td></tr>
</table>

NSAIDs, Nonsteroidal anti-inflammatory drugs.

TABLE IV. Selected immunotherapeutic strategies

Therapy	Immune rationale	Benefits	Observations to date
Standard subcutaneous immunotherapy (native allergens)	Antigen presentation in nonmucosal site results in T_H1 skewing	Proved for venom and respiratory allergy, possible benefit (pollen) for oral allergy syndrome	Primarily avoided for risk of anaphylaxis (eg, peanut)
Sublingual/OIT	Antigen presentation to mucosal site provides desensitization and might induce tolerance	Natural foods, reduced risk of systemic anaphylaxis compared with injections	Mounting evidence for desensitization and relative safety; unclear effect on tolerance
Modified protein vaccine	Reduced IgE activation by mutation of IgE-binding epitopes	A safer form of immunotherapy compared with injection of native protein	Murine models show promise, human studies are planned
Peptide vaccine (overlapping peptides)	Peptides are less likely to cross-link IgE, avoiding mast cell activation	No requirement for IgE epitope mapping/mutation	Limited
Conjugation of immune stimulatory sequences to allergen and additional adjuvant methods	Enhance T_H2 response by activating innate immune receptors (using specific sequences or whole bacteria)	Increased efficacy, possibly improved safety	Preclinical studies
Plasmid DNA-encoded vaccines	Endogenous production of allergen might result in tolerance	Possible 1-dose treatment	Murine models reveal strain-specific response
Anti-IgE antibodies	Targeted toward Fc portion of antibody, can inactivate IgE with reduced risk for activating mast cells	Not food specific Some response in eosinophilic gastroenteropathy (pilot study)	Preliminary study showed improved threshold overall but did not show uniform protection
Chinese herbal medicine	Mechanism unknown	Not food specific	Murine models show efficacy Human safety studies are underway
Cytokine/anti-cytokine (eg, anti–IL-5)	To interrupt inflammatory signals	Might allow directed interruption of inflammatory processes without need for food restriction	Preliminary study shows benefit for eosinophilic esophagitis.

important to recognize that the key treatment for food-induced anaphylaxis is prompt administration of epinephrine.

PREVENTION

There are limited data on primary prevention of food allergy through dietary means, although numerous studies possessing various limitations have addressed outcomes of atopic disease, such as atopic dermatitis and asthma. Based on review of the available literature, professional organizations[82,83] have generally concluded that there is insufficient evidence regarding reduced atopic disease to recommend maternal avoidance of allergens during pregnancy or lactation, although there is some evidence that allergen avoidance during lactation might be related to reduced atopic dermatitis. For infants with a family history of atopy placing them at increased risk, data primarily support the practice of exclusive breast-feeding for at least 4 months compared with feeding intact cow's milk formula to decrease the cumulative incidence of atopic dermatitis and cow's milk allergy in the first 2 years. Similarly, avoidance of solid foods for the first 4 to 6 months is associated with reduced risk of atopic dermatitis. Additionally, for infants not being exclusively breast-fed, whole protein formula (cow's milk or soy) compared with the use of studied extensively or partially hydrolyzed formulas in the first few months appears to be associated with increased risks for atopic dermatitis. After 4 to 6 months, there are insufficient studies/data that specific allergen avoidance alters atopy outcomes.

FUTURE THERAPIES

Future therapeutic options for food allergy include strategies that target specific foods and ones that block allergic responses and are not food specific.[48,84,85] Table IV summarizes some of the current strategies. Of note, immunotherapeutic approaches now under study attempt to avoid serious adverse effects that would otherwise be triggered by injection of native allergens, as noted in a study of injection immunotherapy for peanut allergy,[86] by changing the route of administration or by modifying (engineering) the treatment proteins. The approach undergoing the most current research is oral immunotherapy (OIT), in which doses of the food protein are given in gradually increasing amounts toward a maintenance dose. Jones et al[87] enrolled 39 children with peanut allergy in an open study of OIT; the study did not use initial OFCs, but after therapy for 4 to 22 months, initially aiming for 300 mg as a maintenance dose, 27 of 39 children completing the maintenance phase tolerated the targeted 3.9-g open peanut food challenge (18 of them without symptoms). Immune parameters followed during the study revealed a decrease in skin test and basophil activation, a decrease in peanut-specific IgE levels, and an increase in IgG levels.[4] In a first double-blind trial of milk OIT by Skripak et al,[88] 20 children (12 completed active treatment and 7 received placebo) underwent a regimen of an initial escalation day (aiming for 50 mg), 8 weekly updosings to a final dose of 500 mg, and maintenance for 3 to 4 months. The median dose eliciting a reaction at baseline was 40 mg, which increased to 5,140 mg (range, 2,540-8,140 mg) in the treated group but was unchanged in the placebo group. OIT is presumed to restore or induce a tolerant state. However, a distinction must be made between desensitization, in which the allergen is ingested without symptoms during treatment but requires daily ingestion, and tolerance, in which the food might be ingested without allergy symptoms despite periods of abstinence. Studies to date indicate that OIT induces desensitization, but it remains unclear whether tolerance is achieved.[89] Staden et al[90] randomized children to egg or

milk OIT (n = 25) or observation during dietary elimination (n = 20); after OFCs at about 21 months on therapy, the treatment group discontinued daily therapy for 2 months and were rechallenged. Although 64% of the treatment group had a good or at least partial response to OIT while on treatment, food challenges performed 2 months off treatment revealed only 36% continued to have true tolerance, a percentage that exactly matched tolerance achieved in untreated control subjects. More studies are required to assess safety,[91] efficacy, and mechanisms.

SUMMARY

Food allergies are common, result in both acute and chronic disease, might be increasing in prevalence, affect quality of life, and can be severe and potentially fatal. Diagnosis currently relies on a careful history and an appreciation of epidemiologic aspects of the disorder, the role and limitation of simple diagnostic tests, and, if needed, the use of an OFC to confirm allergy or tolerance. Treatment currently relies on avoidance of triggers and appropriate prompt response to allergic reactions, such as using epinephrine for anaphylaxis. Insights on pathophysiology are leading to the development of improved methods for prevention, diagnosis, and management, including clinical studies that are currently underway that might reduce risks for allergic subjects or possibly cure these allergies.

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Drug allergy

David A. Khan, MD,[a] and Roland Solensky, MD[b] *Dallas, Tex, Corvallis, Ore*

Drug allergy is one type of adverse reaction to drugs and encompasses a spectrum of hypersensitivity reactions with heterogeneous mechanisms and clinical presentations. A thorough history is essential to the management of drug allergy. Laboratory testing has a very limited role in the management of drug allergy. Graded dose challenges and procedures to induce drug tolerance might be required in patients with drug allergy when there is a definite need for a particular agent. Management of reactions to specific agents, including β-lactam antibiotics, sulfonamides, local anesthetics, radiocontrast media, angiotensin-converting enzyme inhibitors, nonsteroidal anti-inflammatory drugs, and biologic modifiers, will be discussed in further detail. (J Allergy Clin Immunol 2010;125:S126-37.)

Key words: *Drug allergy, adverse drug reactions, drug hypersensitivity, graded challenge, desensitization, tolerance, penicillin, cephalosporin, carbapenem, sulfonamide, local anesthetic, radiocontrast media, angiotensin-converting enzyme inhibitors, nonsteroidal anti-inflammatory drug, biologic modifiers*

Abbreviations used

ACE-I:	Angiotensin-converting enzyme inhibitor
ADR:	Adverse drug reaction
AERD:	Aspirin-exacerbated respiratory disease
ASA:	Acetylsalicylic acid
DILE:	Drug-induced lupus erythematosus
DRESS:	Drug rash with eosinophilia and systemic symptoms
NSAID:	Nonsteroidal anti-inflammatory drug
NSF:	Nephrogenic systemic fibrosis
PPL:	Penicilloyl-polylysine
RCM:	Radiocontrast media
SJS:	Stevens-Johnson syndrome
TEN:	Toxic epidermal necrolysis
TMP-SMX:	Trimethoprim-sulfamethoxazole

EPIDEMIOLOGY AND CLASSIFICATION OF ADVERSE DRUG REACTIONS

Adverse drug reactions (ADRs) are defined by the World Health Organization as any noxious, unintended, and undesired effect of a drug that occurs at doses used for prevention, diagnosis, or treatment. ADRs are commonly encountered in both inpatient and outpatient settings. In a meta-analysis of inpatient ADR prospective studies, 15.1% of patients sustained ADRs during their hospitalizations, and 6.7% of patients experienced serious ADRs.[1] In a 4-week prospective cohort study of outpatients followed in primary care clinics, 25% of patients reported ADRs, 13% of which were serious.[2]

ADRs are categorized into predictable (type A) and unpredictable (type B) reactions. Predictable reactions are usually dose dependent, related to the known pharmacologic actions of the drug, and occur in otherwise healthy subjects. Predictable reactions account for about 80% of all ADRs and are subdivided into overdose, side effects, secondary effects, and drug interactions. Unpredictable reactions are generally dose independent, are unrelated to the pharmacologic actions of the drug, and occur only in susceptible subjects. Unpredictable reactions are subdivided into drug intolerance (an undesirable pharmacologic effect that occurs at low and sometimes subtherapeutic doses of the drug without underlying abnormalities of metabolism, excretion, or bioavailability of the drug), drug idiosyncrasy (abnormal and unexpected effect, usually caused by underlying abnormalities of metabolism, excretion, or bioavailability), drug allergy (immunologically mediated ADRs [including IgE-mediated drug allergy]), and pseudoallergic reactions (also called anaphylactoid reactions, which are due to direct release of mediators from mast cells and basophils rather than IgE antibodies).

The Gell and Coombs system of hypersensitivity is the most common method of classifying immunologically mediated ADRs. It is comprised of immediate-type reactions mediated by drug-specific IgE antibodies (type I), cytotoxic reactions mediated by drug-specific IgG or IgM antibodies (type II), immune complex reactions (type III), and delayed-type hypersensitivity reactions mediated by cellular immune mechanisms (type IV). Type IV reactions can be subdivided into 4 categories involving activation and recruitment of monocytes (type IVa), eosinophils (type IVb), $CD4^+$ or $CD8^+$ T cells (type IVc), and neutrophils (type IVd).[3]

The pharmacologic interaction with immune receptors concept is a recently proposed addition to drug hypersensitivity classification. In this scheme a drug binds noncovalently to a T-cell receptor, which can lead to an immune response through interaction with an MHC receptor. In this scenario no sensitization is required because there is direct stimulation of memory and effector T cells analogous to the concept of superantigens.[4] Although these mechanistic classifications of drug-induced allergic reactions are useful, not all drug-induced allergic reactions can be categorized based on these limited mechanisms of hypersensitivity.

CLINICAL MANIFESTATIONS OF IMMUNOLOGICALLY MEDIATED ADRS

Drug-induced allergic reactions can affect numerous organ systems and manifest in a variety of reactions, including various drug-induced allergic syndromes, and many drug-induced allergic reactions can have more than 1 mechanistic pathway (Table I).

From [a]the University of Texas Southwestern Medical Center and [b]The Corvallis Clinic.

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Reprint requests: David A. Khan, MD, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, TX 75390-8859. E-mail: dave.khan@utsouthwestern.edu.

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TABLE I. Heterogeneity of drug-induced allergic reactions

Organ-specific reactions	Clinical features	Examples of causative agents
Cutaneous		
Exanthems	Diffuse fine macules and papules Evolve over days after drug initiation Delayed-type hypersensitivity	Allopurinol, aminopenicillins, cephalosporins, antiepileptic agents, and antibacterial sulfonamides
Urticaria, angioedema	Onset within minutes of drug initiation Potential for anaphylaxis Often IgE mediated	IgE mediated: β-lactam antibiotics Bradykinin mediated: ACE-I
Fixed drug eruption	Hyperpigmented plaques Recur at same skin or mucosal site	Tetracycline, NSAIDs, and carbamazepine
Pustules	Acneiform Acute generalized eczematous pustulosis (AGEP)	Acneiform: corticosteroids, sirolimus AGEP: antibiotics, calcium-channel blockers
Bullous	Tense blisters Flaccid blisters	Furosemide, vancomycin Captopril, penicillamine
SJS	Fever, erosive stomatitis, ocular involvement, purpuric macules on face and trunk with <10% epidermal detachment	Antibacterial sulfonamides, anticonvulsants, oxicam NSAIDs, and allopurinol
TEN	Similar features as SJS but >30% epidermal detachment Mortality as high as 50%	Same as SJS
Cutaneous lupus	Erythematous/scaly plaques in photodistribution	Hydrochlorothiazide, calcium-channel blockers, ACE-Is
Hematologic	Hemolytic anemia, thrombocytopenia, granulocytopenia	Penicillin, quinine, sulfonamides
Hepatic	Hepatitis, cholestatic jaundice	Para-aminosalacylic acid, sulfonamides, phenothiazines
Pulmonary	Pneumonitis, fibrosis	Nitrofurantoin, bleomycin, methotrexate
Renal	Interstitial nephritis, membranous glomerulonephritis	Penicillin, sulfonamides, gold, penicillamine, allopurinol
Multiorgan reactions		
Anaphylaxis	Urticaria/angioedema, bronchospasm, gastrointestinal symptoms, hypotension IgE- and non–IgE-dependent reactions	β-Lactam antibiotics, mAbs
DRESS	Cutaneous eruption, fever, eosinophilia, hepatic dysfunction, lymphadenopathy	Anticonvulsants, sulfonamides, minocycline, allopurinol
Serum sickness	Urticaria, arthralgias, fever	Heterologous antibodies, infliximab
Systemic lupus erythematosus	Arthralgias, myalgias, fever, malaise	Hydralazine, procainamide, isoniazid
Vasculitis	Cutaneous or visceral vasculitis	Hydralazine, penicillamine, propylthiouracil

Cutaneous manifestations are the most common physical manifestation of drug-induced allergic reactions; however, many other organ systems can be involved, including hematologic abnormalities, hepatitis, pneumonitis, lymphadenopathy, or arthralgias. Although drug-induced allergic reactions might present with noncutaneous physical findings, these findings are generally nonspecific and are not nearly as helpful in diagnosis and management decisions. Numerous cutaneous eruptions have been attributed to drug-induced allergic reactions and have been reviewed elsewhere.[5]

Because certain drug eruptions are associated with specific immunologic reactions, it is important to characterize the type of eruption in regard to determining the cause, further diagnostic tests, and management decisions. The most common cutaneous manifestation of drug-induced allergic reactions is a generalized exanthem (also know as a maculopapular eruption). Urticaria, angioedema, or both is another common cutaneous drug reaction that can be due to IgE-mediated reactions, serum sickness, pseudoallergic reactions, or other mechanisms (eg, bradykinin mediated). The most severe form of cutaneous drug reactions are Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN). The drug rash with eosinophilia and systemic symptoms (DRESS) syndrome is another cutaneous, drug-induced, multiorgan inflammatory response that can be life-threatening. First described in conjunction with anticonvulsants, it has since been ascribed to a variety of other drugs. DRESS is atypical from other drug-induced allergic reactions in that the reaction develops later, usually 2 to 8 weeks after therapy is started; symptoms can worsen after the drug is discontinued; and symptoms can persist for weeks or even months after the drug has been discontinued.[6]

EVALUATION: HISTORY TAKING

A thorough history is an essential component in the evaluation of patients with suspected drug allergies. The history helps guide the clinician in the choice of diagnostic tests and whether it might be safe to reintroduce the medication. If possible, the original medical record that describes the drug reaction should be reviewed. The most important components of a drug allergy history are as follows.

- *What is the name of the medication?* Although obvious, not uncommonly, patients are unable to provide this basic piece of information. Reasons for this include passage of time and the fact that names of many medications sound similar, and patients who reacted to multiple drugs might confuse which drug caused which reaction.
- *How long ago did the reaction occur?* The time elapsed is important because some allergies, such as to penicillin, wane over time.

- *Which systems (eg, cutaneous, respiratory, and gastrointestinal) were involved in the reaction, and what were the characteristics?* If a cutaneous eruption occurred, what kind was it (eg, urticarial, morbilliform, bullous, or exfoliative)? Showing the patient pictures of different types of rashes might be helpful.
- *When during the course did the reaction occur?* Alternatively, was the onset of symptoms after the course was completed?
- *Why was the medication prescribed?* The indication is important because symptoms of the underlying disease might be misattributed to the medication (eg, a truncal rash during penicillin therapy for streptococcal pharyngitis).
- *Was the patient taking concurrent medications at the time of the reaction?* Antibiotics are usually blamed for reactions, but drugs such as opiates and nonsteroidal anti-inflammatory drugs (NSAIDs) are frequently coadministered and might be responsible instead.
- *What was the therapeutic management required secondary to the reaction?* Self-discontinuation of a medication suggests a milder reaction than if a patient required hospitalization. Some patients recall treatment they received more readily than the characteristics of the reaction itself.
- *Had the patient taken the same or a cross-reacting medication before the reaction?* Most allergic reactions require a period of sensitization, typically during a previous course that was tolerated.
- *Has the patient been exposed to the same or similar medication since the reaction?* For instance, some patients with a history of penicillin allergy report that later they tolerated a course of amoxicillin clavulanate (Augmentin; GlaxoSmithKline, London, United Kingdom), not realizing the latter is a penicillin-class compound.
- *Has the patient experienced symptoms similar to the reaction in the absence of drug treatment?* The most common situation is chronic recurrent idiopathic urticaria, which can be confused for drug allergy.
- *Does the patient have an underlying condition that favors reactions to certain medications?* Examples of such conditions include mononucleosis for ampicillin reactions and HIV infection for trimethoprim-sulfamethoxazole (TMP-SMX) reactions.

DIFFERENTIAL DIAGNOSIS IN DRUG ALLERGY

Drug-induced allergic reactions can present in numerous ways, affecting single organs or with multiorgan involvement. However, each clinical presentation is not unique or specific to drug-induced allergic reactions, and therefore other conditions might need to be considered based on the presentation. For example, a morbilliform eruption occurring in a child receiving amoxicillin for an upper respiratory tract infection might indeed be due to a viral exanthem and not a drug-induced allergic reaction. In addition, patients with multiple drug allergies might actually have an underlying chronic disease and are inappropriately labeled with multiple drug allergies. This frequently occurs in patients with underlying chronic urticaria or anxiety disorders but can also occur with other conditions, such as asthma, vocal cord dysfunction, idiopathic anaphylaxis, or rarely even mastocytosis.

LABORATORIES IN DRUG ALLERGY

Routine laboratory evaluation appropriate to the clinical setting might be useful for the evaluation of a patient with a suspected drug reaction, depending on the history and physical examination findings. Although eosinophilia is often suggestive of a drug-induced allergic reaction, most patients with drug-induced allergic reactions do not have eosinophilia, and therefore the absence of eosinophilia clearly does not exclude a drug-induced allergic cause. Autoantibodies might be helpful in the evaluation of drug-induced vasculitis (eg, antinuclear cytoplasmic antibody) and drug-induced lupus erythematosus (DILE). In the case of systemic DILE, antihistone antibody levels are frequently positive, whereas in patients with cutaneous DILE, anti-Ro/SSA, anti-La/SSB, or both levels are frequently positive.[7]

In cases of suspect anaphylaxis, a diagnosis of anaphylaxis might be made by detecting an increase in serum total tryptase levels above baseline values or in serum mature tryptase (also known as β-tryptase) levels, which peak 0.5 to 2 hours after drug administration and then decrease with a half-life of about 2 hours.[8] Additional methods for detecting systemic mast cell mediator release include obtaining 24-hour urine collections for major urinary metabolites of histamine or prostaglandin D_2.

For immediate hypersensitivity reactions mediated by IgE antibodies, demonstration of the presence of drug-specific IgE is usually taken as sufficient evidence that the patients is at significant risk of having a type I reaction if the drug is administered. This is helpful in the case of high-molecular-weight agents. In the case of small-molecular-weight drugs, validated and reliable skin test reagents are only available for penicillin. Haptenation of the β-lactam ring of penicillin to a protein (eg, penicilloyl-polylysine [PPL]) enhances the immunogenicity, with resultant improvement in the detection of specific IgE. The negative predictive value of penicillin skin testing (with PPL, penicillin G, and penicilloate and/or penilloate) for serious immediate-type reactions approaches 100%. However, insufficient knowledge about drug degradation products, metabolites, or both and how they are conjugated with body proteins has been an impediment to developing either skin or *in vitro* assays for assessing immune responses to most other small-molecular-weight drug chemicals. Specific IgE *in vitro* assays (eg, RASTs, ImmunoCAP, and Immulite) are available, although most are not adequately validated with unclear specificity and sensitivity and lack internal positive controls. In addition, *in vitro* assays for IgE to drugs are hampered because of difficulties with binding of drug allergens to solid-phase matrices.

The basophil activation test is a recently described method of evaluating expression of CD63 or CD203C on basophils after stimulation with an allergen. There are very limited data using this method to evaluate patients with possible drug allergies to β-lactam antibiotics, NSAIDs, and muscle relaxants,[9] and further confirmatory studies, especially with commercially available tests, are needed before its general acceptance as a diagnostic tool. Drug patch testing might be useful for certain types of cutaneous drug reactions, including maculopapular exanthems, acute generalized exanthematous pustulosis, and fixed drug eruptions, but generally is not helpful for SJS or urticarial eruptions.[10] In complex cases in which multiple drugs are involved without a clear-cut temporal relationship, a skin biopsy might be useful. However, there are no absolute histologic criteria for the diagnosis of drug-induced eruptions, and a skin biopsy might not definitively exclude alternative causes.

TABLE II. Induction of drug tolerance procedures

Type of drug tolerance	Duration	Initial dose	Mechanisms	Example
Immunologic IgE (drug desensitization)	Hours	μg	Antigen-specific mediator depletion, downregulation of receptors	Penicillin Carboplatin, cisplatin, oxaliplatin
Immunologic non-IgE	Hours to days	mg	Unknown	TMP-SMX
Pharmacologic	Hours to days	mg	Metabolic shift, internalization of receptors	Aspirin
Nonimmunologic mast cell activation	Hours	μg	Unknown	Paclitaxel
Undefined	Weeks	μg-mg	Unknown	Allopurinol

INDUCTION OF DRUG TOLERANCE AND GRADED CHALLENGE PROCEDURES

In situations in which there is a definite medical need for a particular agent, no suitable alternative agent exists, and testing with high negative predictive value does not exist, there are primarily 2 options for the patient with a drug allergy. On the one hand, a procedure to induce temporary drug tolerance can be performed to allow the patient to take the drug safely. In contrast, a test dose or graded challenge can be administered to determine whether the patient is currently allergic to the particular drug.

The term *drug desensitizatio*n has been widely used and is defined as a procedure that modifies a patient's immune response to a drug, allowing him or her to take the drug temporarily in a safe manner. In cases such as IgE-mediated drug allergy (eg, to penicillin), the term drug desensitization is accurate in that patients are indeed sensitized to penicillin before the procedure and afterward typically have diminished or absent skin test reactions and hence are less sensitive or desensitized.[11] However, the term drug desensitization has also been used to describe a number of different protocols for patients with non–IgE-mediated drug allergies who in many cases are not truly sensitized initially but might react to the drug through various non–IgE-mediated or even nonimmune mechanisms. Recently, the term *induction of drug tolerance* has been proposed as a more appropriate term to encompass not only IgE-mediated desensitization procedures but other non–IgE-mediated desensitizations as well.[12] The term *drug tolerance* is defined as a state in which a patient with a drug allergy will tolerate a drug without an adverse reaction. Drug tolerance does not indicate either a permanent state of tolerance or that the mechanism involved was immunologic tolerance. Drug desensitizations for IgE-mediated drug allergy are indeed a form of immunologic drug tolerance. Induction of drug tolerance procedures modify a patient's response to a drug (through immunologic or other nonimmunologic mechanisms) to temporarily allow treatment with it safely. Induction of drug tolerance can involve IgE immune mechanisms, non-IgE immune mechanisms, pharmacologic mechanisms, and undefined mechanisms (Table II).

All procedures to induce drug tolerance involve administration of incremental doses of the drug but vary considerably over the starting dose and duration of the procedure. Through various mechanisms, these procedures induce a temporary state of tolerance to the drug, which is maintained only as long as the patient continues to take the specific drug. Therefore this procedure would need to be repeated in the future if a patient requires the drug again after finishing a prior therapeutic course.

Graded challenge, or test dosing, is defined as a procedure to determine whether a patient will have an adverse reaction to a particular drug by administering lower than therapeutic doses over a period of time with observation for reactions. The rationale for starting with a lower dose is based on the concept that a smaller dose of allergen will result in a less severe and more easily treated reaction. Unlike induction of drug tolerance procedures, a graded challenge does not modify a patient's immunologic or nonimmunologic response to a given drug. Although it is not possible to be absolutely certain that a patient is not allergic to a drug because valid diagnostic tests are not available for most drugs, graded challenges are intended for patients who, after a full evaluation, are unlikely to be allergic to the given drug. Furthermore, the benefit of treatment with the drug should outweigh the risk of performing the graded challenge. The starting dose for graded challenge is generally higher than for induction of drug tolerance procedures, and the number of steps in the procedure might be 2 or several. The time intervals between doses are dependent on the type of previous reaction, and the entire procedure can take hours or days to complete. After a successful graded challenge and therapeutic course of the drug, future courses of the drug can be started without another challenge.

A typically safe starting dose for an IgE immune induction of drug tolerance (desensitization) procedure is about twice the dose used in the puncture or intradermal skin test used to document the IgE-mediated allergy. A typical starting dose for a graded challenge is 1/100th of the final treatment dose. This is in contrast to the starting dose for an IgE immune induction of drug tolerance, in which case the starting dose is often 1/10,000th of the final dose. Caution should be exercised when a graded challenge consisting of more than 4 or 5 steps is performed because it might inadvertently induce modifications of immune effector cells and therefore induce drug tolerance in the patient. In these circumstances future administrations of the drug should be made cautiously.

The choice of whether to introduce a clinically indicated drug through a graded challenge or through induction of drug tolerance mainly depends on the likelihood that the patient is allergic at the time of the procedure. Patients who, based on their history, diagnostic test results, or both, are unlikely to be allergic to a drug can undergo graded challenge. For example, if penicillin skin testing is unavailable and a patient with a history of a mild pruritic rash during penicillin treatment 20 years ago requires penicillin therapy, it would be reasonable to administer penicillin through a graded oral challenge. Patients who have a relatively higher likelihood of being allergic to a drug should undergo an induction of drug tolerance procedure. For example, if penicillin skin testing is unavailable and a patient with a recent history of penicillin-induced anaphylaxis requires penicillin, it should be administered through induction of drug tolerance. Graded challenge (or induction of drug tolerance) should almost never be performed if the reaction history is consistent with a severe non–IgE-mediated reaction, such as SJS, TEN, DRESS, hepatitis, or hemolytic anemia.

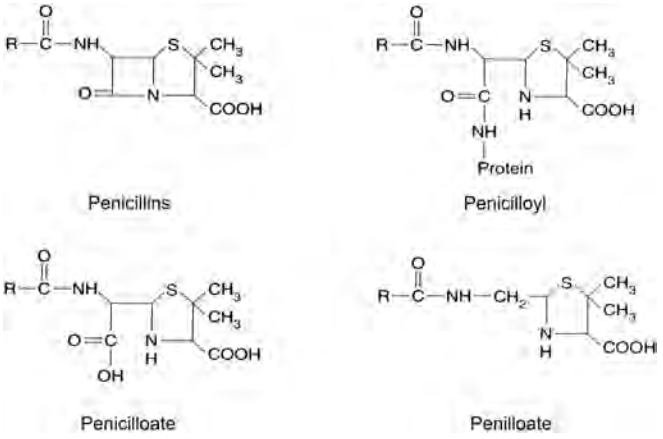

FIG 1. Chemical structures of major and minor penicillin allergenic determinants. The R-group distinguishes different penicillin compounds.

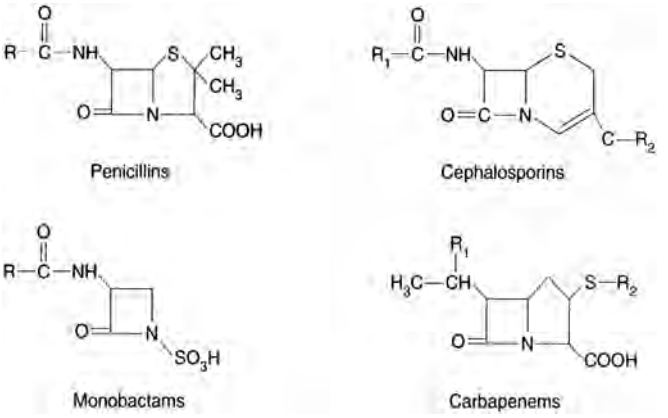

FIG 2. Chemical structures of β-lactam antibiotics.

MANAGEMENT OF COMMON ALLERGIC REACTIONS TO SPECIFIC AGENTS

β-Lactam antibiotics: Penicillins

Penicillin is the most prevalent medication allergy, with about 10% of patients reporting being allergic. When evaluated, however, approximately 90% of patients with a history of penicillin allergy are able to tolerate penicillins.[13,14] This observation is partly due to the fact that penicillin-specific IgE antibodies wane over time and many (but not all) patients outgrow their penicillin allergy. In addition, many patients were probably mislabeled as being allergic at the time of their reaction because symptoms and signs of an underlying illness can be confused for a penicillin-induced reaction. Patients labeled as allergic to penicillin are more likely to be treated with more expensive and broad-spectrum antibiotics (eg, quinolones and vancomycin),[15] which contributes to the development and spread of multiple drug-resistant bacteria and leads to higher health care costs.

The immunochemistry of penicillin was elucidated in the 1960s.[16] Under physiologic conditions, penicillin spontaneously degrades to a number of reactive intermediates that act as haptens and covalently bind to self-proteins, which then can elicit an immune response. Approximately 95% of penicillin degrades to the penicilloyl moiety, which is referred to as the major antigenic determinant (Fig 1). The remaining portion of penicillin degrades to several derivatives, and of these, penicilloate and penilloate are the most important to induce allergic responses. These 2 compounds, along with penicillin itself, are collectively known as the minor antigenic determinants, and they cover all clinically relevant allergenic determinants not covered by penicilloyl.

Less commonly, the R-group side chain, which distinguishes different penicillin compounds, can also serve as an allergenic determinant (Fig 2). This type of allergy results in patients who selectively react to amoxicillin, for example, but are able to tolerate other penicillins.[17] In contrast, patients allergic to the core β-lactam portion of penicillin cross-react to various penicillins. Selective allergy to amoxicillin or ampicillin is relatively common in parts of Southern Europe and quite infrequent in the United States; the reason for these differences in unknown.

Insight into the immunochemistry of penicillin has allowed for the development of validated skin test reagents to detect penicillin-specific IgE antibodies.[13,14] PPL was commercially available as Pre Pen from 1974 until 2004 and is expected to return to the market in 2009. Of the minor determinants, only penicillin G is commercially available. Some medical centers synthesize penicilloate and penilloate for local use. Amoxicillin or ampicillin should be included in the skin-testing panel when patients report reactions to these antibiotics.

The negative predictive value of penicillin skin testing is very high. In large-scale studies 1% to 3% of patients with negative skin test responses (with both major and minor determinants) had mild and self-limiting reactions on being challenged with the drug.[13,14] Some studies report that about 10% to 20% of patients with penicillin allergy have skin test reactivity only to penicilloate or penilloate.[13,14,18] The clinical significance of these findings is uncertain. Penicillin challenges of subjects with negative skin test responses to PPL and penicillin G[19] have similar reaction rates compared with those in subjects with negative skin test responses to the full set of major and minor penicillin determinants.[13,14]

Reaction history is a poor predictor of who will demonstrate a positive penicillin skin test response. Overall, about one third of patients with positive penicillin skin test responses report vague reaction histories.[20] Therefore any patient with a history of a possible IgE-mediated reaction to penicillin is a candidate for skin testing. Elective skin testing (when patients are well and not in immediate need of antibiotic therapy) should be considered. The medical care of patients labeled as having penicillin allergy can be compromised because of use of inappropriate antibiotics.[15] Patients who have positive responses should receive penicillins only through an induction of drug tolerance procedure. For patients with negative skin test responses, clinicians should consider a challenge with penicillin because without it, many patients are subsequently not treated with β-lactams because of fear on either the part of the patient or treating physician.

Resensitization after oral treatment with penicillin is rare in both pediatric and adult patients, including after repeated courses.[21,22] Hence routine repeat penicillin skin testing is not indicated in patients with a history of penicillin allergy who have tolerated 1 or more courses of oral penicillin. Consideration can be given to retesting individuals with recent or particularly severe previous reactions. Resensitization after high-dose parenteral treatment with penicillin might be more likely, but data are limited. Nevertheless, repeat penicillin skin testing in this situation might be warranted.[23]

TABLE III. Summary of studies of cephalosporin challenges in patients with a history of penicillin allergy without preceding penicillin allergy testing

Reference	History of penicillin allergy	No history of penicillin allergy	Cephalosporins administered
Dash, 1975[E1]	25/324 (7.7%)	140/17,216 (0.8%)	Cephalexin and cephaloridine
Petz, 1978[E2]	57/701 (8.1%)	285/15,007 (1.9%)	Cephalexin, cephaloridine, cephalothin, cefazolin, and cefamandole
Goodman et al, 2001[E3]	1/300 (0.3%)	1/2,431 (0.04%)	Cefazolin (in all but 1 patient)
Daulat et al, 2004[E4]	1/606 (0.17%)	15/22,664 (0.07%)	First generation (42%), second generation (21%), third/fourth generations (37%)
Fonacier et al, 2005[E5]	7/83 (8.4%)	Not reported	First generation (59%), second generation (8.4%), third generation (25%), fourth generation (7%)

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Without penicillin skin testing, the approach to patients with a history of penicillin allergy is based on the reaction history and likelihood of needing treatment with penicillins. Patients with a low likelihood of being allergic (eg, those with distant [> 10 years] or vague reaction histories) might receive penicillins through cautious graded challenge. On the other hand, patients with severe reaction histories (eg, anaphylaxis) or recent reactions should receive penicillins only through an induction of drug tolerance procedure.

β-Lactam antibiotics: Penicillin/cephalosporin cross-reactivity

Retrospective studies of administration of cephalosporins to patients with a history of penicillin allergy, without prior penicillin skin testing, showed much higher reaction rates in the 1970s compared to recently (Table III). Before 1980, cephalosporins were contaminated with trace amounts of penicillin, which would overestimate the cross-reactivity. Studies that rely on patient history to diagnose penicillin allergy are problematic because about 90% of these patients do not have penicillin allergy at the time of treatment with cephalosporins. Furthermore, some patients with severe penicillin reaction histories might have been denied treatment with cephalosporins.

Table IV summarizes studies in which patients with positive penicillin skin test responses were challenged with cephalosporins. Although these studies are of higher quality by virtue of proving type I penicillin sensitization before cephalosporin challenge, they still have limitations, including lack of control groups (eg, patients challenged with placebo or challenged with non–β-lactam antibiotics) and the fact that the challenges were not blinded. Patients might have an underlying propensity to react to unrelated drugs,[24] which can account for some reactions to cephalosporins in patients with penicillin allergy. In patients with documented allergic-like reactions to penicillins, the relative risk for allergic-like reactions was increased for both cephalosporins and sulfonamides.[25]

Ideally, management of cephalosporin administration to patients with a history of penicillin allergy includes penicillin skin testing (when available). About 90% of patients have negative penicillin skin test responses and can safely receive cephalosporins (as well as other β-lactams). Patients with positive penicillin skin test responses have a slightly increased risk of reacting to cephalosporins, and therefore they should be administered through graded challenge or an induction of tolerance procedure. When penicillin skin testing is not available, cephalosporins might be given through a full-dose or graded challenge, depending on the reaction history and the likelihood the patient has penicillin allergy. The reaction risk is very low, but rarely, anaphylactic reactions have been described.

Allergic cross-reactivity between amoxicillin and cephalosporins that share identical R-group side chains is higher than for patients with positive penicillin skin test responses. Twelve percent to 38% of patients proved to be selectively allergic to amoxicillin (ie, able to tolerate penicillin) reacted to cefadroxil.[26,27] Therefore patients with amoxicillin allergy should avoid cephalosporins with identical R-group side chains (cefadroxil, cefprozil, and cefatrizine) or receive them through induction of drug tolerance procedures. Similarly, patients with ampicillin allergy should avoid cephalexin, cefaclor, cephradine, cephaloglycin, and loracarbef or receive them through induction of drug tolerance procedures.

β-Lactam antibiotics: Penicillin/carbapenem cross-reactivity

Data on allergic cross-reactivity between penicillin and carbapenems are similar to those for penicillin/cephalosporins. Table V summarizes retrospective studies of carbapenem administration to patients with a history of penicillin allergy (no penicillin skin testing performed). The carbapenem reaction rate is somewhat higher in patients with a history of penicillin allergy. Table V also summarizes studies in which patients with positive penicillin skin test responses were challenged with carbapenems, and no patients experienced reactions (3 patients were not challenged because of positive carbapenem skin test responses).

The approach to carbapenem administration in patients with a history of penicillin allergy is analogous to that for cephalosporins. Patients with negative penicillin skin test responses can receive carbapenems safely. Patients with positive penicillin skin test responses should receive carbapenems through graded challenge, given that the chance of reacting is less than 1%. Without penicillin skin testing, carbapenems can be administered through graded challenge. Skin testing with carbapenems can be considered in patients with positive penicillin skin test responses or when penicillin skin testing is not performed.

Sulfonamides

Sulfonamides are defined as drugs with an SO_2-NH_2 moiety. Sulfonamide antibiotics also contain an aromatic amine at the N_4 position and a substituted ring at the N_1 position, whereas nonantibiotic sulfonamides do not. Beside penicillins, sulfonamide

TABLE IV. Summary of patients with positive penicillin skin test responses challenged with cephalosporins, not including patients with positive skin test responses to only amoxicillin or ampicillin (and not to major, minor, or both penicillin determinants)

	Cephalosporin			
Reference	**No. of patients**	**No. of reactions**	**Skin testing**	**Comment**
Girard, 1968[E6]	23	2 (8.7%)	No	Both reactions to cephaloridine
Assem and Vickers, 1974[E7]	3	3 (100%)	No	All reactions to cephaloridine
Warrington et al, 1978[E8]	3	0	Yes	
Solley et al, 1982[E9]	27	0	No	
Saxon et al, 1987[E10]	62	1 (1.6%)	No	Cephalosporin not noted
Blanca et al, 1989)[E11]	16	2 (12.5%)	No	Both reactions to cefamandole
Shepherd and Burton, 1993[E12]	9	0	No	
Audicana et al, 1994[E13]	12	0	Yes	
Pichichero and Pichichero, 1998[E14]	39	2 (5.1%)	No	Reaction to cefaclor and ?
Novalbos et al, 2001[E15]	23	0	Yes	
Macy and Burchette, 2002[E16]	42	1 (2.4%)	No	Reaction to cefixime
Romano et al, 2004[E17]	75	0	Yes	
Greenberger and Klemens, 2005[E18]	6	0	No	
Park et al, 2006[E19]	37	2 (5.4%)	No	Cephalosporins not noted

Please see the Online Repository at www.jacionline.org for complete reference citations.

TABLE V. Summary of carbapenem challenges in patients with a history of penicillin allergy without preceding penicillin allergy testing and in patients with positive penicillin skin test responses

	Carbapenem reaction rate			
Reference	**History of penicillin allergy (no penicillin ST)**	**No history of penicillin allergy**	**History of penicillin allergy (+ penicillin ST)**	***P* value**
McConnell et al, 2000[E20]	4/63 (6.3%)	NA	NA	NA
Prescott et al, 2004[E21]	11/100 (11%)	3/111 (2.7%)	NA	.024
Sodhi et al, 2004[E22]	15/163 (9.2%)	4/103 (3.9)	NA	.164
Cunha et al, 2008[E23]	0/110 (0%)	NA	NA	NA
Romano et al, 2006[E24]	NA	NA	0/110*	NA
Romano et al, 2007[E25]	NA	NA	0/103*	NA
Atanaskovic et al, 2008[E26]	NA	NA	0/107*	NA

Please see the Online Repository at www.jacionline.org for complete reference citations.
ST, Skin test response.

antibiotics are the most common cause of drug-induced allergic reactions.[28] They most commonly cause delayed cutaneous maculopapular/morbilliform eruptions, and IgE-mediated reactions are relatively infrequent. Sulfonamides are by far the most common cause of SJS and TEN.[29]

Patients infected with HIV have a greatly increased risk of cutaneous reactions from sulfonamide antibiotics, which is probably related to immunologic factors and frequent exposure to these antibiotics. The typical reaction to TMP-SMX in HIV-positive patients consists of a generalized maculopapular eruption that occurs during the second week of treatment and is usually accompanied by pruritus and fever. The incidence of skin rashes to TMP-SMX in healthy subjects is 3% to 5%, whereas reaction rates of 40% to 80% have been reported in patients with HIV.[28] Because TMP-SMX is the drug of choice for a number of HIV-associated infections (most notably prophylaxis and treatment of *Pneumocystis carinii*–induced pneumonia), it is not uncommon for HIV-positive patients with a history of reacting to sulfonamides to require treatment with the antibiotic. Consequently, various induction of drug tolerance procedures have been devised to safely administer TMP-SMX to HIV-positive patients with histories of reacting to the antibiotic.[30] The protocols vary greatly in terms of the starting dose, the incremental increase between doses, the time interval between doses, and the total duration of the desensitization; however, the success rates are comparable. Two studies compared the effectiveness of induction of tolerance versus rechallenge (single dose) in HIV-positive patients with documented reactions to TMP-SMX, and there were no differences in the success rates.[31,32] These results place into question the validity of previously reported induction of tolerance procedures that did not include a control group of patients who received full-dose TMP-SMX.

The N_4 aromatic amine is critical for the development of delayed reactions to sulfonamide antibiotics (through oxidation to hydroxyamines and nitroso compounds), and based on more limited data, the N_1 substituted ring appears to be important for IgE-mediated reactions.[28] Because nonantibiotic sulfonamides lack these structural components, they would not be expected to cross-react with sulfonamide antibiotics. Several clinical studies demonstrated no increased risk of reactions to nonantibiotic sulfonamides in patients with a history of allergy to sulfonamide antibiotics.[33]

Local anesthetics

IgE-mediated reactions to local anesthetics are extremely rare,[34] yet many patients are labeled allergic to all "caines" and denied access to these drugs. Most adverse reactions to local

anesthetics are due to nonallergic factors, such as vasovagal responses; toxic or idiosyncratic reactions caused by inadvertent intravenous epinephrine; or anxiety.[35] Local anesthetics are grouped into benzoate esters and amides. Based on patch testing, there is cross-reactivity among the benzoate esters (which do not cross-react with amides) but not among the amides. It is not known what, if any, relevance this has on immediate-type reactions to local anesthetics. If the reaction history is consistent with a possible type I reaction, skin testing followed by graded challenge tests can be performed with the same (epinephrine-free) local anesthetic that is intended to be used. Although there are differences in reported graded challenge procedures, a rapid and convenient protocol is as follows.[36] Skin prick testing is first performed with the undiluted anesthetic. If the response is negative after 20 minutes, an intradermal test is performed with 0.04 mL of 1:100 dilution of local anesthetic. If the response is negative after 20 minutes, a 1.0-mL subcutaneous injection of saline as a placebo is administered. If there is no reaction after 20 minutes, 1.0 mL of local anesthetic is administered, and the patient is observed for 20 minutes.

False-positive intracutaneous test results can occur in some patients.[37] Also, very rare patients can have positive skin test responses to methylparabens in local anesthetics, and some of these can be false-positive.[36] In these situations preservative-free local anesthetic should be used for skin testing/graded challenge.

Radiocontrast media

Anaphylactoid (non–IgE-mediated anaphylaxis) reactions occur in about 1% to 3% of patients who receive ionic radiocontrast media (RCM) and less than 0.5% of patients who receive nonionic agents.[38] Severe life-threatening reactions are less common: 0.22% of patients receiving ionic RCM and 0.04% of patients receiving nonionic agents.[39] The fatality rate from RCM is about 1 to 2 per 100,000 procedures, and it is similar for both ionic and nonionic agents.[40] Risk factors for anaphylactoid reactions to RCM include female sex, asthma, and a history of a previous anaphylactoid reaction to RCM; β-blocker exposure, the presence of cardiovascular conditions, or both are associated with greater risk for more serious anaphylactoid reactions.[41]

The pathogenesis of anaphylactoid reactions is unrelated to "seafood allergy" (attributed to high iodine content); patients with food allergy require no special precautions before receiving RCM. RCM reactions are generally not mediated by specific IgE antibodies. RCM likely has direct effects on mast cells and basophils, leading to degranulation and systemic mediator release, which accounts for the clinical manifestations of anaphylactoid reactions. Complement activation might account for some reactions. A recent European trial suggests that some RCM reactions might be IgE mediated because approximately half of patients with immediate-type reactions to RCM had positive skin test responses, which were highly specific.[42]

Management of patients who require RCM and experienced prior anaphylactoid reactions includes the following: (1) determine whether the study is essential; (2) determine that the patient understands the risks; (3) ensure proper hydration; (4) use a nonionic, iso-osmolar RCM, especially in high-risk patients (asthmatic patients, patients taking β-blockers, and those with cardiovascular disease); and (5) use a pretreatment regimen that has been documented to be successful in preventing most reactions but is less successful in preventing recurrence of severe reactions.[43] Pretreatment is defined as the administration of medications before administration of a drug to lessen the likelihood and severity of a drug-induced allergic reaction. Medications used for pretreatment are thought to be effective because of blockade of receptors for mast cell mediators or through reduction in mast cell mediator release (mast cell stabilization). A typical pretreatment regimen consists of 50 mg of prednisone 13, 7, and 1 hour before the procedure; 50 mg of diphenhydramine 1 hour before the procedure; and either 25 mg of ephedrine or 4 mg of albuterol 1 hour before the procedure. However, the latter agents might not be favorable from a risk/benefit standpoint in patients with cardiovascular disease. The use of H_2 antagonists in the pretreatment regimen is controversial because it can increase the RCM reaction rate.[43]

Delayed reactions to RCM, defined as those occurring between 1 hour and 1 week after administration, occur in approximately 2% of patients.[44] These reactions most commonly manifest as mild, self-limited cutaneous eruptions and do not require any treatment.[44] The mechanism of delayed skin reactions to RCM appears to be T-cell mediated.[45] Rarely, more serious and life-threatening delayed reactions to RCM have been described, such as SJS and TEN.[45]

Anaphylactoid reactions to gadolinium occur less frequently than to contrast materials used for computed tomographic scans.[46] Premedication regimens consisting of corticosteroids and antihistamines have been successfully used.[47] Nephrogenic systemic fibrosis (NSF), also called gadolinium-associated systemic fibrosis, is a recently described devastating progressive systemic fibrosing disorder that afflicts patients with renal dysfunction who recently received gadolinium.[48] The mechanism of NSF has not been elucidated, but it is hypothesized that dechelation of gadolinium chelates attracts $CD34^+$, $CD45^+$, procollagen-positive circulating fibrocytes.[48] Gadolinium has been found in biopsy specimens of skin lesions. Pre-existing renal failure might facilitate the reaction by delaying the excretion of gadolinium chelates. There is no effective treatment for NSF, and affected patients have increased mortality.[48]

Angiotensin-converting enzyme inhibitor: Cough and angioedema

Angiotensin-converting enzyme inhibitors (ACE-Is) have 2 major adverse effects: cough and angioedema. The incidence of cough from ACE-Is ranges from 5% to 35%.[49] Cough occurs more commonly in women, nonsmokers, and Chinese patients. The cause for ACE-I–induced cough is unclear but might be related to bradykinin, substance P, or other mechanisms. ACE-I–induced cough is typically dry and might be associated with a tickling sensation in the throat. The cough can occur within hours of the first dose or within weeks or months of initiation of therapy. With discontinuation of the ACE-I, the cough usually resolves in 1 to 4 weeks and rarely lingers up to 3 months.[49] In patients for whom cessation of ACE-I therapy is not desirable, several pharmacologic agents have been reported in small case series to reduce coughing, including cromolyn, theophylline, NSAIDs, amlodipine, nifedipine, and ferrous sulfate.[49] ACE-I–induced cough is not dose related, and angiotensin II receptor blockers are not associated with an increased incidence of cough.[50]

The incidence of angioedema to ACE-Is is estimated to occur in 1 to 7/1,000 patients, and this risk is higher in African-Americans compared with that seen in whites.[51] ACE-I–induced

TABLE VI. Hypersensitivity reactions to aspirin and NSAIDs and cross-reactivity

Type of reaction	Underlying disease	Cross-reactivity with COX-1 inhibitors
Respiratory (AERD)	Rhinitis, nasal polyps, sinusitis, asthma	Yes
Urticaria/AE	Chronic urticaria	Yes
Urticaria/AE	None	Yes or no
Anaphylaxis	None	No

The cross-reactivity patterns depicted in this table are generally true, but exceptions can occur.
AE, angioedema.

angioedema is often unrecognized because its manifestation can occur anywhere between a few hours to 10 years after an ACE-I is first taken. A recent retrospective study found a mean of 1.8 years from initiation of an ACE-I until the onset of angioedema.[52] ACE-I–induced angioedema accounts for approximately one third of all patients presenting to the emergency department for angioedema.[53] Characteristically, ACE-I–induced angioedema involves the head and neck primarily, especially the lips and tongue; concomitant urticaria and pruritus are rare. In some cases laryngeal edema can cause fatalities. Reports of angioedema of the intestinal tract caused by ACE-Is have also been described. Bradykinin is a prominent mediator in both hereditary angioedema and ACE-I–induced angioedema.[54] ACE-Is are contraindicated in patients with hereditary angioedema. In patients with ACE-I–induced angioedema, angiotensin II receptor blockers are often used as alternative medications. Limited data suggest that in patients with angioedema, when taking an ACE-I, the risk of persistent angioedema when subsequently switched to an angiotensin II receptor blockers is less than 10%.[55] Treatment includes discontinuing the medication and careful management of the airway, and in some cases fresh frozen plasma has been useful.

Acetylsalicylic acid/NSAID reactions

Acetylsalicylic acid (ASA) and NSAIDs can cause a spectrum of drug-induced allergic reactions, including exacerbation of underlying respiratory disease, urticaria, angioedema, anaphylaxis, and rarely pneumonitis and meningitis. Some of these drug-induced allergic reactions exhibit cross-reactivity to other NSAIDs and aspirin, whereas some reactions might be drug specific (Table VI).

Aspirin-exacerbated respiratory disease (AERD) is a clinical entity characterized by ASA/NSAID-induced respiratory reactions in patients with underlying chronic respiratory diseases, such as asthma, rhinitis, sinusitis, and/or nasal polyposis. AERD has been previously referred to by a number of different terms, including aspirin sensitivity, aspirin intolerance, aspirin idiosyncrasy, aspirin-induced asthma, and aspirin or Samter's triad. AERD does not fit precisely into a specific category of ADRs, although it has often been referred to as a type of pseudoallergic reaction. AERD affects up to 20% of adult asthmatic patients, is more common in women, has an average age of onset around the age of 30 years, and usually starts with rhinitis, progressing to hyperplastic sinusitis and nasal polyposis.[56] Asthma might be present since childhood or might develop *de novo*, on average 2 years after the onset of nasal congestion and polyposis.

Fundamental to the pathophysiology of AERD is excessive production of cysteinyl leukotrienes, increased numbers of inflammatory cells expressing cysteinyl leukotriene 1 receptors, and greater airway responsiveness to cysteinyl leukotrienes. In addition, a number of genetic polymorphisms involving the leukotriene pathway have been reported to be associated with AERD, including the leukotriene C_4 promoter, the cysteinyl leukotriene 1 receptor promoter, and prostanoid and thromboxane receptor–related genes.[57] Administration of ASA leads to inhibition of COX-1, with a resultant decrease in prostaglandin E_2 levels. Prostaglandin E_2 normally inhibits 5-lipoxygenase, but with a loss of this modifying effect, arachidonic acid molecules are preferentially metabolized in the 5-lipoxygenase pathway, resulting in increased production of cysteinyl leukotrienes.

Within minutes of ingestion of therapeutic doses of ASA or NSAIDs, patients with AERD typically have both rhinoconjunctivitis and bronchospasm. The bronchospasm induced can be severe and result in respiratory failure with a need for intubation and mechanical ventilation. Gastrointestinal symptoms and urticaria are rare extrapulmonary manifestations of AERD. Patients with AERD will react to ASA and NSAIDs that inhibit COX-1. Selective COX-2 inhibitors almost never cause reactions in patients with AERD and can typically be taken safely.

There is no diagnostic *in vitro* or skin test for AERD. The diagnosis is usually established based on history, but when a definitive diagnosis is required, a controlled oral provocation challenge with ASA can be performed. A recent study showed that 100% of patients with a history of a severe reaction to aspirin (poor response to albuterol with need for medical intervention) had positive oral aspirin challenge esults.[58] Management of patients with AERD involves avoidance of aspirin and NSAIDs and aggressive medical, surgical, or both types of treatment of underlying asthma and rhinitis/sinusitis. A pharmacologic induction of drug tolerance procedure (also known as aspirin desensitization), during which tolerance to aspirin can be induced over a few days and then maintained chronically, is an important therapeutic option for patients with AERD and improves clinical outcomes for both upper and lower respiratory tract disease.[59,60]

Several other drug-induced allergic reactions to ASA or NSAIDs can occur. Patients with chronic urticaria/angioedema might have exacerbation of their urticaria/angioedema with ingestion of NSAIDs that inhibit COX-1 but typically tolerate COX-2 inhibitors. Patients without a history of underlying chronic urticaria/angioedema can have acute urticaria/angioedema with ingestion of aspirin or NSAIDs. Some of these patients demonstrate cross-reactivity to other COX-1 inhibitors, whereas others have selective reactions to a particular NSAID. Anaphylactic reactions to NSAIDs are typically drug specific, and these patients typically tolerate other NSAIDs.[61] Finally, some patients are not easily categorized who have blended reactions with overlap of various clinical features from the above well-described ASA/NSAID reaction syndromes.

HIV medications

Patients infected with HIV have an increased frequency of drug-induced allergic reactions, and the reasons behind this are likely multifactorial.[62] Drug exanthems from TMP-SMX are among the most common drug-induced allergic reactions in patients with HIV, as previously discussed. Antiretroviral medications have also been associated with numerous drug-induced allergic reactions, ranging from mild exanthems to life-threatening reactions, such as SJS or TEN.

Although many antiretroviral medications can cause drug-induced allergic reactions, abacavir deserves special mention because of the successful implementation of a pharmacogenetics approach to management. Abacavir is a nucleoside reverse transcriptase inhibitor that is associated with a hypersensitivity reaction in approximately 4% of treated patients, with an estimated mortality rate of 0.03%.[63] This multiorgan reaction includes symptoms such as fever, rash, malaise/fatigue, gastrointestinal symptoms, and respiratory symptoms. In 90% of cases, hypersensitivity reactions occurred within the first 6 weeks after initiation of abacavir. Rechallenge with abacavir resulted in recurrence of symptoms within hours of re-exposure, including hypotension in 25% of those rechallenge reactions. Because of the severity of reactions on rechallenge, hypersensitivity to abacavir is a contraindication to subsequent treatment with any formulation that includes it.

Investigations into genetic risk factors associated with these reactions discovered that several HLA alleles, most notably HLA-B*5701, were strongly associated with risk of abacavir hypersensitivity reactions.[64,65] The prevalence of HLA-B*5701 varies considerably by ethnicity and geography, with estimated US prevalences of 8% for whites, 1% for Asians, and 2.5% for African Americans.[66] A double-blind, prospective, randomized study of 1,956 predominantly white patients with HIV from 19 countries was performed to evaluate the utility of genetic screening before initiation of abacavir therapy.[67] Subjects were randomly assigned to undergo prospective HLA-B*5701 screening with exclusion for abacavir treatment if screened positive. Screening for HLA-B*5701 reduced the risk of hypersensitivity reaction to abacavir, with reaction rates of 3.4% in the screened group versus 7.8% in the control group. A North American study with a more racially diverse population demonstrated that genetic screening decreased the rate of abacavir hypersensitivity to less than 1%, which is lower than historical rates.[68] The ability to identify genetic predispositions to drug-induced allergic reactions and implement genetic screening tests, as in the case of abacavir hypersensitivity, might hold promise for preventing other drug-induced allergic reactions in susceptible persons.[69]

Cancer chemotherapeutic agents

Hypersensitivity reactions have been reported for most cancer chemotherapeutic agents.[70] The severity of reactions can range from mild cutaneous reactions to fatal anaphylactic reactions. Taxanes, such as paclitaxel and docetaxel, can cause anaphylactoid reactions (non–IgE-mediated anaphylaxis), frequently with the first administration. Pretreatment with antihistamines and corticosteroids will prevent reactions in greater than 90% of cases.[71] Platinum compounds, such as cisplatin, carboplatin, and oxaliplatin, typically cause hypersensitivity reactions after several treatment courses. Results of skin testing have been found to be positive in the majority of patients with immediate reactions to platinum-containing compounds, suggesting an IgE-mediated mechanism. Cetuximab is an mAb used to treat colorectal cancer and squamous cell carcinoma of the head and neck and has been associated with anaphylactic reactions. IgE antibodies to cetuximab have been found in the majority of anaphylactic reactions and are specific for an oligosaccharide, galactose-α-1,3 galactose, which is present on the Fab portion of the cetuximab heavy chain.[72] Procedures to induce drug tolerance have been reported to be successful and safe in platinum-containing compounds, taxanes, and other chemotherapeutics.[73]

Biologic modifiers

In the past decade, a number of biologic immune modulatory agents have been developed to treat various inflammatory diseases and tumors. They are comprised of proteins such as cytokines, mAbs, and fusion proteins of solubilized receptors. These agents differ from other drugs in that they are not small-molecular-weight compounds but large potentially immunogenic proteins. Their metabolism is different, many are naturally occurring proteins, and all have inherent immunologic effects. Because of all of these differences, a separate type of classification for adverse reactions to biologic agents has been proposed based on the mechanism of reactions.[74] High-dose reactions are related to high cytokine levels administered directly or from cytokines released (eg, capillary leak syndrome). Hypersensitivity reactions can be either antibody or cell mediated. Immune or cytokine dysregulation can result in secondary immunodeficiency, autoimmunity, or allergic/atopic disorders. Cross-reactive reactions can occur when the biologic agent is intended for a pathologic cell type but cross-reacts with normal cells. Finally, biologic agents can also result in nonimmunologic side effects. Interferons are an example of biologic agents capable of causing most of the above reactions, including high-dose flu-like symptoms, hypersensitivity reactions of urticaria, autoimmune reactions (including thyroid disease and psoriasis), and nonimmunologic effects, such as depression.

Capillary (vascular) leak syndrome is a rare but potentially fatal condition that has been attributed to a number of biologic agents, including IL-2, GM-CSF, and granulocyte colony-stimulating factor.[75] Clinical and biochemical findings can include fever, edema (peripheral, pulmonary, ascites, and pleural/pericardial effusions), weight gain, hypotension, hypoalbuminemia, and multiorgan failure. The mechanism of the endothelial damage with subsequent fluid and protein extravasation is unclear but appears to be related to the inherent biologic effects of these cytokines.

TNF-α antagonists include humanized and fully human mAbs to TNF-α (infliximab and adalimumab) and TNF-receptor fusion proteins (etanercept). Acute infusion reactions are a relatively common adverse reaction to infliximab, often after the first dose, usually occurring within 4 hours of the infusion, and characterized by symptoms including hypotension/hypertension, chest pain, dyspnea, fever, and urticaria/angioedema.[76] The pathophysiology of these reactions is not known but is usually not IgE mediated, although several cases of anaphylaxis have been reported. The majority of patients can continue the infusion with reduction in rate or with premedication.[77] Delayed serum sickness–like reactions with symptoms of fever, urticaria/angioedema, and myalgias have also been reported but are much less common. The presence of antibodies to infliximab has correlated with both acute and delayed infusion reactions to infliximab. Etanercept and, less commonly, adalimumab are associated with delayed injection site reactions that typically peak at 2 days, usually occur in the first 2 months of therapy, and rarely cause discontinuation of treatment. Recall injection site reactions at the sites of previous injections can also occur and can be T-cell mediated delayed-type hypersensitivity reactions.[78] In addition to the above-mentioned infusion- or injection-related reactions, a number of other immunologic adverse reactions have been reported with TNF-α

antagonists, including vasculitis, systemic lupus erythematosus, psoriasis, interstitial lung disease, ocular autoimmune diseases, sarcoidosis, and hepatitis.[79]

Omalizumab is an mAb to human IgE approved for the treatment of asthma. Anaphylactic reactions have been reported with omalizumab in less than 0.1% of treated patients.[80] Most, but clearly not all, anaphylactic reactions occur to the first 3 doses and within 2 hours of the injection.

Finally, although not often considered a biologic therapy, intravenous gamma globulin has been associated with a variety of infusion reactions. The most common infusion-related reactions include symptoms of headache, fever, chills, tachycardia, anxiety, nausea, dyspnea, arthralgia, and myalgias and rarely more serious signs, such as hypotension. Most infusion reactions are mild and rate related and occur 6 to 24 hours after an infusion. The mechanisms causing these reactions are postulated to involve activation of complement by immunoglobulin aggregates, antigen-antibody complexes, and contaminant vasoactive proteins.[81]

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Allergic skin diseases

Luz S. Fonacier, MD,[a] Stephen C. Dreskin, MD, PhD,[b] and Donald Y. M. Leung, MD, PhD[c] *Mineola, NY, and Aurora and Denver, Colo*

The skin is one of the largest immunologic organs and is affected by both external and internal factors, as well as innate and adaptive immune responses. Many skin disorders, such as atopic dermatitis, contact dermatitis, urticaria, angioedema, psoriasis, and autoimmune blistering disorders, are immune mediated. Most of these diseases are chronic, inflammatory, and proliferative, in which both genetic and environmental factors play important roles. These immunologic mechanisms might have implications for potential targets of future therapeutic interventions. (J Allergy Clin Immunol 2010;125:S138-49.)

Key words: *Allergic contact dermatitis, autoimmune blistering disease, atopic dermatitis, eczema, immune-mediated skin disorders, irritant contact dermatitis, psoriasis, urticaria*

The skin is one of the largest immunologic organs and is often a target for allergic and immunologic responses. Many skin disorders, such as atopic dermatitis (AD), contact dermatitis (CD), urticaria, angioedema, psoriasis, and autoimmune blistering disorders, are immune mediated, with abnormalities in innate and adaptive immunity. Most of these diseases are chronic, inflammatory, and proliferative, in which both genetic and environmental factors play important roles. These immunologic mechanisms might have implications for potential targets of future therapeutic interventions. This review will examine some recent research advances in allergic and immunologic skin diseases.

Abbreviations used

ACD:	Allergic contact dermatitis
AD:	Atopic dermatitis
AMP:	Antimicrobial peptide
ASST:	Autologous serum skin test
BHR:	Basophil histamine release
BP:	Bullous pemphigoid
CAPB:	Cocoamidopropyl betaine
CD:	Contact dermatitis
CIU:	Chronic idiopathic urticaria
CU:	Chronic urticaria
DC:	Dendritic cell
EH:	Eczema herpeticum
FDA:	US Food and Drug Administration
HBD:	Human β-defensin
ICD:	Irritant contact dermatitis
IDEC:	Inflammatory dendritic epidermal cell
IVIG:	Intravenous immunoglobulin
LC:	Langerhans cell
NACDG:	North American Contact Dermatitis Group
PDC:	Plasmacytoid dendritic cell
PPD:	Paraphenylenediamine
PV:	Pemphigus vulgaris
ROAT:	Repeat open application test
SCD:	Systemic contact dermatitis
TLR:	Toll-like receptor
T.R.U.E. TEST:	Thin layer rapid use epicutaneous test
TSLP:	Thymic stromal lymphopoietin

CONTACT DERMATITIS

Allergists and clinical immunologists are seeing increasing numbers of patients with eczema and CD and are performing more patch testing. Cohort population-based studies in Europe showed point prevalence rates of 0.7% to 18.6% for allergic contact dermatitis (ACD).[1,2] Allergists trained in patch testing are more confident about the clinical relevance of such testing, especially for the differential diagnosis of the common eczematous diseases.[3]

Pathophysiology

CD can be allergic (20%) or irritant (80%). The morphology, severity, and location of CD is affected by the innate allergenicity or irritancy of the allergen, the site and degree of contact, the thickness and integrity of the skin involved, exposure time, environmental conditions, the immunocompetency of the patient, and even genetics.

ACD is the prototype of the type IV cell-mediated hypersensitivity reaction. The allergens in ACD are usually small-molecular-weight molecules or haptens that conjugate with proteins in the skin and induce activated epidermal keratinocytes to release proinflammatory cytokines. The Langerhans cells endocytose, process, and combine specific hapten peptides with HLA class I molecules and then migrate to the draining regional lymph nodes, where they activate and sensitize naive $CD4^+$ T cells (T_H0 cells). Activated T_H cells then proliferate and generate clones of hapten-specific $CD4^+CD25^+$ regulatory and $CD8^+$ effector cells, which become either memory or effector cells. This is known as the afferent limb of the immune reaction. The $CD4^+$ regulatory/effector and $CD8^+$ effector cells then "home" to the original skin site and there function as the efferent limb of the immune response. Both $CD4^+$ and $CD8^+$ sensitized effector cells release proinflammatory cytokines/cytotoxins (INF-γ, TNF-α, GM-CSF, IL-2, perforin, and granzyme), which cause an intense perivascular inflammatory

From [a]Winthrop University Hospital, Mineola; [b]University of Colorado Denver, Aurora; and [c]National Jewish Health, Denver.

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Reprint requests: Donald Y. M. Leung, MD, PhD, National Jewish Health, 1400 Jackson St, Room K926i, Denver, CO 80206. E-mail: leungd@njhealth.org.

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infiltrate and spongiosis. $CD4^+$ CCR10 chemokine–expressing memory lymphocytes are retained for long periods of time in the original ACD sites, accounting for the shortened latent period (anamnesis) on subsequent exposure. In mice mast cells at the site of ACD have been shown to recruit polymorphonuclear leukocytes to the site through the release of the mediators TNF-α and IL-8.

Irritant contact dermatitis (ICD) is the result of nonimmunologic, multifactorial, direct tissue reaction. T cells activated by means of nonimmune, irritant, or innate mechanisms release inflammatory cytokines (TNF-α, IL-1, IL-8, and GM-CSF) that contribute to the dose-dependent inflammation seen in patients with ICD.[4] There is usually a higher concentration of offending agents, such as solvents, detergents, chemicals, and alcohol. Lesions with erythema, edema, desquamation, and fissures are sharply demarcated typically and limited to the area in direct contact with the offending agent. They can burn or sting. Scratching, scrubbing, washing, excessive wetness, improper drying, perspiration, and extremes of temperature contribute to the reaction (Table I).

ACD and ICD frequently overlap because many allergens at high enough concentrations can also act as irritants. Impairment of the epidermal barrier layer, such as fissuring, can increase allergen entry into the epidermis.

TABLE I. Differentiation between ICD and ACD

Criteria	IICD	ACD
At risk	Anyone, especially if repeated exposure and occupational exposure	Previously sensitized and genetically predisposed
Mechanism	Nonimmunologic, direct tissue damage	Immunologic, delayed-type hypersensitivity reaction
Concentration of offending agent	Usually high, dose effect	Might be low threshold dose, all or nothing
Common causative agents	Water, soaps, solvents detergents, acids, bases, saliva, urine, stool	Poison ivy, poison oak, poison sumac, metals, cosmetics, medications, rubber, resins, adhesives
Risk if atopic	Increased	Decreased
Symptoms	Burning, stinging, soreness	Itching
Morphology	Erythema, edema, desquamation, fissures	Erythema, edema, vesicles, papules, lichenification
Demarcation	Usually sharp, limited to area in contact with agent	Sometimes sharp
Typical onset	Minutes to hour	Hours to days
Histology	Spongiosis, primarily neutrophilic infiltrates	Spongiosis, primarily lymphocytic infiltrates

Clinical evaluation

The diagnosis of ACD is suspected from the clinical presentation of the rash and the possible exposure to a contact allergen.

Face and eyelid. Fifty-five percent to 63.5% of eyelid dermatitis might be from ACD, 15% from ICD, less than 10% from AD, and 4% from seborrheic dermatitis.[5] Data collected by the North American Contact Dermatitis Group (NACDG) showed that in 193 (72%) of 268 patients with only eyelid dermatitis, gold was the most common allergen. Fragrances, preservatives, nickel, thiuram (rubber), cocamidopropyl betaine (CAPB) and amidoamine (shampoos), and tosylamide formaldehyde resins (nail polish) are other allergens to consider in the evaluation of eyelid dermatitis.[6] In patients with mixed facial and eyelid dermatitis, nickel, Kathon, and fragrance had the most positive patch test responses.[7,8]

Hands and feet. Hand dermatitis can be due to ICD, ACD, AD, dyshidrosis, or psoriasis. It can be extremely difficult to distinguish the cause of hand dermatitis, particularly because of tremendous overlap. Neither ACD nor ICD has pathognomonic clinical or histologic features. A thorough medical, work, and recreational history, together with a physical examination, ancillary laboratory tests, and patch tests, is critical in the evaluation of patients with hand eczema. Patch tests in patients with hand eczema showed that the relevant allergens include nickel sulfate (17.6%), potassium dichromate (7.2%), rubber elements (19.6%, including thiuram mix, carba mix, paraphenylenediamine [PPD], and mercaptobenzothiazole), and cobalt chloride (6.4%).[9] A Swedish study of 5700 patients showed that patients whose entire hands were involved were more likely to react to thiuram mix, PPD, chromate, and balsam of Peru. Patients with involvement of the fingers and interdigital spaces and those with palm involvement reacted more to nickel, cobalt, and 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one.[10]

The prevalence of hand eczema in patients with AD is 2- to 10-fold higher than that seen in nonatopic subjects, and 16% had nail dystrophy. The increasing prevalence of hand involvement with increasing age is probably due to increased water exposure and occupational insults, along with coexisting irritant dermatitis. Certain morphologic features can help distinguish the different contributing factors to hand eczema. Involvement of the dorsal hand and finger combined with the volar wrist suggest AD as a contributing causative factor.[11] ICD commonly presents as a localized dermatitis without vesicles in the webs of fingers; it extends onto the dorsal and ventral surfaces ("apron" pattern; Fig 1), dorsum of the hands, palms, and ball of the thumb. On the other hand, ACD often has vesicles and favors the fingertips, nail folds, and dorsum of the hands and less commonly involves the palms. ICD often precedes ACD, and therefore some pattern changes, such as increasing dermatitis from web spaces to fingertips or from palms to dorsal surfaces, should prompt patch testing or a repeat of it.[12]

Although ICD can cause dermatitis of the feet, ACD seem to be more common. ACD of the feet is usually located on the dorsum of the feet and toes (especially the hallux) but can also involve the sole and calcaneus. The interdigital areas are rarely involved. Humidity, heat, friction, and AD can contribute to or facilitate the development of CD of the feet. The most common positive patch test reactions in patients with ACD exclusively on the feet are rubber compounds (mercaptobenzothiazole mix, thiuram mix, carba mix, and PPD mix), with some patients sensitive to more than 1 of the agents. Other chemicals in footwear (eg, leather, adhesives, glues, and dyes) or in topical agents used for treatment (eg, creams, ointments, and antiperspirants) can cause ACD. Chemical agents used in the tanning and dyeing processes of leather (chrome), glues (colophony) used in soles and insoles, and nickel sulfate used in footwear buckles, eyelets, and ornaments[13] can be sensitizing agents.

Systemic contact dermatitis

Systemic contact dermatitis (SCD) is a systemic disease that demonstrates the inherent role of the skin as an immunologic organ. In the event that the suppressor function is inadequate, systemic administration of an allergen can lead to a full-blown effector

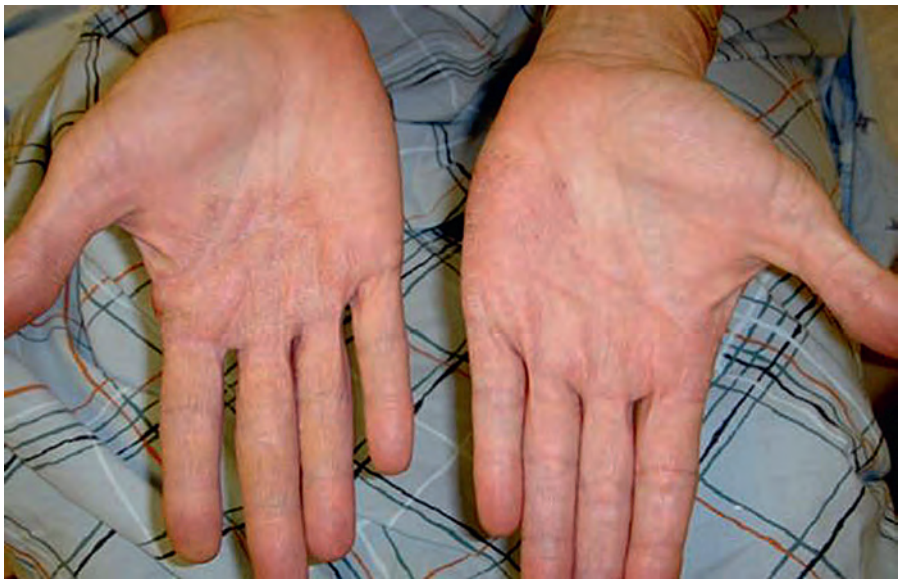

FIG 1. Irritant contact dermatitis of the hands: localized dermatitis without vesicles of webs of fingers extending onto the dorsal and ventral surfaces ("apron" pattern).

response. SCD is a localized or generalized inflammatory skin disease in contact-sensitized individuals exposed to the hapten orally, transcutaneously, intravenously, or by means of inhalation. It manifests as dermatitis at the cutaneous site of exposure, at previously sensitized sites (eg, an old lesion or the site of a previously positive patch test response), or in previously unaffected areas.[14] A variety of metals (cobalt, copper, chromium, gold, mercury, nickel, and zinc) have been found to cause SCD. The exposure type, duration, and environmental conditions (sweat and oxygen) in proximity to the metal are critical for mobilization of the ions inducing immune reactions. Medications reported to cause SCD include corticosteroids, antihistamines (diphenhydramine, ethylenediamine, hydroxyzine, and doxepin), miconazole, terbinafine, neomycin, gentamicin, erythromycin, pseudoephedrine, cinchocaine, benzocaine, tetracaine, oxycodone, intravenous immunoglobulin (IVIG), aminopenicillins, 5-aminosalicylic acid, naproxen, allopurinol, mitomycin C, 5-fluorouracil, and suxamethonium. In drug-induced SCD the clinical picture is frequently that of symmetric drug-related intertriginous and flexural exanthema. The criteria for the diagnosis of symmetric drug-related intertriginous and flexural exanthema include the following:

1 exposure to a systemic drug at first or repeated dosing (contact allergens excluded);
2 erythema of the gluteal/perianal area, V-shaped erythema of the inguinal/perianal area, or both;
3 involvement of at least 1 other intertriginous/flexural localization;
4 symmetry of affected areas; and
5 absence of systemic signs and symptoms.[15]

With alternative medicine's increasing popularity, more patients are using herbal preparations, homeopathy, and herbs in food, spices, and cosmetics that might contain plants and botanical extracts. Ragweed, chamomile, feverfew *(Tanacetum parthenium)*, *Arnica* species, marigold, *Echinacea* species, mugwort, cinnamon oil, vanilla oil, and balsam of Peru have been reported to cause SCD.

Occupational exposure

CD in the occupational setting can be benign and short lived or lead to severe disabling dermatitis. Patients with severe cases have poorer prognosis despite improvements in general working conditions, better availability of diagnostic patch testing, improved understanding of cutaneous biology, and treatment with topical and systemic steroids. ACD to nickel or chromium, a history of chronic dermatitis, delay of adequate treatment, a history of AD, and poor understanding by the worker of his or her disease is associated with a worse prognosis. Treatment of ACD in the workplace includes a timely diagnosis, identification of allergens or irritants, elimination of causal exposures, patient education, and use of therapeutic agents. AD is an important factor in susceptibility to persistent postoccupational dermatitis, and potential involvement of genetic predisposition to chemicals is observed. Two genomic screens[16,17] showed areas of genetic linkage on several chromosomes.

Patch testing. Patch testing is the only practical, scientific, and objective method for the diagnosis of ACD. It is indicated in patients with a chronic, pruritic, eczematous, or lichenified dermatitis in whom ACD is suspected. Patch test reactions are affected by oral corticosteroids (>20 mg of prednisone per day or its equivalent), cancer chemotherapy, or immunosuppressive drugs but not by antihistamines. Topical corticosteroids on the patch test site should be discontinued for 5 to 7 days before patch testing.

Sources of allergens. Commercially available, standardized patch testing allergens have been calibrated with respect to nonirritant concentrations and compatibility with the test vehicle. Test systems currently available are the thin-layer rapid-use epicutaneous test (T.R.U.E. TEST) and certain screening panels that are not US Food and Drug Administration (FDA) approved but conform to the standards of care recommended by CD experts. Commercial sources of customized patch testing materials include Smart Practice Canada (Calgary, Alberta, Canada); Hermal Pharmaceutical Laboratories, Inc (Hawthorne, NY); Dormer Laboratories, Inc (Rexdale, Ontario, Canada); and Trolab

Allergens (Omniderm Pharma Canada, Inc, Vaudreuil-Dorion, Quebec, Canada). The standardized allergens are loaded in Finn chambers or AllergEaze patch testing chambers (Haye's Service B.V., Alphen aan den Rijn, The Netherlands).

Number of allergens. Although the usefulness of patch testing is enhanced with the number of allergens tested, the ideal number of patch tests to be applied remains controversial. The T.R.U.E. Test contains 29 allergens, and the NACDG series ranges from 65 to 70 allergens. Studies show that the T.R.U.E. Test has higher false-negative reactions to neomycin, thiuram mix, balsam of Peru, fragrance mix, cobalt, and lanolin. Also, gold, bacitracin, methyldibromoglutaronitrile/phenoxyethanol, propylene glycol, bromonitropropane, cinnamic aldehyde, DMDM hydantoin, and ethylene urea/melamine formaldehyde have a prevalence of more than 2% in the NACDG 2004 but are not included in the current T.R.U.E. Test. Allergens not found on commercially available screening series in the United States frequently result in relevant reactions, and personal products are a useful supplement, especially in facial or periorbital dermatitis. The T.R.U.E. Test might serve as a triage or screening tool in an allergists' practice, but occupational exposures can benefit from early referral for supplemental testing.

Patch test technique. Patches are applied to upper or middle back areas (2.5 cm lateral to a midspinal reference point) free of dermatitis and hair and kept in place for 48 hours. Test results are read 30 minutes after removal of the patches to allow resolution of erythema caused by the tape, chamber, or both if present. A second reading should be done 3 to 5 days after the initial application. Thirty percent of relevant allergens eliciting negative reactions at the 48-hour reading elicit positive reactions in 96 hours. Irritant reactions tend to disappear by 96 hours. Metals (gold, potassium dichromate, nickel, and cobalt), topical antibiotics (neomycin and bacitracin), topical corticosteroids, and PPD can elicit positive reactions after 7 days. More than 50% of positive gold test results are delayed for about 1 week.

Nonstandardized patch tests, such as with the patient's personal products, allergens from cosmetics, or industrial allergens, might be needed. Leave-on cosmetics (makeup, perfume, moisturizer, and nail polish), clothing, and most foods are tested "as is," whereas wash-off cosmetics (soap and shampoo) are tested at 1:10 to 1:100 dilutions. Household and industrial products should only be tested after ascertaining their safety and patch test concentrations in the MSDS information.

Determining clinical relevance. The relevance of positive reactions to clinical ACD can only be established by carefully correlating the history, including sources of antigen in the patient's environment. A positive patch test reaction might be relevant to present or previous dermatitis, multiple true-positive results can occur, and mild responses can still represent an allergic reaction. A positive patch test reaction is considered to be a "definite" reaction of ACD if the result of a "use test" with the suspected item was positive or the reaction to patch testing with the object or product was positive, "probable" if the antigen could be verified as present in known skin contactants and the clinical presentation was consistent, and "possible" if skin contact with materials known to contain allergen was likely. Multiple sensitivities can occur when different allergens are present in different products used simultaneously. Likewise, concomitant sensitization of allergens can occur when multiple allergens are present in the same product; both processes induce sensitization. Cross-sensitization can also occur. Common combinations of positive patch test results are PPD and benzocaine (cross-sensitize); thiuram mix, carba mix, and mercapto mix (rubber products); quaternium 15 and paraben (quarternium-15, a formaldehyde releaser and formaldehyde are frequently combined and cosensitize); and cobalt and nickel (cobalt used in alloys with nickel and chromium and cosensitized). Patients older than 40 years are prone to multiple sensitivities.

TABLE II. Nickel content of certain foods

>50 μg	Soybean, boiled, ~1 cup: 895 μg Cocoa, 1 tbsp: 147 μg Cashew, ~18 nuts: 143 μg	Figs, ~5: 85 μg Lentils, ½ cup cooked: 61 μg Raspberry: 56 μg
20-50 μg	Vegetables, canned ½ cup: 40 μg Lobster, 3 oz: 30 μg Peas, frozen ½ cup: 27 μg	Asparagus, 6 spears: 25 μg Oat flakes, ⅔ cup: 25 μg Pistachios, 47 nuts: 23 μg
<20 μg	Strawberries, 7 medium: 9 μg Wheat bread, 1 slice: 5 μg Poultry, 3.5 oz: 5 μg Carrots, 8 sticks: 5 μg Apple, 1 medium: 5 μg	Cheese, 1.5 oz: 3 μg Yogurt, 1 cup: 3 μg Mineral water, 8 fl oz: 3 μg Mushroom, raw ½ cup: 2 μg Corn flakes, 1 cup: 2 μg

The repeat open application test (ROAT) might confirm the presence or absence of ACD. The suspected allergens are applied to the antecubital fossa twice daily for 7 days and observed for dermatitis. The absence of reaction makes CD unlikely. If eyelid dermatitis is considered, ROAT can be performed on the back of the ear.

SELECTED CONTACT ALLERGENS

Metals

Nickel. The prevalence rate of a positive patch test reaction to nickel in North America is consistently increasing. The most recent patch test data from the NACDG[18] reported that 18.7% of patients evaluated for ACD had a positive patch test reaction to nickel. Female subjects' sensitization to nickel is higher because of increased ear piercing. Laws regulating nickel products (eg, limiting the migration limit of nickel, the nickel ion release threshold of nickel-plated objects in prolonged contact with the skin, and the nickel content of post assemblies) in Europe appear to decrease sensitization in the younger population.

Evidence supports the contribution of dietary nickel to vesicular hand eczema.[19] A meta-analysis of SCD estimated that about 1% of patients with nickel allergy would have systemic reactions to the nickel content of a normal diet. Ten percent would react to exposures to 0.55 to 0.89 mg of nickel.[20] Foods with higher nickel content include soybean, fig, cocoa, lentil, cashew, nuts, and raspberry (Table II).

Gold. Previous NACDG data reported that 389 (9.5%) of 4101 patients had positive patch test reactions to gold. The most common sites of dermatitis were in the hands (29.6%); the face, with seborrheic distribution (19.3%); and the eyelids (7.5%).[21] Although mostly used for fashion appeal, gold is also an anti-inflammatory medication, is used in the electroplating industry, and is part of dental appliances. Patients with gold dental appliances (especially if present for more than 10 years) can present with oral symptoms. A subset of patients with facial dermatitis clear

with gold avoidance, mostly women with titanium dioxide in facial cosmetics, which adsorbs gold released from jewelry. Patients with gold allergy and eyelid dermatitis have cleared by not wearing gold jewelry, and therefore a trial of gold avoidance might be warranted with positive patch test reactions to gold. The avoidance period required for demonstrating benefit is long and might only be partially mitigating.[22]

Cosmetics

An individual is exposed to more than 100 chemical contactants in a typical day. Common allergens in these products include fragrances, preservatives, excipients, glues, and sun blocks.

Fragrance. Fragrance, the allergen of the year for 2007, is the most common cause of ACD from cosmetics and results in positive patch test reactions in 10.4% of patients. There are more than 2800 fragrance ingredients listed in the database of the Research Institute for Fragrance Materials, Inc,[23] and more new chemicals and botanical extracts are frequently used as fragrances. A manufacturer's label of "unscented" might erroneously suggest absence of fragrance when, in fact, a masking fragrance is present. "Fragrance-free" products are typically free of classic fragrance ingredients and are generally acceptable for the allergic patient. However, botanical extracts can be added to improve odor characteristics.

Because fragrances are complex substances, a perfume can contain hundreds of different chemicals that are difficult to identify individually. Fragrance mix I contains allergens found in 15% to 100% of cosmetic products[23] and might detect approximately 85% of subjects with fragrance allergy.[24] The addition of other commonly used fragrance ingredients (ylang ylang oil, narcissus oil, sandalwood oil, and balsam of Peru) increases the yield to 96%. The actual fragrance mix widely used in cosmetics and household products are seldom used in patch testing by the NACDG. Thus a positive patch test reaction to fragrance must correlate with distribution of the dermatitis and an evaluation of clinical relevance, such as a positive ROAT reaction.

Preservatives and excipients. Lanolin is a common component of consumer products. Unfortunately, its composition has not been fully characterized. Medicaments containing lanolin are more sensitizing than lanolin-containing cosmetics. It is a weak sensitizer on normal skin but a stronger sensitizer on damaged skin. Thus patients with chronic dermatitis, especially stasis dermatitis, are at higher risk of lanolin sensitivity.[25]

Cosmetic preservatives can be grouped into formaldehyde releasers and non–formaldehyde releasers. Paraben, a non–formaldehyde releaser, is the most commonly used preservative in cosmetics, as well as in pharmaceutical and industrial products, because of its broad spectrum of activity against yeasts, molds, and bacteria. Type I immediate hypersensitivity reactions (contact urticaria) and SCD from ingestion of paraben-containing medications or foods have been reported.[26]

Hair products. Hair products are second only to skin products as the most common cause of cosmetic allergy.

PPD is currently the most common cause of CD in hairdressers. In hair dye users the dermatitis often spares the scalp and usually involves the face near the hairline, eyelids, and neck. Nevertheless, generalized eruptions can occur. IgE–mediated contact urticaria and anaphylaxis, as well as lymphomatoid reactions, have also been reported. PPD cross-reacts with other chemicals, such as COX-2 inhibitor (celecoxib), sunscreens, and antioxidants used in the manufacture of rubber products (N-isopropyl-N′-phenyl-*p*-phenylenediamine). Theoretically, once oxidized, the PPD is no longer allergenic, but in reality, it is likely that PPD is never completely oxidized.[27] The FDA-required labeling and home-user tests appear to be predictive of PPD sensitization.[28] New hair dyes that contain FD&C and D&C dyes have very low levels of cross-reactivity with PPD and its other chemically related oxidative dyes (eg, Elumen Hair Color; Goldwell Cosmetics, Linthicum Heights, Md).

CAPB is an amphoteric surfactant often found in shampoos, bath products, and eye and facial cleaners. CAPB allergy typically presents as eyelid, facial, scalp, and/or neck dermatitis. Contaminants, such as amidoamine and dimethylaminopropylamine, which occur in the manufacture of CAPB, are thought to be allergens causing ACD. Positive patch test reactions to CAPB are often clinically relevant.[29]

Glycerol thioglycolate is the active ingredient in permanent wave solution. Unlike PPD, the thioglycolates might remain allergenic in the hair long after it has been rinsed out. Thus skin eruptions can continue for weeks after application of the permanent wave solution, and hairdressers allergic to it might be unable to cut permanent waved hair.

Medications

Antibiotics and antiseptics. Neomycin and nitrofurazone are potent sensitizers. Neomycin sulfate can cross-sensitize with gentamicin, kanamycin, streptomycin, spectinomycin, tobramycin, and paromomycin.

Corticosteroids. Although type I hypersensitivity reactions have been observed to corticosteroids, delayed-type hypersensitivity is by far the most common.[30] ACD to topical corticosteroids is rarely suspected from the history or from the appearance of the dermatitis, probably because of its anti-inflammatory action. Thus patients with a long-standing nonhealing dermatitis (eg, AD, stasis dermatitis, or chronic hand eczema) and patients with worsening of a previous dermatitis or an initial improvement followed by a deterioration of the dermatitis after application of corticosteroids should be evaluated for corticosteroid allergy. Patch tests for corticosteroid allergy should include the groups of simultaneously or cross-reacting corticosteroids,[31] as well as the vehicle and preservatives in the preparations. There is a 7-fold increase in frequency of a positive patch test reaction within a corticosteroid group. Cross-reactivity between groups A and D2 and groups B and D2 also has been reported.[32]

The optimal patch test concentration has not been worked out for most corticosteroids. A high patch test concentration of a potent corticosteroid might result in a false-negative test result on early readings because of its anti-inflammatory action. In such cases a lower concentration can be used if there is a strong suspicion of ACD, including corticosteroid-treated asthma and rhinitis. Patch tests to corticosteroids should include the patient's own commercial product. Thirty percent of the cases of ACD to corticosteroids might be missed if a delayed 7-day reading is not done.[33]

Surgical implant devices

The use of nickel in biomedical devices, especially in joint prostheses and endovascular stents, has led to increasing concern about the safety of permanent or semipermanent metal medical devices in suspected nickel-sensitized patients. Presently, there is high variability of care in terms of testing, recommendations, and, in some cases, selection of more expensive and less optimal

options. Unfortunately, there are no large, evidence-based, prospective case studies or expert panel consensus guidelines on this issue.[19]

In patients with ACD to orthodontics, nickel is the most common allergen. Stainless-steel arch wires are thought to release less amounts of nickel compared with flexible titanium-nickel arch wires. In a retrospective study of 131 patients suspected of coronary in-stent restenosis 6 months after 316L stainless-steel stent placement and patch testing 2 months after angioplasty, there were 11 positive patch test reactions in 10 (8%) of the patients. All 10 patients with a positive patch test reaction to metal (7 to nickel and 4 to molybdenum) had in-stent restenosis associated with clinical symptoms and a higher frequency of restenosis than seen in patients without metal allergy, suggesting that allergy to metals, nickel in particular, plays a relevant role in inflammatory fibroproliferative restenosis.[34] A prospective study of 174 patients with stents noted that patients with a recurrence of in-stent restenosis, although not after initial stent placement, had significantly greater positive patch test reactions to metals, most commonly nickel and manganese.[35] To date, the evidence for complications caused by nickel allergy is weak; proved cases are rare and remain on the case report level. The need for patch testing is controversial, and patch tests are not reliable in predicting or confirming implant reaction. A negative patch test reaction is reassuring for the absence of a delayed hypersensitivity reaction.

CD and patch testing in children

Although ACD is more common in teenagers, children as young as 6 months can be sensitized to contact allergen. The relevant allergens in children are similar to those in adults, with nickel, fragrance, and rubber being common sensitizers. The increasing rate of sensitization might be due to new trends in body piercing, tattooing, and use of cosmetic products. Adolescents constitute a significant portion of the population allergic to nickel. Kütting et al[36] recommend that ear piercing be delayed until after 10 years of age, presumably to allow for the development of immune tolerance. Children can tolerate the same patch test concentrations as adults, and polysensitization is common. Children with and without AD have the same rate of positive patch test reactions.

Treatment and prevention

Allergen identification to improve contact avoidance can be challenging, especially in work-related CD. Alternatives and substitutes to cosmetics should be offered to the patient to increase compliance. For patients with nickel allergy, barriers such as gloves and covers for metal buttons and identification of nickel by using the dimethyl-glyoxime test can be prescribed.

For supportive care and relief of pruritus, cold compresses with water or saline, Burrow solution (aluminum subacetate), calamine, and colloidal oatmeal baths might help acute oozing lesions. Excessive handwashing should be discouraged in patients with hand dermatitis, and nonirritating or sensitizing moisturizers must be used after washing.

A topical corticosteroid is the first-line treatment for ACD. For extensive and severe CD, systemic corticosteroids might offer faster relief. Studies on calcineurin inhibitors are limited, and their efficacy in patients with ACD or ICD has not been established. Oral antihistamines can be tried for pruritus, but oral diphenhydramine should not be used in patients with ACD to Caladryl (diphenhydramine in a calamine base) and hydroxyzine hydrochloride (Atarax) in an ethylenediamine-sensitive patient. Other modes of therapy are UV light treatment and immunomodulating agents, such as methotrexate, azathioprine, and mycophenolate mofetil.

OTHER IMMUNE-MEDIATED SKIN DISORDERS

Psoriasis

Psoriasis and AD have many similarities. They are common, chronic, inflammatory, and proliferative skin disorders in which both genetic and environmental factors play important roles. Psoriasis is primarily T_H1 mediated, whereas AD is generally thought to be T_H2 mediated. T cells in both diseases are triggered by conventional antigens and superantigens. Some studies suggest that group A streptococcus is a superantigen for acute guttate psoriasis, which is often preceded by or concurrent with infection[37,38] and is associated with an increase in serum antistreptococcal titers. Symptoms in patients with guttate psoriasis and AD frequently improve with systemic antibiotic therapy.

Although both psoriasis and AD have been associated with increased numbers of dendritic cells (DCs) in the skin, differences in DC populations, as well as the chemokine and cytokine environment, might have implications on potential targets for future therapeutic interventions. In patients with AD, myeloid DCs upregulate CCL17 and CCL18, which is in contrast to TNF-α and inducible nitric oxide synthase in patients with psoriasis.[39]

T_H17 cells have been implicated in the pathogenesis of psoriasis and other autoimmune inflammatory diseases.[37] Keratinocytes produce 2 T_H17 cytokines: IL-17A and IL-22. IL-23, which is overproduced by DCs and keratinocytes in patients with psoriasis, stimulates survival and proliferation of T_H17 cells within the dermis and drives keratinocyte hyperproliferation.

Autoimmune bullous diseases

Autoimmune blistering diseases are associated with antibodies against structural components of the skin and mucous membranes that maintain cell-to-cell and cell-to-matrix adhesion. In pemphigus vulgaris (PV), autoantibodies target the desmoglein adhesion molecule in the intercellular junctions and produce intraepithelial blisters. In bullous pemphigoid (BP) and epidermolysis bullosa acquisita, subepidermal blistering is associated with autoantibodies against the anchoring complex at the junction of the dermis and epidermis. Autoantibodies in patients with BP are formed against the basement membrane hemidesmosomal glycoproteins BP230 and BP180 and preferentially recognize phosphoepitopes in collagen XVII. In most subepidermal autoimmune blistering conditions, autoantibodies form deposits that cause the release of proteolytic enzymes through activation of the complement cascade, which destroys the basement membrane.[40]

Drugs containing sulfhydryl groups that cleave epidermal intercellular substances have resulted in the production of antibodies and blistering skin diseases. Penicillamine, furosemide, captopril, penicillin and its derivatives, sulfasalazine, salicylazosulfapyridine, phenacetin, nalidixic acid, and topical fluorouracil have been implicated.

The diagnosis of autoimmune bullous diseases requires the detection of tissue-bound and circulating serum autoantibodies by using various immunofluorescence methods, such as immunoblotting, ELISA, and immunoprecipitation.[41]

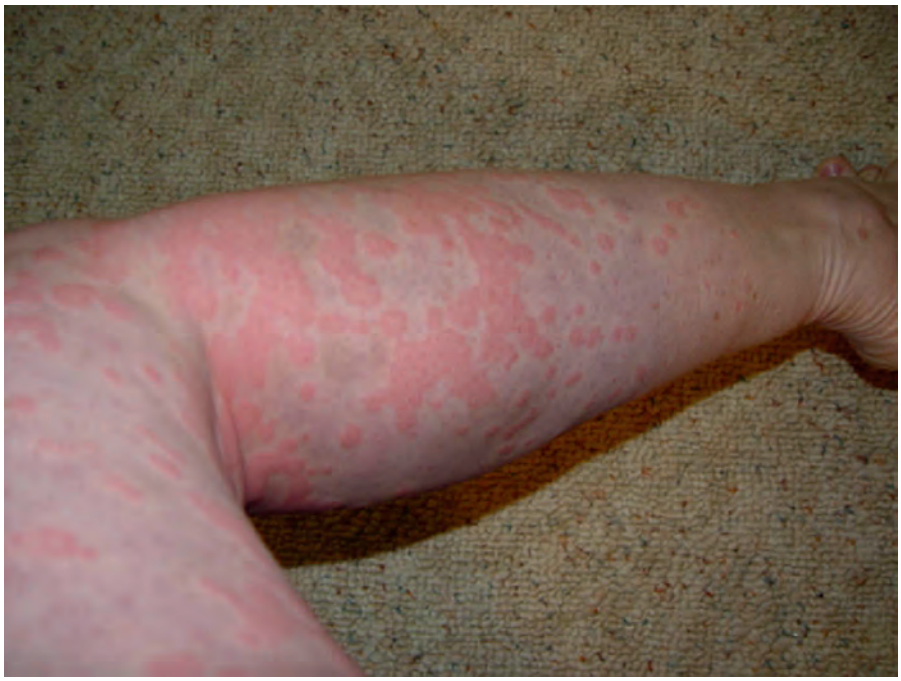

FIG 2. Urticarial skin lesions.

Treatment of PV includes long-term use of systemic corticosteroids to diminish autoantibody production, and only about 10% of patients achieve complete remission after initial treatment. Azathioprine, cyclophosphamide, methotrexate, mycophenolate, hydroxychloroquine, gold, and dapsone are other potential options. Rituximab, alone or in combination with IVIG, appears to be an effective therapy for patients with refractory PV and pemphigus foliaceus.

The mainstay of therapy in most patients with BP is oral corticosteroids at the lowest maintenance dose that will prevent new lesion formation and allow alternate-day therapy. A randomized trial suggests that patients with BP might have improved outcomes with topical rather than systemic corticosteroids, even in the presence of extensive disease.[42] Steroid-sparing agents, such as azathioprine, mycophenolate mofetil, cyclophosphamide, methotrexate, dapsone, and tetracycline, can be used in combination with prednisone. In patients with cicatricial pemphigoid with involvement of the eyes, esophagus, or larynx, IVIG, etanercept, and infliximab have been used.

URTICARIA

Significant advances have occurred in our understanding of chronic urticaria (CU) since the last publication of the primer in 2003, but our understanding of this challenging illness is still imperfect. This review will cite pertinent recent review articles, and the reader is encouraged to find primary citations within these reviews. During the last 5 years, further evidence has accumulated that quality of life is severely affected in patients with CU, and these patients deserve our unqualified attention.[43,44] The most significant recent conceptual advances in our understanding of CU have been (1) the deepening appreciation that there is evidence of autoimmunity for a substantial number of patients and (2) a better understanding of the implications of diminished basophil function.[45-48]Nevertheless, it is still unclear whether the detected autoimmune phenomena or defects in basophil signaling contribute to the pathophysiology of CU and, if they do participate, what pathways are involved. Therapy of CU has also advanced, with more evidence supporting the efficacy of immunomodulatory drugs.[49,50]

Background

Urticarias are pruritic, edematous erythematous lesions of variable size that blanch under pressure (Fig 2). An episode of urticaria is a common phenomenon affecting 15% to 25% of individuals during their lives.[51] Most of these cases are acute in nature and are easily managed, but about 30% of patients continue to have frequent episodes of hives for more than 6 weeks and are considered to have chronic disease.[44] Approximately 40% of patients also have angioedema, swelling of the subdermis, that accompanies the urticarial lesions. In a smaller number, approximately 10%, angioedema is present without visible urticaria.[44] CU occurs more often in adults and affects women (75%) more than men. Based on the results of history, physical examination, laboratory testing, and provocative testing, CU has been further divided into IgE-mediated urticaria (approximately 1% to 5%) or the physical urticarias (approximately 20%) and idiopathic urticaria (75% to 80%). The idiopathic cases, chronic idiopathic urticaria (CIU), include 30% to 60% who have an autoimmune phenotype, but the evidence that autoimmunity is pathophysiologic in the same way that physical stimuli are considered directly related to the development of hives is not generally accepted.[44,47,52] For the purpose of this discussion, patients with autoantibodies will be considered to have idiopathic urticaria with evidence of autoimmunity.

Pathogenesis

The primary effector cells in patients with urticaria are mast cells, which are present in high numbers throughout the body,

including the subcutaneous tissue. Activated mast cells produce a wide variety of proinflammatory and vasodilatory substances, including the immediate (<10 minutes) release of histamine from granules and the production of leukotriene C_4 and prostaglandin D_2 from membrane phospholipids. There is also more delayed (4-8 hours) production and secretion of inflammatory cytokines, such as TNF-α, IL-4, and IL-5. The immediate products are responsible for pruritus, swelling, and erythema, whereas the later products lead to an influx of inflammatory cells.[47]

Lesions of acute urticaria are characterized by subcutaneous edema with widened dermal papillae and rare inflammatory cells. Lesions of CU, in addition to the presence of edema, are characterized by a perivascular inflammatory infiltrate consisting of $CD4^+$ and $CD8^+$ T lymphocytes, eosinophils, basophils, and neutrophils. A small number of patients with urticarial vasculitis present with atypical clinical features and have histologic evidence of vascular destruction.[47]

In small subgroups of patients, CU is driven by IgE/allergen interactions stimulating the high-affinity receptor for IgE (FcεRI) or by physical stimuli acting through nonspecific pathways. For CIU, the pathophysiology is still unclear. The earliest observations suggestive of an autoimmune mechanism was by Grattan and Humphreys,[43] who reported in 1986 that sera from a subset of patients with CIU could cause a wheal-and-flare reaction when injected intradermally into their own (autologous) skin. The results of this autologous serum skin test (ASST) are positive in approximately 40% of patients with otherwise idiopathic CU and generally in less than 5% of control subjects.[52] Two other *in vitro* tests of serum-derived activity that activates basophils have been published. Assay of serum-mediated expression of CD63 on donor basophils correlates with the basophil histamine release (BHR) assay and assay of serum-induced expression of the surface marker CD203c, which is correlated with both the BHR assay and the size of the ASST reaction. There is general consensus that these assays detect IgG autoantibodies to the α-chain of FcεRI (90%) or IgE.[46,52] These "functional" autoantibodies are distinct from immunochemical detection of IgG recognizing FcεRI (α-chain) in an ELISA or on immunoblotting because autoantibodies measured in this fashion are found in many healthy subjects.[45-48,52]

Although at first glance the importance of functional autoantibodies to FcεRI appears to be a good conceptual framework, there are a number of limitations.[45,48] The finding that some donor basophils work better than others and that mast cells and basophils do not always work with the same serum is a mystery and undermines the general applicability of these assays.[45,48]. Although *in vitro* tests are dependent on IgG in the serum, some sera that result in a positive ASST response can still produce a positive ASST response after removal of IgG by protein G, suggesting the presence of other histamine-releasing factors.[45,48] Discrepancies have been reported between *in vivo* ASST and *in vitro* BHR tests. For example, only about 50% of sera from patients with CIU who have a positive ASST response have a positive BHR response with single-donor basophils, whereas the correlation is better if the study is done with those who have the strongest ASST responses and more than 1 donor of basophils/mast cells is used in the assay.[48] If the autologous test is performed with plasma, 86% of patients have positive responses compared with 40% of those when the test is performed with serum, suggesting that the coagulation pathway might play a role.[45,48] The importance of autoantibodies has been questioned because there are only small differences between the clinical course of those with and those without evidence of functional autoantibodies and the autoantibodies can be detected in patients who are in remission.[47]

An entirely different view of the mechanisms underlying CIU comes from the observation that patients with CIU tend to be basopenic and that the basophils that are present are relatively resistant to activation by anti-IgE. This has led Brodell et al[47] to divide patients with CIU into 2 groups: responders and nonresponders. Although the patients with the responder phenotype complain of more itching, these defects resolve as disease activity lessens, and there are only modest differences in the clinical course of these 2 populations.[48] An additional interesting finding is that these subgroups do not segregate with the subgroups with and without evidence of autoimmunity.[48] As in the case of the subpopulation with evidence of autoantibodies, the knowledge of these subgroups has not resulted in changes in therapy.

Diagnosis

This discussion will focus on recently described laboratory tests for patients with CIU that either lead to a specific treatment regimen or allow the physician to reassure the patient that their hives are due to an intrinsic process and not an extrinsic cause. Several general approaches to the workup of patients with CU have been recently published.[43,44]

Many specialists look for evidence of autoimmunity. The most common tests ordered are those for anti-thyroid antibodies because results on these tests are abnormal in 15% to 20% of patients with otherwise idiopathic urticaria. Other tests for autoimmunity include the ASST and 2 new *in vitro* tests for antibodies that activate target basophils: the BHR test and a test for upregulation of the basophil surface marker CD203c. As mentioned above, patients with evidence of autoantibodies have been reported to have more severe disease, but the effect is small.[43,44] In patients who are desirous of knowing what might be contributing to their CU, knowledge of these autoantibodies might help them accept that CIU is a skin disease and is not caused by an exogenous trigger.

Yet another area of controversy is the detection of infection with *Helicobacter pylori*. This common infection is found in a minority of patients with CU. A meta-analysis of 10 studies provided evidence that eradicating *H pylori* from patients with CIU who have evidence of this infection is beneficial at resolving the urticaria.[43] The pathophysiologic link between infection with *H pylori* and urticaria is uncertain, leaving the general idea that low levels of immune complexes might be causative.[44]

Measurement of the ability of *ex vivo* basophils from patients with CIU to release histamine when triggered with anti-IgE is still a research test that might become clinically useful in the future.[48]

Treatment

For many patients with acute urticaria and for a few patients with CU, a specific trigger can be identified, and avoidance can be an effective approach. This is not the case for some patients with acute urticaria and most with CU. A generally accepted approach for those with acute urticaria is to suppress the hives with H_1-type antihistamines, with preference for low-sedating and nonsedating agents on a daily basis and potentially sedating antihistamines for rescue and at night. For some patients with severe acute urticaria

who are unresponsive to antihistamines, a brief course of oral corticosteroids is warranted.

Treatment of patients with CIU is much more complicated. Low-sedating and nonsedating type 1 antihistamines remain the mainstay of therapy, and their efficacy has been shown to be greater than that of placebo in multiple double-blind, placebo-controlled trials. Many specialists believe that it is important to treat underlying immunologic and infectious conditions that have been detected through a detailed history, a thorough physical examination, and selected laboratory evaluations. If the urticaria is controlled with standard doses of H_1 blockade, it is reasonable to continue this treatment for several months, occasionally stopping it briefly to see whether the hives have spontaneously resolved. For patients whose symptoms are not controlled by H_1 blockade, there are a variety of opinions as to what to do next. A brief course of oral corticosteroids might be warranted, but systemic corticosteroids are not an acceptable long-term treatment. The only treatment beyond antihistamines that has been proved to be effective in a double-blind, placebo-controlled fashion is cyclosporin A.[43,44,49] Because this is a fairly aggressive treatment, some specialists try a variety of other interventions before prescribing cyclosporine. The most common approach is to push the H_1 blockade by using these agents at 2 to 4 times the FDA-approved dose.[44] Other approaches include adding an H_2 blocker, a leukotriene pathway modifier, or both. Commonly tried immunomodulatory agents with a better side-effect profile than cyclosporine include hydroxychloroquine, sulfasalazine, colchicines, dapsone, mycophenolate, and omalizumab (anti-IgE); however, none of these have been formally proved to be effective. The decision to treat patients with CIU who have anti-thyroid antibodies and normal thyroid-stimulating hormone levels with L-thyroxine is controversial.[43,52] Antimetabolites, such as methotrexate, azathioprine, and the anti–B-cell drug rituximab have also been used. Reviews of these treatments and specific guidelines for use of many of these agents and for monitoring risks and side effects have been published recently.[43,44,49,50]

AD

The reader is referred to a number of excellent recent reviews on the pathophysiology and treatment of AD.[51,52] This section will therefore primarily focus on advances in our understanding of AD since the last primer was published.

Skin barrier dysfunction

During the past year, it has become well accepted that skin barrier dysfunction plays a critical role in the development of AD. This is largely because loss-of-function null mutations in the skin barrier gene filaggrin have been repeatedly demonstrated to be a major risk factor for the development of AD.[53-56] Filaggrin gene mutations are associated with persistent and more severe eczema, early onset of AD, and an increased risk of asthma in patients with a previous history of eczema.[57,58] Therefore this mutation contributes to the atopic march by enhancing systemic allergen sensitization through the skin. Defects in skin barrier function, however, likely result from a combination of factors, including a deficiency of skin barrier proteins, increased peptidase activity, the lack of certain protease inhibitors, and lipid abnormalities.[59-61] Furthermore, T_H2 responses have also been found to reduce filaggrin gene and protein expression.[62]

Innate immune response

Study of the innate immune response in patients with AD has been an active area of investigation. There is now considerable evidence that a defective innate immune response contributes to increased bacterial and viral infections in patients with AD.[63] Pattern-recognition receptors play a critical role in sensing the environment for invading pathogens. Toll-like receptors (TLRs) are prototypic pattern-recognition receptors that discriminate between diverse pathogen-associated molecular patterns. A polymorphism within the *TLR2* gene has been shown to be associated with severe forms of AD prone to recurrent bacterial infections and has been linked to TLR2 dysfunction.[64]

Keratinocytes and DCs in the epidermis represent the key cells involved in the skin innate immune response. AD skin contains an increased number of IgE-bearing Langerhans cells (LCs). Binding of IgE to LCs occurs primarily through high-affinity IgE receptors. In contrast to mast cells and basophils where the FcεRI is a tetrameric structure, the receptor on LCs consists of the α-chain, which binds IgE and γ-chain dimers containing an immunoreceptor tyrosine–based activation motif for downstream signaling, but lacks the classic β-chain.[65] Allergens that invade the skin are taken up by IgE molecules bound to FcεRI-expressing LCs for allergen presentation to T_H2 cells. The clinical importance of these IgE receptors is supported by the observation that the presence of FcεRI-expressing LCs bearing IgE molecules is required to provoke eczematous skin lesions through application of aeroallergens to uninvolved skin of patients with AD.

Human plasmacytoid dendritic cells (PDCs) are the only professional IFN-producing cells, and their responses to viral antigens are important for effective host defense against viral infections.[66] Human PDCs bear TLR7 and TLR9 on their cell surfaces. Furthermore, they express FcεRI. Because of a close interaction of FcεRI with TLR9, the amount of IFN-α and IFN-β released in response to TLR9 stimulation is profoundly downregulated in PDCs after FcεRI aggregation and allergen challenge *in vitro*.[67,68] Compared with psoriasis, CD, or lupus erythematosus, the frequency of PDCs in patients with AD is decreased.[69,70] This might account for the increased propensity of patients with AD to have disseminated viral skin infections.

Keratinocytes play an important role in the skin innate immune response by producing antimicrobial peptides (AMPs) in response to stimulation by invading pathogens and inflammation or trauma to the skin. Defensins and cathelicidins are broad-spectrum AMPs that act as natural antibiotics to kill a wide variety of bacterial, viral, and fungal pathogens.[63] Chronic inflammatory skin diseases, such as psoriasis or CD, demonstrate marked upregulation of cathelicidin and defensin expression in their skin lesions. In contrast, AD skin lesions are associated with very weak upregulation of human β-defensin (HBD) 2 and 3 and LL-37.[71] Expression of T_H2 cytokines, such as IL-4, IL-13, and IL-10, have been shown to downregulate AMP expression *in vitro* and might account for low AMP levels in the skin of patients with AD.[71,72] Moreover, reduced mobilization of human HBD3 accounts for defective killing of *Staphylococcus aureus* in patients with AD.[73] In addition to the propensity for bacterial infections caused by low HBD2, HBD3, and LL-37 expression, cathelicidin and HBD3 deficiency in patients with AD also contributes to severe viral infections, such as eczema vaccinatum caused by orthopoxvirus[74] and eczema herpeticum (EH).[75] In support of this concept, lower levels of cathelicidin are detected

in skin lesions of patients with AD with 1 or more episodes of EH in their history compared with patients with those seen in patients with AD without EH.

The adaptive immune response in patients with AD

Systemic immune response. Most patients with AD have peripheral blood eosinophilia and increased serum IgE levels. This is reflected in an increased frequency of peripheral blood skin-homing T_H2 cells producing IL-4, IL-5, and IL-13 but little IFN-γ. This might be due to selective apoptosis of circulating memory/effector T_H1 cells in patients with AD.[76]The decreased IFN-γ levels produced by T cells from patients with AD might be the result of reduced production of IL-18.[77] Furthermore, an inverse relationship between skin colonization with *S aureus* and spontaneous T cell–derived IFN-γ production has been observed.[78]

Biphasic T_H2-T_H1 cytokine skin response. Acute AD skin lesions are associated with the infiltration of T_H2 cells expressing increased levels of IL-4, IL-13, and IL-31, a pruritogenic T_H2 cytokine that correlates with severity of AD.[79] A number of determinants support T_H2 cell development in patients with AD. These include the cytokine milieu in which the T-cell development is taking place, the costimulatory signals used during T-cell activation, and the antigen-presenting cells. IL-4 promotes T_H2 cell development, whereas IL-12 induces T_H1 cells. AD keratinocytes participate in the adaptive immune response by expressing high levels of the IL-7–like cytokine thymic stromal lymphopoietin (TSLP), which activates myeloid DCs to promote T-cell expression of IL-5 and IL-13.[80] Skin-specific overexpression of TSLP in a transgenic mouse resulted in an AD-like phenotype, with the development of eczematous lesions containing inflammatory dermal cellular infiltrates, an increase in T_H2 $CD4^+$ T cells expressing cutaneous homing receptors, and increased serum levels of IgE,[81] suggesting an important role of TSLP in AD. DCs primed by TSLP might convert to strong inducers of T-cell responses of the T_H2 type *in vitro*,[82] so that enhanced TSLP release triggered by frequent allergen challenge might initiate and microbes might perpetuate T_H2 immune responses in patients with AD.

LCs bearing FcεRI are the major myeloid DC population present in nonlesional and acute AD skin. After IgE binding and internalization of the allergen, LCs migrate to peripheral lymph nodes, present the processed allergen to naive T cells, and initiate a T_H2 immune response with sensitization to the allergen. Concomitantly, aggregation of FcεRI on the surface of LCs *in vitro* promotes the release of chemotactic factors, which *in vivo* is thought to contribute to the recruitment of IgE receptor–bearing inflammatory dendritic epidermal cells (IDECs) into the epidermis. IDECs mainly present at inflammatory sites, produce high amounts of proinflammatory cytokines after FcεRI cross-linking, display a high stimulatory capacity to T cells, and serve as amplifiers of the allergic inflammatory immune response.[83] Moreover, stimulation of FcεRI on the surface of IDECs induces the release of IL-12 and IL-18 and enhances the priming of naive T cells into IFN-γ–producing T_H1 or T_H0 cells. These mechanisms might contribute to the switch from the initial T_H2 immune response in the acute phase to the T_H1 immune responses in the chronic phase of AD.

Regulatory T cells

Other T-cell types can also contribute to the magnitude and persistence of AD-related skin inflammation. Recent studies have examined the potential role of regulatory T cells. Mice deficient in forkhead box protein 3–positive regulatory T cells spontaneously have eczema.[84] Although one report found an absence of resident regulatory T cells in AD skin lesions,[85] another found increased numbers of regulatory T cells in AD skin.[86] Reefer et al[87] analyzed the properties of $CD25^{hi}$ T-cell subtypes in patients with AD associated with increased serum IgE levels. $CD25^{hi}$ T cells expressing regulatory T-cell markers (forkhead box protein 3, CCR4, and cutaneous lymphocyte-associated antigen) were increased in patients with AD compared with those seen in control subjects with low serum IgE levels. This phenomenon was linked to disease severity. Two subtypes of $CD25^{hi}$ T cells were identified on the basis of differential expression of the chemokine receptor CCR6. Activated $CCR6^{neg}$ cells secreted T_H2 cytokines, and coculture with effector T cells selectively enhanced IL-5 production. Moreover, induction of a T_H2-dominated cytokine profile on activation with bacterial superantigen was restricted to the $CCR6^{neg}$ subtype. These studies indicate that despite a regulatory phenotype, activated $CD25^{hi}$ T cells that lack expression of CCR6 promote T_H2 responses and might therefore contribute to the atopic immune response.

T_H17 cells

T_H17 cells have also been identified in AD skin lesions and might therefore contribute to skin inflammation in patients with AD.[88] However, their expression is significantly less than that seen in patients with psoriasis.[89] Furthermore, T_H2 cytokines, such as IL-4 and IL-13, inhibit IL-17–induced effects on generation of AMPs by keratinocytes.[90]

Management

Recent approaches to the management of AD have focused on the development of improved skin barrier creams, early dietary interventions, and novel immunomodulators that can reduce skin inflammatory responses.[51,91] There remains interest in treating infants with hydrolyzed infant formulas[92] or probiotics to control eczema early in life by directing allergic responses.[93-96] Perhaps the most interesting development is the observation that oral supplementation with vitamin D augments the innate immune response in patients with AD.[97] There also remains interest in the use of topical calcineurin inhibitors as an anti-inflammatory therapy. Promising novel anti-inflammatory therapies are also gaining attention. Although these require further controlled trials, they include lymphocyte function-associated molecule 3/IgG fusion protein[98] and anti-CD20.[99]

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Environmental and occupational allergies

David Peden, MD,[a] and Charles E. Reed, MD[b] *Chapel Hill, NC, and Rochester, Minn*

Airborne allergens are the major cause of allergic rhinitis and asthma. Daily exposure comes from indoor sources, chiefly at home but occasionally at schools or offices. Seasonal exposure to outdoor allergens, pollens, and molds is another important source. Exposure to unusual substances at work causes occupational asthma, accounting for about 5% of asthma in adults. Indoor and outdoor air pollutants trigger airway inflammation and increase the severity of asthma. Diesel exhaust particles increase the production of IgE antibodies. Identification and reduction of exposure to allergens is a very important part of the management of respiratory allergic diseases. The first section of this chapter discusses domestic allergens, arthropods (mites and cockroaches), molds, and mammals (pets and mice). Indoor humidity and water damage are important factors in the production of mite and mold allergens, and discarded human food items are important sources of proliferation of cockroaches and mice. Means of identifying and reducing exposure are presented. The second section discusses outdoor allergens: pollens and molds. The particular plants or molds and the amount of exposure to these allergens is determined by the local climate, and local pollen and mold counts are available to determine the time and amount of exposure. Climate change is already having an important effect on the distribution and amount of outdoor allergens. The third section discusses indoor and outdoor air pollution and methods that individuals can take to reduce indoor pollution in addition to eliminating cigarette smoking. The fourth section discusses the diagnosis and management of occupational asthma. (J Allergy Clin Immunol 2010;125: S150-60.)

Key words: *Allergens, indoor environment, mites, cockroaches, mice, pets, molds, pollens, humidity, water damage, air pollution, occupational asthma, climate change*

Abbreviations used
DEP: Diesel exhaust particle
ETS: Environmental tobacco smoke
HEPA: High-efficiency particulate air
NO_2: Nitrogen dioxide
SO_2: Sulfur dioxide

Two key factors influence the development and severity of allergic disease: host factors and environmental factors. Environmental factors include the specific allergens that are the targets of the IgE-mediated immune response, those elements of the environment that influence the presence of those allergens, and indoor and outdoor air pollutants. Also, environmental stimulants of innate immunity influence the development of allergic responses. Although pharmacologic treatments focus on host factors, interventions directed at environmental factors are critical for optimal management of allergic disease, as well as its prevention. Environments can be defined as domestic, outdoors, and occupational, and this chapter will focus on the identification of environmental exposures and methods of intervention for their control.

INDOOR DOMESTIC ALLERGY

Background

The primary indoor allergens that contribute to allergic disease include arthropod allergens, mammalian allergens (from either pets or pests), and fungal allergens.[1-5] Additionally, indoor pollutants can also influence host response to allergens and should be considered when developing environmental interventions.[6] Seasonal outdoor allergens can also play a role in the indoor environment when they penetrate into the indoor setting.[6]

Pathogenesis: Allergens

There is overwhelming evidence that indoor domestic allergens play a key role in allergic disease. The primary arthropod allergens associated with allergic disease are house dust mites and cockroaches.

House dust mite allergen. The 2 primary species of house dust mite associated with asthma are *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*. The prevalence of IgE sensitization to mites varies with the local environment; arid environments are associated with low-level sensitization (5%), whereas up to 60% of the population can be sensitized in humid locales. Exposure to mite allergens has been associated not only with the severity of allergic disease but also with disease pathogenesis.[3,4,6-8]

These microscopic arachnids do not bite humans or other animals but feed on human and animal dander and are found in bedding, upholstery, and carpeting. House dust mites require humid environments because they directly absorb water from the air, with critical relative humidity ranging from 55% to 75% depending on the ambient temperature. There are 2 major groups of mite allergens, with group 1 being derived from proteins found in the mite gut and group 2 being primarily male reproductive glycoproteins. A major source of mite allergens is mite fecal pellets. These allergens are found on particles that range from 10 to 20 μm in size, which means they tend to settle on surfaces and are not suspended in ambient air.[9,10]

From [a]the Department of Pediatrics, University of North Carolina, Chapel Hill, and [b]the Department of Medicine, Mayo Clinic, Rochester.

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Reprint requests: Charles E. Reed, MD, 9193 Bald Eagle Rd, PO Box 158, Boulder Junction, WI 54512. E-mail: cereed@centurytel.net.

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Cockroach allergen. Cockroaches represent another significant source of allergens, with the German cockroach *(Blatella germanica)* and the American cockroach *(Periplaneta americana)* being the most frequently encountered species in American homes. Cockroach allergy plays a critical role in asthma pathogenesis in the inner city, with the degree of sensitization being linked to the likelihood of requiring urgent or emergency treatment for asthma in urban populations. It has been reported that up 40% of urban children and 20% of suburban children are sensitized to cockroach allergens.[9,10]

Cockroaches tend to feed on discarded human food items. Thus they are attracted to locations in which such materials are readily available. Although they are found in single-family homes, they are more successful in townhomes and multifamily dwellings, which have a higher concentration of persons and, consequently, more discarded food. Cockroaches live in confined spaces, often in walls and between floors in large buildings, and are more active at night.[11]

Cockroach allergens derive from the bodies and feces of these insects. Like mite allergens, cockroach allergens tend to be found on larger particles (10-40 μm in diameter) and thus are more likely to be found in settled house dust rather than on suspended particles in ambient air. Cockroach allergen can be found in high concentrations on floors, carpets, counters, and other flat surfaces, especially in rooms that contain discarded or stored food. Cockroach allergens have also been reported in bedding, although this might be from passive transport of allergens from floor dust to the bed by persons living in cockroach-infested locations.[9,11]

Mouse allergy. Rodent allergens are also important allergens in the inner city, with mice being more common in domestic settings than rats because rats tend to stay outdoors. Mouse allergen exposure has been associated with decreased asthma control in inner-city residents. Mouse allergen has also been found in suburban settings and single-family homes but at levels that are typically 100- to 1,000-fold less than those reported in inner-city dwellings.

Mouse allergens are present in urine and are associated with pheromones and the mating behavior of these animals. Rodent urine is easily aerosolized, and thus rodent allergens can be found in smaller particles (<10 μm in diameter), which can be suspended in ambient air. Like cockroaches, feral mice tend to nest in small hidden spaces and are active primarily at night. Thus it is relatively rare to encounter these animals during the day. These animals are also attracted to discarded human food materials, and thus mouse allergen might be found in greater concentrations in areas where garbage is stored before disposal. In the event that mice are kept as pets, exposure to mouse allergen is similar to that of animal handlers and is principally in the bedding of the cages in which the animals are kept.[7,12-17]

Pet allergy. Mammalian pets are also a source of allergens, with dogs and cats being by far the most common pets in the United States. Common allergens derived from dogs and cats include Can f 1 and Fel d 1 and can be derived from saliva, dander, or other secretions. Like rodents, dog and cat allergens are found in small aerosolized particles (<10 μm in diameter) and can be found suspended in ambient air.[4,18-21] These allergens, especially those from cats, can be carried to other locations on the clothing of persons who own cats. It has also been reported that dog and cat allergens are found in house dust of homes with and without animals. Thus community exposure to these and probably many other domestic allergens likely contributes to exposure to these allergens outside of the home.[7,9]

There is a paradox that has developed with regard to the role that pets have in asthma and atopic disease pathogenesis. It has been argued that many persons with pet allergy do not have cats in their homes, and conversely, many persons who live with mammalian pets do not have clinical disease.[22,23] It has been reported that owning mammalian pets might actually be protective against the development of atopy.[24] Whether this is due to associated increases in domestic endotoxin levels (which, according to the hygiene hypothesis, would protect against atopy) or development of immunologic tolerance is unclear.[24] However, there is strong consensus that in persons with IgE sensitization to mammalian pets and clinical disease, increased exposure to pet allergen is deleterious.

Mold allergy. Mold is a term that encompasses hundreds of species of saprophytic fungi that can be found in the indoor environment. Molds require high humidity and moisture, adequate temperature, and nutrients. It is clear that IgE sensitization can occur to molds, and there is great interest in the role these allergens play in asthma exacerbation and pathogenesis. Quantifying mold exposure can be very complex and is not standardized for clinical practice. Methods for this include culture of spores from recovered environmental samples, spore counts, and assessment of fungal allergens in recovered house dust or other fungal products (eg, 1-3 β-glucans, which themselves exert health effects). Of note, mold spores are between 2 and 10 μm in size and thus can remain in ambient air for extended periods of time.

The variety of measures used in mold quantification has complicated the study of the role of indoor fungi in asthma. However, it is known that *Alternaria* species in outdoor settings is linked to increased asthma severity and airway reactivity. In the Inner City Asthma Consortium Studies indoor mold levels correlated well with outdoor levels, emphasizing that the outdoor environment plays an important role in establishing indoor mold levels. The National Academy of Science reviewed the relationship of mold and fungal exposures to asthma exacerbation and pathogenesis and stated that there was sufficient evidence that fungal allergen exposure caused disease exacerbation in sensitized subjects but that the existing data were inconclusive regarding the role of fungal exposures on disease pathogenesis.

Pathogenesis: Nonallergens

Indoor combustion. Combustion of biological matter results in notable indoor air pollution and often is due to burning of tobacco, wood, and other plant fuels.[25-28] Byproducts of plant-fuel combustion include particulates, which are rich in polyaromatic hydrocarbons, and other constituents that are converted intracellularly to a number of oxidant species.[29] Burning of wood in indoor stoves and fireplaces generates a number of particulates and oxidant gases and is strongly associated with increased respiratory tract illness. However, environmental tobacco smoke (ETS; side-stream smoke from the burning end of a cigarette and exhaled mainstream smoke from a smoker) is the most significant and remediable indoor air pollutant in the United States. An example of the effect of ETS on indoor particulate levels is shown in a study of 11 hospitality locations (primarily restaurants) in which smoking and nonsmoking sections were maintained. The

average concentration of particulate matter with a diameter in the range of 2.5 μm in smoking areas was 177 $\mu g/m^3$ versus 87 $\mu g/m^3$ in the nonsmoking section, which is still 29 times higher than that in truly smoke-free air and 6 times higher than that of local outdoor air.[30]

Exposure to ETS is unequivocally associated with exacerbation of asthma and is a notable contributing factor to disease severity and pathogenesis, with numerous reviews outlining the effect of ETS on asthma exacerbation and sensitization to allergens.[1,25,29,31-38] Experimental exposure to ETS augments nasal responses to allergen in atopic human subjects, with investigators reporting increased allergen-induced specific IgE and IgG4 levels; increased IL-4, IL-5, and IL-13 levels; decreased IFN-γ levels; and increased amounts of postallergen histamine in nasal lavage fluid.[39] Taken together, these studies provide initial mechanistic support to the epidemiologic reports suggesting that ETS exposure enhances the development of atopy and asthma.[29,40,41]

Another significant indoor pollutant is nitrogen dioxide (NO_2), which derives from use of natural gas appliances, especially if they are poorly maintained or poorly vented. Increased levels of NO_2 in domestic settings are associated with increased respiratory symptoms, such as cough, wheeze, production of phlegm, and bronchitis in exposed children, as well as an enhancement of the effect of viral infection in patients with asthma.[42-46]

Biological agents. Biological contaminants certainly contribute to poor air quality, including indoor endotoxin and products from gram-positive bacteria, and 1,3–β-glucans from molds might also affect airway inflammation in both atopic and nonatopic subjects. There are clearly 2 sides to the role that indoor biological agents might play in asthma because a great many articles have described the apparent protective effect that endotoxin and other agents have in the development of asthma. However, others have shown that increased indoor endotoxin levels are associated with increased respiratory tract illness in both allergic and nonallergic persons in both domestic[47] and occupational[48-50] settings. Endotoxin exposure seems to protect infants from asthma but increases it in adults. In domestic settings the number of animals (dogs, cats, and evidence of rodents) and persons living in the home correlate with the amount of endotoxin present.

Humidity. Indoor relative humidity is increasingly recognized as an important factor in determining asthma severity. Decreased levels of humidity are associated with decreased severity of asthma.[51-53] In a large cross-sectional study of fourth-grade schoolchildren in Munich, Germany, Nicolai et al[54] identified 234 children with active asthma, with 155 of these children undergoing lung function and nonspecific airway reactivity tests within a 3-year span. Dampness was associated with increased nighttime wheeze and shortness of breath but not with persisting asthma. Risk factors for bronchial hyperreactivity in adolescence included allergen exposure and damp housing conditions. Mite antigen levels were examined from homes of 70% of the asthma cohort and found to significantly correlate with dampness and bronchial hyperreactivity. However, the effect of dampness was not due to mite allergen alone because bronchial hyperreactivity remained significantly correlated with humidity, even when adjusting for mite allergen levels.

Diagnosis

General considerations. The items outlined in this diagnosis section have been reviewed extensively elsewhere.[7,9,55-57] Evaluation of environmental allergy involves a number of important elements: a clinical history consistent with allergic airway disease, the presence of IgE sensitization to suspect allergens, and determination of exposure to increased levels of environmental allergens, as well as nonallergenic factors that contribute to disease. Frequently, the clinical history includes a number of general points found in most patients with allergic airway disease. These include a history of recurrent respiratory disease, nighttime cough, exercise intolerance caused by cough or wheeze that occurs after aerobic exercise, and exacerbations associated with viral illness. However, other elements of the history might suggest strong environmental factors. This can include improvement of symptoms on vacation or other periods when the patient is away from his or her primary home or, conversely, worsening of symptoms when visiting a new environment. Although much of this discussion has focused on asthma, symptoms of allergic rhinitis and conjunctivitis can also increase at these times.

An environmental health history can be complicated by a number of factors. Many persons, especially children in dual custodial families, might live in more than 1 location on a regular or intermittent basis. Additionally, many patients might not be forthcoming regarding environmental factors in the home that could be relevant. Such factors can include increased symptoms with the addition of a new pet, smoking behavior of a parent (or the patient), or the presence of cockroaches or mice in the home. Patients can also be exposed to allergens in other settings in which they have less control, such as school buildings or work sites. For instance, it has been shown that allergen levels in day care settings might frequently exceed those levels shown to induce symptoms in domestic settings.

It is also important to establish that IgE-mediated processes are viable candidate mechanisms for a given subject's allergic disorders. Skin or serologic testing of allergen-specific IgE to appropriate allergens should be carried out for all patients presenting with a history consistent with allergic disease. Mite allergen testing should be conducted for most patients living in all but the most arid locations, many of which are above 5,000 feet in elevation. Testing for cockroach allergen should be considered for all patients but especially for those patients who live in multifamily dwellings or other institutional housing settings (eg, military barracks, colleges, and detention centers).

Testing for pet allergens should be considered for persons who own a pet or are going to move to a location in which a dog or cat has been owned by the previous occupants. Rodent allergy testing should be considered for those with indications for cockroach allergy. However, mouse allergens might be more widespread than previously thought. Mold allergens should be considered, especially for those living in damp environments. Although there are hosts of molds one might assess, *Alternaria*, *Aspergillus*, and *Penicillium* species are perhaps the most common indoor fungi. This list should also be expanded based on local mold populations. If there is doubt that respiratory symptoms are due to allergic asthma, other evaluations, including chest and sinus imaging, methacholine testing, and perhaps exercise testing should be considered.

Environmental history for mites. There are specific questions that are especially helpful to establish that specific allergen exposures might be contributing to disease. As noted above, house dust mites require humid environments and reasonably warm ambient temperatures. Additionally, if the amount of animal and human dander available to the mites is increased (many persons in a given bed, persons with eczema, and not

TABLE I. Domestic environmental interventions by allergen or pollutant source

	HEPA filter	Dehumidification and air conditioning	Washing bedding in hot water	Professional extermination	Removal of allergen source or contaminant	Cleaning of walls and floors	Securing food waste	Inspect crawlspace	Repair wall and floor cracks
House dust mites		xx	xx			xx		xx	
Cockroach				xx		xx	xx		xx
Pets	xx				xx	xx			
Mice	xx			xx		xx	xx		xx
Molds	xx	xx				XX		xx	
Tobacco smoke	xx				xx				

changing or washing sheets frequently), then the chance of mite allergen exposure is increased. Non–air-conditioned homes also have increased humidity, and this is an increased risk for mite allergen exposure. Indeed, it is not uncommon for persons to actively humidify the bedroom of an asthmatic subject, thinking that this intervention will be helpful. In fact, it often is exactly the wrong thing to do. Recently, home kits have been developed for use by homeowners to determine whether they have increased exposure to mite allergens.

Environmental history for cockroaches. Factors that might increase cockroach exposure include living in multifamily dwellings, the presence of available (open-pail or undisposed) waste food, and infestation with cockroaches in neighboring units of an apartment or condominium. Surveying the living space for cockroaches at night (they are less active during the day), especially in kitchen areas and places where food is consumed, is useful to confirm that cockroach infestation (and thus exposure) has occurred. Additionally, adhesive bait traps can be set, with recovered cockroaches serving as an indicator of total cockroach burden in the dwelling. If it remains unclear whether cockroach infestation has occurred, then a professional exterminator or entomologist can be consulted.

Environmental history for mice. Discovery of rodent droppings is the most common sign of an infestation. However, one might need to inspect crawlspaces, attics, and other hidden areas of the home to find mouse nests. Occasionally, mice can be found moving at night, and therefore nocturnal inspections might be helpful. Scratching sounds can also be heard with mouse infestation.

Environmental history for mold. Determining whether mold exposure is playing a role in a patient's disease is not standardized and can be frustrating. Demonstration of fungal colonies on drywall, caulking, and floor spaces suggests that molds might be playing an important role. Additionally, moisture plays a significant role in supporting mold populations. Homes that have been flooded or have been water damaged are more likely to harbor mold. Examination of plumbing for leaks might reveal an area that has been colonized by mold. Many environmental contractors offer testing for mold spore counts, often by sampling the ambient atmosphere and then determining how many fungal cultures are present. Unfortunately, such tests are not standardized, and it is difficult, if not impossible, to know what level of mold spores in ambient air represent a health risk. However, if one is interested in establishing whether a specific humidity intervention is useful, one might get a baseline assessment and undertake it again when the work is done.

Humidity and pollution. As noted above, there are a number of nonallergenic factors that can affect disease. Humidity and moisture control is one of these factors, and it has been briefly discussed with regard to mold exposures. The best way to determine whether the relative humidity is too high or too low is to measure it with a hygrometer or relative humidity gauge. Mechanical or electronic hygrometers can be purchased at a hardware store or building-supply store and will provide a good assessment of indoor relative humidity levels. Use of air conditioning and dehumidification are essential elements of humidity control in most temperate climates. Ideally, relative humidity should be no higher than 50% to 55% in the summer and 30% in the winter. Fireplaces can also be sources of water vapor, as well as other gases and particulates.

If persons who live in the house are smokers, this will be an important source of indoor pollutants. Although it is preferable that one does not smoke, there is reduced particulate pollution if smokers truly smoke outside. Many indoor air cleaners that are touted to decrease ambient air tobacco smoke are not very effective. Other indoor sources of pollution include gas stoves, furnaces, and artificial logs. Questions should focus on how well these devices are maintained and whether the exhaust is adequately ventilated.

Treatment

Interventions for environmental allergy can be focused on decreasing host reactivity to allergens (medically with inhaled or nasal corticosteroids, leukotriene inhibitors, antihistamines, short- and long-acting β-agonists, or allergen immunotherapy) and decreasing exposure to environmental allergens and adjuvants (Table I). Recent studies suggest that environmental interventions are most effective when an integrated approach is used in which the patient's specific allergen sensitivities and all of the appropriate environmental factors are simultaneously and appropriately addressed.

As noted in preceding sections, control of indoor humidity and moisture is essential for control of many allergens, including house dust mites and fungi, which are very sensitive to humidity. Air conditioning and dehumidification can be useful in decreasing humidity. Appropriate venting of kitchen and bathroom spaces is also an important intervention, as is checking for leaking plumbing fixtures and appropriate vapor shields in crawlspaces.

Indoor sources of combustion should also be assessed for their effect on indoor air quality. Fireplaces are sources of water vapor, particulates, and various gases, including carbon monoxide and nitrogen oxides, in homes. Gas stoves and furnaces can be sources of NO_2 and carbon monoxide. It is important that these sources of pollutants be well ventilated. There is also some evidence that smoking cigarettes only outside the home might decrease indoor particulate levels, although promotion of truly smoke-free homes is the optimal solution.

Some indoor activities have been associated with increased indoor pollutant and allergen levels, including use of humidifiers, gas cooking, sweeping, and smoking. Additionally, as noted

earlier, using air conditioning, keeping windows closed, and staying indoors decreases the likelihood that outdoor environmental agents (humidity, pollens, molds, ozone, and particulate matter) will infiltrate the indoor setting.

High-efficiency particulate air (HEPA) filters might be useful in decreasing exposure to certain allergens or pollutants. They are most helpful for agents found on particles small enough to be suspended in ambient air (generally <10 μm in diameter) and include allergens from mammalian pets or vermin, fungal spores, and particulates derived from wood or tobacco burning (although HEPA filtration should not be the preferred method of decreasing ETS exposure).

Measures used to control house dust mites depend on decreasing humidity, washing bedding in hot water (>130°F), and using mite-impermeable sheets, pillow covers, and mattress covers. However, some studies question the effectiveness of this latter approach. Cockroach control should involve professional extermination, removal of food sources, and checking walls, floors, and plumbing fixtures for holes or gaps and filling these to prevent these insects from entering the building again.[58-62] Ironically, control of mouse allergens includes many of the same concepts as control of cockroach allergen but should also include inspection of crawlspaces and other hidden areas for nests. Because of the large size of the particles that contain most mite and cockroach allergens, HEPA filters are not useful interventions for these exposures, although they can be useful for rodent allergen control.

Optimal control of pet allergen exposure involves removal of the pet and thorough cleaning of the home. However, even with these measures, pet allergens can persist for up to 6 months. Some have reported that washing pets on a regular basis (especially cats) might decrease allergen exposures. In the event that removal of an animal is not feasible, keeping the pet in an area of the home isolated from the patient's bedroom is often recommended.

Although some meta-analyses suggest that there is insufficient evidence to support the use of allergen control measures as a treatment for asthma, many recent studies demonstrate that maneuvers to decrease allergen levels in a domestic setting are effective in decreasing allergens and decreasing asthma severity.[61,62] These studies also suggest that integrated, multifaceted approaches are more effective than one approach alone.[63] An integrated approach includes establishing the IgE sensitization of the patient and designing an allergen control program to account for decreasing the relevant allergen and adjuvant agents that can affect disease.

OUTDOOR ALLERGENS AND CLIMATE CHANGE

Background

Airborne pollens and molds are important causes of allergic rhinitis and asthma and therefore have been a major focus of research since the 19th century. In as much as the details of each local climate determine which plants and molds will grow there, recently, there has been considerable interest in the effect of climate change on outdoor allergens.[64] The dates and amount of exposure to specific allergens at specific locations can be measured by using several methods. The most common is microscopic identification of the individual pollen grains and mold spores using Rotorod samplers or Burkard spore traps. Exposure to particular species of molds can also be determined by culturing of airborne particles. Particle size and allergen concentration can be determined by using filtration samplers with immunochemical assay of the filter.[64-66] Information about outdoor allergen concentrations at many locations is available from the National Allergy Bureau (www.aaaai.org/nab/).

Pollens

Tree pollens are shed in the spring, grass pollens in early summer, and weed pollens (especially ragweed) in late summer and fall. Pollen grains deposit on the nasal mucosa and release allergenic proteins to cause hay fever. Pollen grains are too large to be respirable, and therefore they do not reach the bronchi to cause asthma. Furthermore, the timing of pollen-induced asthma differs from that of hay fever in 2 ways: it starts later in the season and persists after the season ends. Also, it is worse during thunderstorms. Many of the important allergens of pollens lie on the outside of the cell membrane, the exine. They are not produced by the pollen cell itself but are stuccoed onto the exine by other cells of the male flower. A considerable amount of these allergens remain behind for weeks after the pollen is shed. Respirable bits of this part of the plant become airborne, especially from gusts of wind during thunderstorms. It is also possible that allergens extracted from pollen grains by raindrops can become airborne dust particles after drying.[67] This is one reason that asthma symptoms begin after hay fever symptoms and persist longer.

Molds

Allergy to outdoor molds, especially *Alternaria alternata*, is a more important cause of asthma than pollen.[68,69] Other important species include *Cladosporium*, *Penicillium*, *Aspergillus*, and *Helminthosporium*. Because air-sampling methods rely chiefly on mold spores, it is often assumed that the spores are the main source of the allergens. However, a spore is no more the whole organism than an acorn is an oak tree, and *Alternaria* species spores, like pollen grains, are too large to penetrate into the bronchi. More important sources of allergen-containing particles are the hyphae (which are fibrous and therefore stay suspended in the airstream) and dust from the area where the mold was growing and excreting digestive enzymes. This is important because mold proteases are not only allergens but also cause mast cell/eosinophil inflammation and promote IgE to other proteins through stimulation of protease-activated receptors.[64,66,70,71] Unfortunately, unlike the important pollen extracts, commercial mold extracts are not standardized and might not contain all the important allergenic molecules.[72] As a result, *in vitro* tests for IgE antibody to some molds (especially *Aspergillus* and *Penicillium* species but fortunately not *Alternaria* species) are more reliable than skin tests.

Climate change

Global warming is accelerating; an average warming of 1°C to 2°C is certain to occur in this century. If current emissions and land-use trends continue unchecked, increases in the prevalence and severity of asthma and related allergic diseases mediated through worsening ambient air pollution and increased pollen production are anticipated.[64] The sea will rise, and storms and drought cycles will increase. The pattern of change will vary regionally depending on latitude, altitude, rainfall and storms, land-use patterns, urbanization, transportation, and energy production.

TABLE II. What do we know about climate change and asthma?

What do we know?
Ambient air pollution increases the frequency and severity of asthma attacks and the number of symptomatic days.
Pollen, air pollution, and weather interact and affect the clinical expression of allergic disease.
Climate change is unequivocal, accelerating, and largely anthropogenic and will continue through at least the 21st century.
Climate change is measurably affecting the timing, distribution, quantity, and quality of aeroallergens and changing the distribution and severity of allergic disease.
Climate change alters local weather patterns, including minimum and maximum temperatures, precipitation, and storms, all of which affect the burden of allergic disease.
Warming temperatures promote production of ground-level ozone, which worsens asthma.
There are clinical interventions that can be used to minimize climate change–related increases in asthma and allergic disease (secondary prevention).
Greenhouse gas mitigation is the current global recommendation for stabilizing the climate (primary prevention).
What is still unknown?
Future air quality will be determined by energy and transportation choices, economic development, and population growth.
The degree to which human intervention and planning can minimize changes in vegetation and aeroallergen exposure remains unexplored.
The rate and magnitude of climate change in the future will depend on how rapidly and successfully global mitigation and adaptation strategies are deployed.
The outcome of crossing climate tipping points is unknown but potentially very grave for large portions of the global population.
New technologies addressing climate change and air pollution, as well as new medical treatments for asthma, allergic disease, or both could alter current predictions and trends.

Used with permission from Shea et al.[64]

Climate changes have profound effects on vegetation and floristic zones. Between 1990 and 2006, hardiness zones moved substantially northward in the United States because of the warming climate. In urban areas, where CO_2 levels were 30% higher and temperatures were 2°C higher than in matched rural areas, ragweed grew faster and larger and produced more pollen.[64] In general, increased temperatures produce earlier flowering and longer pollen production. Increased CO_2 levels produce pollen production and might cause some plant proteins to become more allergenic. Table II summarizes the effect of climate change on allergic respiratory disease.

Management

In addition to the usual pharmacologic treatment for allergic rhinitis and asthma, avoidance of exposure to outdoor allergens is an important part of management. The patient should be advised to stay indoors in an air-conditioned building as much as possible. Many patients find it practical to take their summer vacation in a location where there is little or no exposure. In exceptional cases in which asthma is unusually severe, such as *Alternaria* species–induced asthma in the Midwest, it might be advisable for the patient to move to a climate where *Alternaria* species is minimal, like the shores of the Pacific Ocean.

AIR POLLUTION AND ASTHMA

Increased exposure to respirable particulate matter (<10 μm in size) is associated with exacerbation of asthma across the world.[74-83] Studies performed in Utah clearly demonstrated the relationship between airborne particulates and occurrence of respiratory disease associated with the activity of a steel mill that was inactive for a year because of a labor dispute.[84,85] Occurrence of asthma and the level of particulates were less during the strike year compared with those during nonstrike years. The relationship of proximity to a roadway, and presumably vehicular traffic, is correlated with increased asthma. In a study of approximately 6,200 German children, traffic counts correlated with active asthma, cough, and wheeze.[51] In a study in the United Kingdom,[86] children less than 5 years old were more likely to be admitted to the hospital for asthma if they lived within 500 m from a heavily traveled road. The effects of specific pollutants are outlined below, and sources for many of these pollutants are listed in Table III.

Diesel exhaust and allergy

Diesel exhaust particles (DEPs) have been shown in numerous animal, *in vitro*, and human challenge studies to skew immune responses toward a T_H2 response.[72,73,87-90] It is thought that this effect of diesel results from oxidative stress generated by the conversion of polyaromatic hydrocarbons to quinones. In human subjects nasal challenge studies have shown that DEPs increased nasal IgE production. In subsequent studies, which are extensively reviewed elsewhere,[29,41,87-89] this group has reported that DEP challenge of the nasal mucosa causes increased T_H2 cytokine production by cells in recovered nasal lavage fluid. DEPs also enhance ragweed-specific IgE and IgG responses to ragweed allergen, which were characterized by increased expression of T_H2 cytokines and decreased expression of IFN-γ and IL-2. DEP challenge can also shift the primary immune responses of the nasal mucosa in human subjects toward a T_H2 phenotype, yielding allergen-specific IgE.[91]

Sulfur dioxide

The effects of sulfur dioxide (SO_2) have been extensively reviewed.[92-95] Total emergency department visits for respiratory problems and increased hospital admission rates have been linked with increased ambient exposure to SO_2. In children decreased lung function has been linked to increases in ambient SO_2 levels, and the likelihood of chronic asthma or obstructive lung disease likewise is associated with lifetime exposure to SO_2. However, in many of these studies, it is difficult to separate the effects of SO_2 from those of particulate air pollutants. Additionally, ambient SO_2 might contribute to acid aerosol (H_2SO_4) formation and might exert effects either as a gas or by contributing to H_2SO_4 particle formation.

TABLE III. Sources for air pollutants that cause asthma (source: http://www.epa.gov/air/emissions/index)

SO_2: Burning of coal, oil, and fossil fuels with a high sulfur content, usually power generation and industrial sites
NO_2: On- and off-road vehicle use, electricity generation, industrial processes, fossil fuel burning
Ozone: Derived from interaction of NO_2 and related nitrogen oxides with sunlight (UV light); thus this depends on vehicle use.
Particulate matter: Uncontrolled fire and planned wood combustion, road dust, electricity generation, and vehicle use

NO_2

There is a strong relationship between ambient air NO_2 levels and changes in lung function. NO_2 challenge enhances airway inflammation, primarily with an influx of airway PMNs. These effects are most notable at higher levels of NO_2 (4.0 ppm) and might affect the airway function of asthmatic subjects.[92-95] SO_2 also has an effect on the response to airway allergen in allergic asthmatic subjects.[96-99] Exposure to 0.4 ppm NO_2 and a combination of 0.2 ppm SO_2 and 0.4 ppm NO_2 have both been shown to enhance immediate bronchial responses of subjects with mild asthma to inhaled allergen. Exposure to NO_2 has also enhanced late-phase responses of asthmatic subjects to inhaled allergen. Likewise, exposure to 0.4 ppm NO_2 for 6 hours increases allergen-induced eosinophil cationic protein levels in the nasal airways of allergic asthmatic subjects. Taken together, these studies demonstrate that NO_2 can augment the acute response to allergen in atopic subjects.

Ozone

There is little debate that increased ambient air ozone levels induce exacerbations of asthma, as measured by hospitalizations, rescue medication use, and symptoms.[92,96-108] These events typically occur 24 to 48 hours after exposure to increased ozone levels. Even very low levels of ozone (less than the current National Ambient Air Quality Standard for ozone) have been linked to increased exacerbations of asthma.[109]

In controlled exposure studies human volunteers experience 2 primary effects of ozone: (1) a temporary restrictive defect characterized by decreased forced vital capacity and FEV_1, which are accompanied by a sensation of chest discomfort with deep breathing and enhanced nonspecific bronchial responsiveness, and (2) development of neutrophilic inflammation, which can be seen as early as 1 hour after exposure but persists for as long as 24 hours after exposure.[109,110] Despite the temporal relationship between these ozone responses, inflammatory and lung function changes do not correlate with each other, suggesting that they are mediated by different mechanisms.

In addition to changes in neutrophilic inflammation, ozone can induce selective increases in macrophages and monocytes,[103] and some investigators have found that ozone induces influx of monocytes and macrophages with increased expression of CD11b and CD14.[104] Overall, it seems likely that monocytes and macrophages might play an important and incompletely understood role in mediating the immunomodulatory effects of ozone. As with NO_2, ozone enhances the response to allergen challenge, with one report suggesting that an ozone exposure as low as 0.12 ppm for 1 hour increased the response to inhaled allergen.[106] Levels of 0.16 and 0.25 ppm ozone have also been shown to increase the response to inhaled allergen,[107,109] as does repeated challenge with ozone at levels o f 0.125 ppm.[109] Air pollution increases airway reactivity and bronchial inflammation.[110-117]

Pharmacologic interventions for the effects of pollutants on airway physiology

Rigorous studies of treatment interventions for environmental lung diseases have not been carried out on a large scale. Thus it is premature to suggest treatment guidelines for prophylaxis of pollutant-induced asthma exacerbation. However, there are reports that examine the effect of pharmacotherapy on responses to pollutants that might provide clues as to important mechanisms by which such agents affect airway disease.

Analgesics. Many investigators have shown in both animal and human studies that COX inhibitors, such as ibuprofen and indomethacin, inhibit ozone-induced decreases in spirometric results, with little effect on the neutrophilic response to ozone or airway hyperreactivity.[118-123] Volunteers treated with sufentanyl (a short-acting narcotic) shortly after ozone exposure were found to have a significant reversal in the ozone-induced decrease in lung function.[115] Taken together, these studies suggest that the immediate decrease in lung function caused by ozone exposure is a pain response, and for those susceptible to this action of ozone, analgesics might be helpful.

Anti-inflammatory agents and ozone. It is not surprising that agents with anti-inflammatory actions have been examined for their effect on the inflammatory response to pollutants, and these studies have been reviewed elsewhere.[116-126] Briefly, cromolyn sodium or nedocromil blunt immediate spirometric responses to SO_2, ETS, and endotoxin[127] in asthmatic volunteers. Inhaled glucocorticoids inhibit the effect of pollutants on airway inflammation. Corticosteroids have been shown to decrease ozone-induced inflammation in allergic asthmatic subjects and healthy volunteers.[124,128]

Antioxidants and ozone. It has been hypothesized that because pollutants exert oxidant stress, antioxidants might be useful interventions in pollutant-induced disease. Studies by Samet et al[129] examining the effect of an ascorbate-rich diet versus an ascorbate-depleting diet in human subjects suggest that antioxidants might be an important defense against the effect of ozone on lung function in healthy volunteers. Trenga et al[130] also examined the effect of vitamin E and C pretreatment on ozone-induced airway responsiveness by using an SO_2 challenge to induce bronchospasm after ozone exposure. Vitamin E and C therapy was also found to have a protective effect on airway function in asthmatic children after ozone exposure in Mexico City, especially in those children with the glutathione-S-transferase Mu null antioxidant genotype.[131,132] These studies suggest that antioxidants might play a role in protection against the effect of pollutants with oxidant activity.

Environmental interventions

One approach that subjects can take to decrease exposure to pollutants is to avoid or minimize outdoor activities during times when ambient air pollutant levels will be increased. The Air Quality Index for "criteria" pollutants can be found on a number of publicly available media sources, including the Web site for the US Environmental Protection Agency, as well as Web sites maintained by many state governmental agencies, and is generally updated on a daily basis. For ozone, the Air Quality Index has generally been

included as a routine part of television and print weather forecasts during the summer months, when ozone levels are increased.

In addition to personal avoidance strategies, public health approaches to decrease air pollutants have been shown to have a measurable effect on health outcomes. One example of this occurred in concert with the 1996 Olympic Games held in Atlanta. Coincident with attempts by the local government to decrease ozone generation by vehicle exhaust, there was not only a decrease in summer ozone levels but also a significant decrease in asthma morbidity noted during this time.[133] Likewise, in Dublin, Ireland, a ban on bituminous coal sales was implemented on September 1, 1990, to improve air quality.[134] In the 72 months after the ban, there was a 70% decrease in black smoke concentrations, a 5.7% decrease in nontrauma death rates, a 15.5% decrease in respiratory death rates, and a 10.3% decrease in cardiovascular death rates when compared with the 72 months preceding the ban.

OCCUPATIONAL ALLERGY

Background

The 2 main occupational allergies are contact dermatitis (see chapter 12 of this Primer)[135] and asthma. Hypersensitivity pneumonitis is uncommon. Farmers' lung has virtually disappeared because silos are no longer used to store food on dairy farms. Occupational asthma is the most common occupational respiratory disorder in industrialized countries, estimated to account for 5% to 15% of asthma cases in adults of working age, especially those with newly developed asthma. More than 250 agents have been reported to cause occupational asthma. The most frequent are isocyanates, flour and grain dust, airborne particles from other foods (especially fish), colophony and fluxes, latex, animals (especially laboratory animals), aldehydes, and wood dust (Table IV).[136-138] Development of asthma is often preceded by allergic rhinitis. Dust or low-molecular-weight compounds released into the outdoor air from the workplace can also cause asthma in the nearby community. Occupational asthma is distinguished from work-enhanced asthma and reactive airway disease syndrome, which are disorders caused by occupational exposure to airborne irritants.

Pathogenesis

High-molecular-weight agents elicit specific IgE antibody responses, and the cellular pathway of pathogenesis is the same as for all other IgE-mediated asthma. The pathogenesis of low-molecular-weight agents, such as isocyanates, is less clear. These patients often exhibit only the late-phase reaction and have more neutrophilia. However, $CD4^+$ lymphocytes do play a role, and some patients might have specific IgE and IgG4 antibodies.[139,140] Concomitant exposure to airborne agents that activate innate immunity enhances the likelihood of occupational asthma.[141,142] Cigarette smoking is another important risk factor, possibly also acting through innate immunity from its contamination with endotoxin.[143] The role of genetic susceptibility is complex and not a useful factor in diagnosis or management at this time.[144] The severity of asthma depends both on the concentration of the allergen in the air and the duration of exposure. Subjects with long-standing heavy exposure often continue to have asthma long after their exposure ceases.

TABLE IV. Some common occupational allergens

High molecular weight	Low molecular weight
Grain dust (including mites)	Diisocyanates (many sources)
Bakery dust	Acid anhydrides
Fish proteins	Western red cedar (plicatic acid)
Laboratory animals	Colophony
Bird proteins	Penicillins
Natural rubber latex	Nickel
Enzymes, especially detergents	Platinum
Mold proteins	Vanadium
Vegetable gums	
Soy bean dust	
Cotton, coffee, and other seed dusts	
Psyllium	

Diagnosis

By far the most important thing is to consider the possibility! Be sure to include details of the patient's occupation in the history of all adult patients with asthma. A history of symptoms improving when the patient is away from work is often more informative than symptoms occurring during work. Occupational asthma is distinct from work-enhanced asthma from exposure to air pollutants at the workplace. Once the diagnosis of occupational asthma is suspected from the history, additional diagnostic procedures include the following[136,145-147]:

- *Skin tests or in vitro tests for IgE antibody to high-molecular-weight allergens*. Unfortunately, standardized reagents are available for only a few occupational allergens, and therefore the material for the test might have to be improvised.
- *Peak flow measurements to correlate obstruction with exposure*. Many cases have a delayed-onset late-phase response and prolonged persistence after exposure, and therefore the peak flow needs to be measured at least 4 times a day for a long period that includes time off work.
- *Correlation of exhaled nitrous oxide concentration, sputum eosinophil counts, or both with exposure*. Again, the inflammation can persist after exposure ceases.

Specific bronchial challenge tests are often considered the gold standard, but for the practicing physician, they have several problems. The reagents are not readily available. Asthma medications inhibit a positive response, and therefore the test is reliable only in patients with mild disease who do not require daily medication. In patients who have not been recently exposed and are asymptomatic, the concentration required to elicit a positive response is 10 to 100 times higher than the concentration that elicits symptoms at work. Provocation tests are more useful for research centers to identify the cause of asthma in the workplace than for the practicing physician to diagnose individual patients' conditions.

Management

The key is avoidance, avoidance, avoidance,[137,145-147] but this is easier said than done.

First, consider the patient. The simplest thing is to change jobs. In fact, many subjects do this themselves, and therefore the prevalence of occupational asthma is often underestimated (ie, the "healthy worker effect").[148] Often, it is possible to continue working for the same employer at a different location, where exposure is less. If changing jobs is not feasible, protective air-fed helmets might be indicated. Simple masks are poorly effective.

The employer is key to avoidance. After the occurrence of occupational asthma at a workplace has been established, management has the responsibility of controlling the exposure, not only for the benefit of the particular subject but also for prevention of asthma in other employees. In many industries this control has been both feasible and effective, and occupational asthma there has been greatly reduced. Of course, the specific changes required depend on the details of the generation of the airborne causative agent. Monitoring of the effectiveness of the control measures involves measurement of the airborne allergen concentration. In those instances in which measurements have been practical (eg, latex and detergent enzymes), the concentration that elicits symptoms is in the range of 100 ng/m^3. Safe concentrations are 1 or at most 10 ng/m^3.

Pharmacologic treatment is the same as for all subjects with asthma. Unfortunately, many subjects with occupational asthma, especially those with more severe disease, continue to be symptomatic long after exposure has ceased. These subjects require the usual pharmacologic management of chronic asthma.

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Anaphylaxis

F. Estelle R. Simons, MD, FRCPC *Winnipeg, Manitoba, Canada*

Anaphylaxis occurs commonly in community settings. The rate of occurrence is increasing, especially in young people. Understanding potential triggers, mechanisms, and patient-specific risk factors for severity and fatality is the key to performing appropriate risk assessment in those who have previously experienced an acute anaphylactic episode. The diagnosis of anaphylaxis is based primarily on clinical criteria and is valid even if the results of laboratory tests, such as serum total tryptase levels, are within normal limits. Positive skin test results or increased serum specific IgE levels to potential triggering allergens confirm sensitization but do not confirm the diagnosis of anaphylaxis because asymptomatic sensitization is common in the general population. Important patient-related risk factors for severity and fatality include age, concomitant diseases, and concurrent medications, as well as other less well-defined factors, such as defects in mediator degradation pathways, fever, acute infection, menses, emotional stress, and disruption of routine. Prevention of anaphylaxis depends primarily on optimal management of patient-related risk factors, strict avoidance of confirmed relevant allergen or other triggers, and, where indicated, immunomodulation (eg, subcutaneous venom immunotherapy to prevent Hymenoptera sting–triggered anaphylaxis, an underused, potentially curative treatment). The benefits and risks of immunomodulation to prevent food-triggered anaphylaxis are still being defined. Epinephrine (adrenaline) is the medication of first choice in the treatment of anaphylaxis. All patients at risk for recurrence in the community should be equipped with 1 or more epinephrine autoinjectors; a written, personalized anaphylaxis emergency action plan; and up-to-date medical identification. Improvements in the design of epinephrine autoinjectors will help to optimize ease of use and safety. Randomized controlled trials of pharmacologic agents, such as antihistamines and glucocorticoids, are needed to strengthen the evidence base for treatment of acute anaphylactic episodes. (J Allergy Clin Immunol 2010;125:S161-81.)

Key words: *Anaphylaxis, allergic reaction, mast cell, basophil, IgE, Fc∈RI, histamine, tryptase, food allergy, medication allergy, venom allergy, epinephrine, adrenaline, H_1-antihistamine*

Abbreviations used
CNS: Central nervous system
COPD: Chronic obstructive pulmonary disease
CVD: Cardiovascular disease
NSAID: Nonsteroidal anti-inflammatory drug
OSCS: Oversulfated chondroitin sulfate
Siglec: Sialic acid-binding immunoglobulin-like lectin

This chapter focuses mainly on anaphylaxis in community settings. It provides an overview of epidemiology, pathogenesis, clinical diagnosis, confirmation of the triggers, and long-term management, including prevention of recurrences and emergency preparedness. It highlights recent advances published since the review of anaphylaxis published in the 2008 Mini-Primer.[1]

Anaphylaxis is currently defined as a serious allergic reaction that is rapid in onset and might cause death.[2] The diagnosis is considered to be highly likely when any one of 3 clinical criteria is fulfilled (Table I)[2]; the presence of reduced blood pressure or shock is not necessarily required. The terms anaphylactoid or pseudoanaphylaxis are no longer recommended for use.

EPIDEMIOLOGY

The lifetime prevalence of anaphylaxis from all triggers is estimated to be 0.05% to 2%.[3] The rate of occurrence appears to be increasing, especially in young people.[4-14] Accurate community-based population estimates are difficult to obtain because of underdiagnosis, underreporting, and miscoding, as well as use of different definitions of anaphylaxis and different methods of case ascertainment in the different populations studied.[15-17] Representative studies of anaphylaxis from all triggers in the general population are summarized in Table II.[3-12]

It is likely that anaphylaxis is underdiagnosed, especially if it is a patient's first episode, if there is a hidden or previously unrecognized trigger, or if symptoms are mild, transient, or both.[15] Patients might not be able to describe their symptoms if awareness, cognition, and judgment are impaired or if they are dyspneic or becoming unconscious. The presence of itching, flushing, hives, and/or angioedema is helpful in making the diagnosis; however, skin and mucosal symptoms and signs are absent or unrecognized in 10% to 20% of all anaphylactic episodes. Hypotension sometimes goes undocumented, especially in infants and young children.[15]

Underreporting and miscoding of anaphylaxis remain important issues.[15] Only 1% of emergency department visits for acute systemic allergic reactions receive the diagnosis of anaphylaxis; many are called acute allergic reactions, or acute hypersensitivity reactions.[16,17] In a recent nationally representative probability sample from hospital emergency departments in the United States, 57% of likely episodes of anaphylaxis to food did not receive an emergency department diagnosis of anaphylaxis.[13]

Death from anaphylaxis is considered rare[8,14,18-23]; however, underreporting of fatalities likely occurs for a variety of reasons.

From the Department of Pediatrics & Child Health, Department of Immunology, Faculty of Medicine, University of Manitoba.

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Address for reprints: F. Estelle R. Simons, MD, FRCPC Room FE125, 820 Sherbrook St, Winnipeg, Manitoba, Canada, R3A 1R9. E-mail: lmcniven@hsc.mb.ca.

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TABLE I. Clinical criteria for diagnosing anaphylaxis

Anaphylaxis is highly likely when any 1 of the following 3 criteria is fulfilled:
1. Acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue, or both (eg, generalized hives, pruritus or flushing, and swollen lips-tongue-uvula) AND at least 1 of the following:
A. Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia)
B. Reduced BP or associated symptoms of end-organ dysfunction (eg, hypotonia [collapse], syncope, incontinence)
2. Two or more of the following that occur rapidly after exposure to a likely allergen for that patient (minutes to several hours):
A. Involvement of the skin–mucosal tissue (eg, generalized hives, itch-flush, swollen lips-tongue-uvula)
B. Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia)
C. Reduced BP or associated symptoms (eg, hypotonia [collapse], syncope, incontinence)
D. Persistent gastrointestinal symptoms (eg, cramping abdominal pain, vomiting)
3. Reduced BP after exposure to a known allergen for that patient (minutes to several hours):
A. Infants and children: low systolic BP (age-specific) or greater than 30% decrease in systolic BP*
B. Adults: systolic BP of less than 90 mm Hg or greater than 30% decrease from that person's baseline

Adapted from reference 2.

BP, Blood pressure; *PEF*, peak expiratory flow.

*Low systolic blood pressure for children is defined as less than 70 mm Hg from 1 month to 1 year, less than (70 mm Hg + [2 × age]) from 1 to 10 years, and less than 90 mm Hg from 11 to 17 years. Normal heart rate ranges from 80 to 140 beats/min at age 1 to 2 years, from 80 to 120 beats/min at age 3 years, and from 70 to 115 beats/min after age 3 years. Infants and young children are more likely to have respiratory compromise than hypotension or shock.

These include incomplete clinical information, including lack of a history of concomitant diseases, concurrent medications, and drug or alcohol abuse, and absence of a detailed death scene investigation (eg, interview of witnesses).[22] Initial symptoms and signs in fatal episodes of anaphylaxis commonly include respiratory distress rather than circulatory collapse.[21] The autopsy findings might be nonspecific, and laboratory test results might be within normal limits; however, this cannot be used to exclude the diagnosis of anaphylaxis.[20-22]

PATHOGENESIS

Triggers of anaphylaxis

Triggers of anaphylaxis in the community are listed in Table III.[24-69] In many countries the most common food triggers are peanut, tree nuts, shellfish, fish, milk, egg, and sesame[24-26]; however, there are important geographic variations, and in some countries other foods, such as chestnut, rice, buckwheat, or chickpea, predominate.[27] Any food can potentially trigger anaphylaxis, including previously unrecognized triggers, such as quinoa,[28] dragon fruit,[29] or some fresh red meats containing carbohydrates.[30] Food triggers can be hidden (eg, substituted foods, cross-reacting foods, and cross-contacting foods).[26] Food triggers also include additives, such as spices, vegetable gums, and colorants (eg, carmine [cochineal])[31]; contaminants, such as dust mites[32]; and parasites, such as the live seafish nematode *Anisakis simplex*.[33]

Medication-triggered anaphylaxis can occur in patients of any age; however, it is particularly common in middle-aged and older adults. Antibiotics, especially β-lactam antibiotics, and nonsteroidal anti-inflammatory drugs (NSAIDs), including aspirin, ibuprofen, and other agents, are often implicated, as are chemotherapeutic agents.[24,25,34-40] Newly recognized medication triggers include loperamide[37]; contaminants in medications, such as oversulfated chondroitin sulfate (OSCS)-contaminated heparin[38]; seemingly innocuous substances, such as vitamins and supplements containing folic acid[39]; and herbal treatments.[40] Perioperative medications,[41] iodinated contrast media[42] and medical dyes are becoming increasingly relevant triggers in community settings. Biological agents that trigger anaphylaxis include monoclonal antibodies (mAbs), such as cetuximab, infliximab, and omalizumab,[43-45] and allergens used in immunotherapy.[46,47] Vaccines to prevent infectious diseases seldom trigger anaphylaxis. If they do, the culprit is seldom the immunizing agent itself.[48-51] Rather, it is likely to be a protein excipient, such as gelatin or egg, or rarely another excipient, such as dextran.[48,51]

Venom from stinging insects (Order Hymenoptera, family Apidae [eg, honeybees]; family Vespidae [eg, yellow jackets, yellow hornets, white-faced hornets, and paper wasps]; and family Formicidae [eg, ants])[52-54] or, less commonly, saliva from biting insects (flies, mosquitoes, ticks, kissing bugs, and caterpillars) can trigger anaphylaxis.[54-57]

In health care settings ongoing efforts to prevent anaphylaxis from natural rubber latex have been relatively successful; however, in the community anaphylaxis is still occasionally reported after direct exposure to latex-containing gloves, condoms, rubber-handled racquets, balloons, latex-padded play pits, infant pacifiers, and bottle nipples. It also potentially occurs after ingestion of foods that cross-react with latex, such as banana, kiwi, papaya, avocado, potato, and tomato.[58]

Occupational allergens,[25] seminal fluid,[59] and, rarely, inhaled allergens, such as animal dander[60] or grass pollen, can also trigger anaphylaxis; some systemic absorption of these allergens likely occurs.

In addition, nonimmune perturbations of mast cells and basophils might lead to anaphylaxis. This potentially occurs after exercise[61,62] and/or exposure to cold air or water, heat, sunlight/UV radiation, insect venom constituents,[52,53] radiocontrast media,[34,42] ethanol, and some medications, including opioids, COX-1 inhibitors, and vancomycin.[24,25,34] In patients with exercise-induced anaphylaxis, food is a common cotrigger[61]; it is hypothesized that in these patients, food-sensitized immune cells are relatively innocuous until they are redistributed into the systemic circulation from gut-associated deposits during exertion.[62]

Idiopathic anaphylaxis is diagnosed when no triggers can be identified based on history, skin tests are negative, and serum specific IgE levels are absent or undetectable. Before this diagnosis is made, however, the possibility of a hidden or previously unrecognized trigger should be ruled out,[24,28-30,32,33,37-40,57] and the patients should be evaluated for mastocytosis and clonal mast cell disorders.[63-67]

Mechanisms

The underlying pathogenesis of human anaphylaxis commonly involves an immunologic mechanism in which IgE is synthesized

TABLE II. Epidemiology of anaphylaxis in the general population: All triggers

Author	Date	Description of study	Key findings	Comments
Yocum et al[4]	1999	Rochester Epidemiology Project, linked indexed medical records of the general population of Olmstead County, MN	During the years 1983-1987, the average annual incidence rate was 21/100,000 person-years, and the most common triggers were foods, medications, and insect stings.	Anaphylaxis frequently was not recognized by patients or physicians.
Simons et al[5]	2002	Dispensing data for all injectable epinephrine formulations over 5 consecutive years in a general population of 1.15 million in which all dispensings are recorded	Of this defined general population, 0.95% had injectable epinephrine dispensed for out-of-hospital treatment.	Dispensing rates were highest in those <17 years of age (1.44%) and lowest in those ≥65 years of age (0.32%). There was a male predominance to age 15 years and a female predominance after age 15 years.
Bohlke et al[6]	2004	Large health maintenance organization in the United States, 1991-1997; cases identified from automated database using ICD-9 codes 995.0, 995.6, 995.4, and 999.4 plus medical records review	The incidence rate was 10.5 anaphylactic episodes per 100,000 person-years.	After review of the sample using the additional ICD-9 codes 708.0, 708.9, 995.1, 995.3, and 695.1, the incidence rate was estimated at 68.4 cases/100,000 person-years.
Helbling et al[7]	2004	Investigated anaphylaxis with circulatory symptoms during a 3-year period, 1996-1998, in Bern, Switzerland (population, 940,000); allergy clinic medical records were reviewed, and emergency departments were contacted to identify additional cases.	Two hundred twenty-six people had 246 episodes of life-threatening anaphylaxis with cardiovascular symptoms, for an incidence rate of 7.9-9.6/100,000 person-years.	There were 3 deaths, resulting in a case fatality rate of 0.0001%.
Lieberman et al[3]	2006	Panel convened to review major epidemiologic studies of anaphylaxis	There was a frequency estimate of 50 to 2,000 episodes/100,000 person-years or a lifetime prevalence of 0.05% to 2%.	The largest number of incident cases were found in children and adolescents.
Poulos et al[8]	2007	Data on hospital admissions for anaphylaxis were extracted for the periods 1993-1994 to 2004-2005, respectively.	There was a continuous increase by 8.8% per year in the incidence rate of ED visits/hospitalizations for anaphylaxis and a steep increase in hospitalizations for food-triggered anaphylaxis in children <5 years of age.	In children, hospitalizations for food-induced anaphylaxis were an increasing concern.
Camargo et al[9]	2007	State-by-state dispensing data (filled prescriptions, including refills) for epinephrine autoinjectors in 2004 in the United States	State-by-state variation: average was 5.71 EpiPens per 1,000 persons (range from 2.7 in Hawaii to 11.8 in Massachusetts).	Regional variation was also noted: the rate was significantly higher in northern states (except Alaska) than in southern states.
Decker et al[10]	2008	Population-based incidence study from 1990-2000 in the Rochester Epidemiology Project (see Yocum et al study above in this table)	Overall age- and sex-adjusted incidence rate of 49.8/100,000 persons; the annual incidence rate increased from 1990 to 2000.	Age-specific rates were highest for ages 0-19 years (70/100,000 person-years).
Lin et al[11]	2008	Characterization of anaphylaxis hospitalizations in New York state in patients <20 years of age	During the study period, 1990-2006, the anaphylaxis hospitalization rate increased by more than 4-fold.	There was overall bimodal age distribution, with peaks in the very young and in teens.
Sheikh et al[12]	2008	Recorded incidence and lifetime prevalence of anaphylaxis in England were investigated by using QRESEARCH, a national aggregated primary health care database containing the records of >9 million patients.	Age/sex standardized incidence of anaphylaxis was 6.7/100,000 person-years in 2001 and increased by 19% to 7.9/100,000 person-years in 2005; lifetime age/sex standardized prevalence of anaphylaxis was 50/100,000 in 2001 and increased by 51% to 71.5/100,000 in 2005.	Adrenaline prescribing increased by 97% over this time.

This table summarizes selected publications during the past decade in which the rate of occurrence of anaphylaxis from all triggers in the general population was estimated. These estimates vary because of different definitions of anaphylaxis, different methods of case ascertainment, and the different populations studied.
ED, Emergency department; *ICD-9*, International Classification of Diseases–Ninth Revision.

in response to allergen exposure and becomes fixed to high-affinity receptors for IgE (FcεRI receptors) on the surface membranes of mast cells and basophils (Fig 1).[1,2,24,25,69-72] Aggregation of receptor-bound IgE molecules occurs on re-exposure to the allergen and results in cell activation and mediator release.[70-72] IgE also contributes to the intensity of anaphylaxis by enhancing the expression of FcεRI on mast cells and basophils.[70-72]

Rarely, other immunologic mechanisms that do not involve IgE are implicated in human anaphylaxis.[73] IgG-mediated

TABLE III. Mechanisms and triggers of anaphylaxis in the community

Immunologic mechanisms (IgE dependent)
Foods, such as peanut, tree nut, shellfish, fish, milk, egg, sesame, and food additives*
Medications, such as β-lactam antibiotics and NSAIDs, and biological agents†
Venoms, such as stinging insects (Hymenoptera)
Natural rubber latex
Occupational allergens
Seminal fluid (prostate-specific antigen)
Inhalants, such as horse, hamster, and other animal danders and grass pollen (rare)
Radiocontrast media‡
Immunologic mechanisms (IgE independent, formerly classified as anaphylactoid reactions)
Dextran, such as high-molecular-weight iron dextran†
Infliximab†
Radiocontrast media‡
Nonimmunologic mechanisms
Physical factors, such as exercise,§ cold, heat, and sunlight/UV radiation
Ethanol
Medications, such as opioids†
Idiopathic anaphylaxis
Consider the possibility of hidden or previously unrecognized allergens‖
Consider the possibility of mastocytosis/clonal mast cell disorder

Adapted from references 24-69.

*Food additives include spices, vegetable gums, colorants (carmine/cochineal), monosodium glutamate, sulfites, papain, and contaminants.

†Medications can potentially trigger anaphylaxis through an IgE-dependent immunologic mechanism, an IgE-independent immunologic mechanism, or direct mast cell stimulation. Biological agents include mAbs (eg, cetuximab and omalizumab), allergens, vaccines, and hormones (eg, progesterone).

‡Radiocontrast media potentially trigger anaphylaxis through an IgE-dependent immunologic mechanism or through activation of complement.

§With or without a food or medication cotrigger.

‖Includes foods, biting insect saliva, other venoms, medications, and biological agents. Save food or food label, insect or other relevant material, and save patient serum sample for customized *in vitro* tests, such as measurement of allergen-specific IgE (see the text for further details).

anaphylaxis has been reported due to high molecular weight iron dextran or infusion of chimeric, humanized, or human therapeutic mAbs, such as infliximab.[44,51] Complement-mediated anaphylaxis occurs in association with hemodialysis, OSCS-contaminated heparin,[38] protamine neutralization of heparin, liposomal drugs, or polyethylene glycols. Direct activation of the innate immune system might also contribute to triggering anaphylaxis.[74]

In addition, as noted previously, nonimmune activation of mast cells and basophils occurs.[24,25,34]

A trigger can lead to anaphylaxis through more than 1 mechanism; for example, radiocontrast media can trigger anaphylaxis through an immunologic IgE-dependent mechanism and through direct mast cell activation.[34,42] OSCS-contaminated heparin triggers anaphylaxis through activation of the complement system, leading to generation of kallikrein, bradykinin, and the complement protein-derived anaphylatoxins C3a and C5a; in addition, factor XII and the coagulation system are involved.[38,75]

Regardless of the immunologic or nonimmunologic triggering mechanisms and regardless of whether FcεRI or other receptors, such as G protein–coupled receptors or Toll-like receptors, are activated, mast cells and basophils play an important role in initiating and amplifying the acute allergic response. After IgE/FcεRI binding and receptor aggregation, multiple tyrosine kinases, including Lyn, Syk, and Fyn, are activated and exert both positive and negative regulation on the signal transduction cascade.[70,71,76] Calcium influx is the essential proximal intracellular event leading to mast cell degranulation and is controlled by both positive and negative regulation through calcium channels.[70,77] Mast cells and basophils release preformed chemical mediators of inflammation, including histamine, tryptase, carboxypeptidase A, and proteoglycans.[68,70,71,78,79] They also release newly generated mediators, such as leukotrienes, prostaglandins, and platelet-activating factor, and cytokines, such as IL-6, IL-33, and TNF-α, which is a late-phase mediator, as well as a preformed mediator.[68,70,71,80-84] Sphingosine-1-phosphate is now recognized as a circulating mediator in anaphylaxis, and in addition, it acts as a signaling component within the mast cell.[85] Once activated, the mast cell response is regulated by the balance of positive and negative intracellular molecular events that extend beyond the traditional kinases and phosphatases.

New discoveries in mast cell biology have the potential to improve the diagnostic and therapeutic approach to human anaphylaxis. For example, stem cell factor and its receptor Kit are fundamentally important in IgE/antigen-induced mast cell activation, and concurrent inhibition of Kit- and FcεRI-mediated signaling achieves coordinated suppression of human mast cell activation.[86] An orally effective compound has been identified that binds to Syk, downregulates the interaction of Syk with some of its macromolecular substrates, and inhibits FcεRI-induced mast cell degranulation *in vitro* and anaphylaxis *in vivo*.[87] Inhibitory sialic acid-binding immunoglobulin-like lectins (Siglecs) are expressed on human mast cells, on which Siglec-8 engagement results in inhibition of FcεRI-dependent mediator release without apoptosis.[88] Anti-IgE antibody potentially plays a therapeutic role by depleting free IgE, with consequent downregulation of FcεRI on mast cells and basophils and deflation of the intracellular activation signal triggered by IgE/FcεRI aggregation.[89] Basophil involvement in anaphylaxis will likely be further elucidated in the future because a monoclonal antibody directed against pro–major basic protein 1 has been identified.[90] The opening of the endothelial barrier through endothelial G_q/G_{11}-mediated signaling has been identified as a critically important process leading to symptoms of anaphylaxis in many body organ systems.[91]

There are few studies of the role of genetic factors in human anaphylaxis. Investigations in this area might improve our

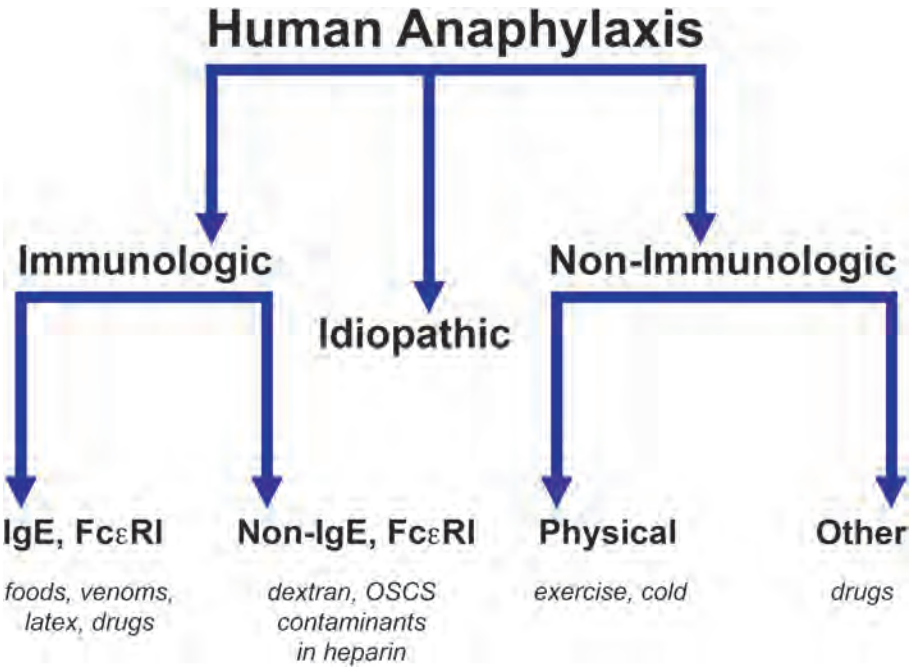

FIG 1. Mechanisms underlying human anaphylaxis. Anaphylaxis is commonly mediated through an immune IgE-dependent mechanism. Rarely, it occurs through another immune mechanism. Uncommonly, it occurs through direct (nonimmune) activation of mast cells. Idiopathic anaphylaxis, currently a diagnosis of exclusion, presents opportunities for identification of previously unrecognized triggers, elucidation of pathophysiologic mechanisms, and identification of patients with mastocytosis or clonal mast cell disorders.[69]

understanding of why anaphylaxis occurs in only a minority of persons who are sensitized to an antigen and why episodes vary greatly in severity from mild with spontaneous remission to severe and fatal.[92,93]

Patient-specific risk factors for severity and fatality

Patients might be at increased risk of anaphylaxis severity and fatality because of age, concomitant disease, concurrent medications, and other factors that are still being delineated (Table IV).[24,25,64-69,93-108]

In infants anaphylaxis is sometimes hard to recognize because they cannot describe their symptoms, and many of the signs of anaphylaxis in infancy, such as flushing and dysphonia after a crying spell, spitting up or loose stools after feeding, and loss of sphincter control, are ubiquitous in the healthy state.[94] Teenagers and young adults are at increased risk of anaphylaxis triggered by foods and possibly other agents because of inconsistent behaviors with regard to avoiding their confirmed relevant triggers and carrying epinephrine autoinjectors.[95] During pregnancy, anaphylaxis places the mother and especially the baby at high risk of fatality or permanent central nervous system (CNS) damage. During the first, second, and third trimesters, potential triggers of anaphylaxis are similar to those in nonpregnant women. During labor and delivery, the most common triggers are penicillins and other β-lactam antibiotics given as prophylaxis against neonatal group B streptococcal infection.[96] Elderly adults are at increased risk of fatality in anaphylaxis because of concomitant diseases, such as chronic obstructive pulmonary disease (COPD), and cardiovascular diseases (CVDs) and the medications used to treat them.[21,97-99]

In patients of any age, diseases that impede prompt recognition of triggers or symptoms potentially place patients at increased risk of anaphylaxis.[24,25,69,93] These include impaired vision or hearing, neurologic disorders, psychiatric disorders (including depression), autism spectrum disorder, developmental delay,[24,69] and use of medications, such as first-generation H_1-antihistamines (eg, diphenhydramine and chlorpheniramine), antidepressants, or CNS-active chemicals, such as ethanol or recreational drugs.[24,69]

Concomitant diseases, such as asthma or other chronic respiratory diseases, especially if severe or uncontrolled,[21,24,25,69] and also CVDs[97-99] and mastocytosis or clonal mast cell disorders,[64-67,100-103] are associated with increased risk of life-threatening or fatal anaphylaxis. Severe allergic rhinitis and severe eczema increase the risk of life-threatening anaphylaxis to some foods.[105] Concurrent medications, such as β-blockers and angiotensin-converting enzyme inhibitors increase the severity of anaphylaxis, and β-blockers potentially make anaphylaxis more difficult to treat.[24,25,98,99103,105]

In some patients severe or fatal anaphylactic episodes might be associated with defects in mediator degradation pathways and intracellular signaling pathways, as reflected, for example, in increased baseline serum tryptase levels (which are strongly associated with insect sting–triggered anaphylaxis),[67,103] increased baseline plasma histamine levels,[104] low serum angiotensin-converting enzyme activity,[105] and reduced platelet-activating factor acetylhydrolase activity.[80]

Other concomitant factors reported to increase the risk of an anaphylactic episode include exercise; exposure to extremes of temperature or humidity or high pollen counts; foreign travel or other disruption of routine; feeling unwell; fever; acute infection,

TABLE IV. Patient factors that increase risk of anaphylaxis severity and fatality

Age*
Infants: Underrecognition, underdiagnosis; no appropriate epinephrine auto-injector dose
Adolescents and young adults: ↑ Risk-taking behavior
Pregnancy: During labor and delivery, antibiotic prophylaxis against neonatal group B streptococcal infection is a common trigger
Elderly: ↑ Risk of fatality from medication and venom-triggered anaphylaxis
Comorbidities*
Asthma and other respiratory diseases, especially if severe or uncontrolled
CVDs, including hypertension
Mastocytosis† and clonal mast cell disorders†
Allergic rhinitis and eczema‡
Depression and other psychiatric diseases (might impair recognition of symptoms)
Thyroid disease (some patients with idiopathic anaphylaxis)
Concurrent medication/chemical use*
Potentially affect recognition of anaphylaxis
Sedatives/hypnotics/antidepressants/ethanol/recreational drugs
Potentially increase anaphylaxis severity
β-Blockers and ACE inhibitors
Other factors*
Exercise
Acute infection, such as upper respiratory tract infection
Menses
Emotional stress
Occupation, such as beekeeping
Priming effect of recent previous anaphylactic episode
Increased baseline plasma histamine levels (hyperhistaminemia)
Increased baseline serum tryptase levels
Reduced level of PAF AH activity, leading to increased PAF levels
Reduced level of ACE activity, leading to increased bradykinin levels

Adapted from references 68, 69, and 94-108.

ACE, Angiotensin-converting enzyme; *AH*, acetylhydrolase; *PAF*, platelet-activating factor.

*In some patients several factors might need to be present concurrently for risk to be increased, such as an elderly person plus cardiovascular disease plus β-blocker medication. In others concurrent triggers might be needed, such as food plus exercise.

†Suggested by increased baseline total tryptase levels.

‡Atopic diseases are a risk factor for anaphylaxis triggered by food, exercise, and latex but not for anaphylaxis triggered by insect stings, β-lactam antibiotics, or insulin.

such as an upper respiratory tract infection; emotional stress; menses (premenstrual and ovulatory phases); and/or ingestion of NSAIDs or ethanol.[20,32,61,62,106-108]

ASSESSMENT OF PATIENTS WITH A HISTORY OF ANAPHYLAXIS

Ideally, patients with a history of an acute anaphylactic episode should be referred to an allergy/immunology specialist with training and experience in risk assessment in anaphylaxis, including confirmation of the diagnosis, verification of the triggers, and evaluation of comorbidities and concurrent medications.

Clinical diagnosis of anaphylaxis

When patients are seen after an acute anaphylactic episode, the history of the episode should be confirmed and relevant emergency medical services and emergency department records should be reviewed.[24,25,68,69,93] The history should focus on recall of exposure to potential triggering agents or events, the minutes or hours elapsed between exposure and symptom onset, and the evolution of symptoms and signs. Involvement of body organ systems varies among patients and even in the same patient from one episode to another; however, review of anaphylaxis case series reveals some general patterns. Skin involvement is reported in 80% to 90% of episodes, respiratory tract involvement in up to 70%, gastrointestinal tract involvement in up to 45%, cardiovascular system involvement in up to 45%, and CNS involvement in up to 15% (Table V).[24,25,69,93]

The differential diagnosis of anaphylaxis includes common entities, such as acute generalized hives, acute asthma, syncope, panic attack, aspiration of a foreign body, and cardiovascular or neurologic events.[24,25] Postprandial syndromes, such as pollen-food syndrome and scombroidosis, also need to be considered, as do excess endogenous histamine syndromes, such as mastocytosis; flush syndromes, including perimenopausal flushing; nonorganic diseases, such as vocal cord dysfunction; and other diagnostic entities, some of which are rarely encountered (Table VI).[24-26,32,33,63-68,109,110] The differential diagnosis is age related to some extent. In infants foreign body aspiration, congenital malformations of the respiratory or gastrointestinal tracts, and apparent life-threatening event/sudden infant death syndrome need to be considered.[94] In middle-aged and elderly adults myocardial infarction, pulmonary embolus, and stroke are important considerations.[21,25,97]

Laboratory tests at the time of the acute anaphylactic episode

In some patients the clinical diagnosis of anaphylaxis can be confirmed by means of a blood test; for example, an increased plasma histamine level or serum total tryptase level. These tests are not specific for anaphylaxis (Table VII).[24,25,68,78,79]

Plasma histamine levels should optimally be measured 15 to 60 minutes after onset of symptoms of anaphylaxis. Special handling of the blood sample is required. Histamine and its metabolite, N-methylhistamine, can also be measured in a 24-hour urine sample.[68] Serum total tryptase levels should optimally be

TABLE V. Symptoms and signs of anaphylaxis

Cutaneous/subcutaneous/mucosal tissue
Flushing, pruritus, hives (urticaria), swelling, morbilliform rash, pilor erection
Periorbital pruritus, erythema and swelling, conjunctival erythema, tearing
Pruritus and swelling of lips, tongue, uvula/palate
Pruritus in the external auditory canals
Pruritus of genitalia, palms, soles
Respiratory
Nose: pruritus, congestion, rhinorrhea, sneezing
Larynx: pruritus and tightness in the throat, dysphonia and hoarseness, dry staccato cough, stridor, dysphagia
Lung: shortness of breath, chest tightness, deep cough, wheezing/bronchospasm (decreased peak expiratory flow)
Cyanosis
Gastrointestinal
Nausea, cramping abdominal pain, vomiting (stringy mucus), diarrhea
Cardiovascular
Chest pain, palpitations, tachycardia, bradycardia, or other dysrhythmia
Feeling faint, altered mental status, hypotension, loss of sphincter control, shock, cardiac arrest
CNS
Aura of impending doom, uneasiness, throbbing headache, dizziness, confusion, tunnel vision; in infants and children, sudden behavioral changes, such as irritability, cessation of play, and clinging to parent
Other
Metallic taste in the mouth
Dysphagia
Uterine contractions in postpubertal female patients

Adapted from references 24, 25, 93, and 94. Sudden onset of symptoms and signs is characteristic of anaphylaxis.

measured from 15 minutes to 3 hours after onset of symptoms. No special handling of the blood sample is required. The total tryptase level is typically increased in patients with anaphylaxis triggered by an injected medication or an insect sting and in those with hypotension and shock but is less likely to be increased in those with anaphylaxis triggered by food or in those who are normotensive.[68,78] Serial measurements of serum total tryptase and comparison with baseline levels obtained after the acute episode or available in stored serum might be more helpful than measurement at a single point in time.[68,78]

Other biomarkers reported to be useful in confirming an acute episode of anaphylaxis include serum mature β-tryptase; mast cell carboxypeptidase A3; chymase; platelet-activating factor; bradykinin; C-reactive protein; cytokines, such as IL-2, IL-6, IL-10, IL-33, and TNF-receptor I; and urinary cysteinyl leukotriene E4 and 9-α-11-β prostaglandin F_2.[68,72,80-84] Many studies of these potential biomarkers have included appropriate control groups, such as patients with severe acute asthma, but some have not. Biomarkers are released at different times after activation of mast cells and basophils, and patients experiencing anaphylaxis in community settings arrive in emergency departments at different time intervals after symptom onset; therefore measurement of a panel of different biomarkers might be useful.[68]

Confirmation of the triggers of anaphylaxis

An important aspect of risk assessment in patients who have experienced anaphylaxis in the community is confirmation of the trigger or triggers identified through a detailed history of antecedent exposures, so that the relevant specific trigger or triggers can be avoided and recurrences of anaphylaxis can be prevented (Table VIII).[24-26,34,52,58,61,68,69,93,111] Skin tests should be performed with validated instruments, techniques, and recording systems, preferably at least 3 to 4 weeks after the anaphylactic episode, to allow time for rearming of skin mast cells and recovery of mast cell releasability.[68] Measurement of allergen-specific IgE levels by using a quantitative method can be performed at any time during or after the acute anaphylactic episode; however, if the blood sample is obtained during or shortly after the episode from patients who have received intravenous fluid resuscitation, levels can be falsely undetectable or low because of the dilutional effect on circulating IgE. Negative tests for sensitization to a trigger in a patient with a convincing history of anaphylaxis from that trigger should be repeated weeks or months later. It is important to note that both positive skin tests and increased specific IgE levels indicate sensitization to the allergens tested but are not diagnostic of anaphylaxis or any other disease.[24-26,34,52,58,68,69]

If indicated, incremental challenge/provocation tests should be conducted in appropriately equipped health care facilities by professionals trained and experienced in patient selection, timing of the challenge, use of challenge protocols, and diagnosing and treating anaphylaxis. Before a challenge is performed, the potential risks and benefits should be discussed with the patient (or, for children, the caregivers) and documented in the medical record.[68,111]

Assessment of patients with food-triggered anaphylaxis. Skin prick tests with foods that elicit a wheal of 3 mm larger than that caused by the negative control (eg. saline) are considered positive. Commercially available food allergen extracts do not contain standardized allergens. Some food allergens, such as fruits and vegetables, are labile and degrade in glycerinated extracts during manufacture and storage; therefore skin prick tests with these allergens are often performed with fresh foods. Intradermal tests to foods are contraindicated because of lack of specificity (false-positive tests) and their potential for triggering anaphylaxis.[26,68,112] An exception to this might be use of intradermal tests to assess sensitization to fresh meat containing the carbohydrate galactose-α-1,3-galactose.[30]

In food-sensitized patients specific IgE levels have predictive values for positive (failed) or negative (passed) food challenge

TABLE VI. Differential diagnosis of anaphylaxis

Common entities	**Nonorganic disease**
Acute generalized hives	Vocal cord dysfunction
Acute asthma	Munchausen syndrome||
Syncope (faint, vasovagal episode)	
Panic attack	
Aspiration of a foreign body	**Shock**
Cardiovascular event (myocardial infarction, pulmonary embolus)	Hypovolemic Cardiogenic
Neurologic event (seizure, stroke)	Distributive (eg, spinal cord injury) Septic (might involve all of the above)
Postprandial syndromes	**Other**
Pollen-food syndrome*	Nonallergic angioedema
Scombroidosis†	Red Man syndrome (vancomycin)
Monosodium glutamate	Urticarial vasculitis
Sulfites	Hyper-IgE urticaria syndrome
	Progesterone anaphylaxis
	Pheochromocytoma
	Idiopathic systemic capillary leak syndrome
Excess endogenous histamine	
Mastocytosis/clonal mast cell disorders‡	
Basophilic leukemia	
Flush syndromes	
Perimenopause	
Carcinoid	
Autonomic epilepsy	
Medullary carcinoma thyroid	

Adapted from references 24-26, 63-68, 109, and 110.

*Pollen-food allergy syndrome, also termed oral allergy syndrome, is elicited by a variety of plant proteins, especially pathogen-related proteins that comprise a large number of class 2 allergenic proteins found in various fruits and vegetables. These plant proteins cross-react with airborne allergens. Typical symptoms include pruritus, tingling, and angioedema of the lips, tongue, palate, throat, and ears after eating raw, but not cooked, fruits and vegetables.

†This disease is due to histamine poisoning from fish, such as tuna, mackerel, saury, mahi-mahi, anchovies, and herring, that are stored at increased temperatures (30°C), at which bacteria such as *Morganella marganii* and *Klebsiella pneumoniae* produce histamine and *cis*-urocanic acid. Symptoms occur from minutes to hours after ingestion of the fish and last for hours. They include flush (especially of the face and neck), angioedema, nausea, vomiting, diarrhea, and hypotension. An important clue to the diagnosis is that more than 1 person eating the fish is usually affected. Skin prick tests to fish are negative, and fish-specific IgE levels are absent or undetectable.

‡Anaphylaxis might be the first manifestation of mastocytosis or a clonal mast cell disorder.

||Nonorganic diseases also include Munchausen syndrome by proxy in a child or other dependent, globus hystericus, and undifferentiated somatoform anaphylaxis.

tests. Allergen-specific IgE levels with greater than 95% predictive risk values of a positive (failed) food challenge result have been identified by using the ImmunoCAP (Phadia, Uppsala, Sweden). These levels are defined for cow's milk (≥15 kU/L), egg (≥7 kU/L), peanut (≥14 kU/L), tree nuts (≥15 kU/L), and fish (≥20 kU/L); in infants lower values have been established for milk (≥5 kU/L) and egg (≥2 kU/L).[26] Predictive values for allergen-specific IgE levels potentially differ from one immunoassay to another, and this can affect management decisions.[26,68,113]

A positive skin test, an increased serum IgE level, or both to a specific food document sensitization to that food. Such tests are not diagnostic of anaphylaxis because sensitization to 1 or more food allergens is common in the general population of healthy people who have no history of anaphylaxis. For example, 60% of young people have a positive skin prick test to 1 or more foods, yet most of those with positive tests have never experienced anaphylaxis from a food.[114] In addition, although positive skin tests and increased allergen-specific IgE levels correlate with an increased probability of clinical reactivity to specific foods, the results of these tests do not necessarily correlate with the risk of future anaphylactic episodes or with the severity of such episodes.[26,68]

Oral food challenge testing was extensively reviewed in the *Journal* in 2009.[111] Patients with a convincing history of anaphylaxis to a specific food and evidence of sensitization to that food should not undergo oral food challenge tests because of their high risk of anaphylaxis from such tests. Others, such as those with an equivocal history, low or moderate evidence of sensitization, or both, might benefit from a physician-monitored incremental oral food challenge. A positive (failed) challenge provides a sound basis for continued avoidance of the food. A negative (passed) challenge allows introduction or reintroduction of the specific food into the patient's diet.[111]

Unproved or disproved diagnostic methods, such as electrodermal skin testing and kinesiology, remain in use for assessment of patients with food allergy.[115]

In the future, *in vitro* tests that will distinguish reliably between sensitization without risk of clinical reactivity versus sensitization with risk of clinical reactivity might be available. These include measurement of allergen-specific basophil reactivity,[116] assessment of sensitization by using recombinant allergens,[117] peptide microassay-based immunoassays to map IgE and IgG_4 binding to sequential allergen epitopes,[117-119] or assessment of allergen-specific cytokine or chemokine production.[68]

Assessment of medication- or biological agent–triggered anaphylaxis. Any medication or biological agent can potentially trigger anaphylaxis. For most agents, the antigenic determinants have not been characterized or validated; indeed, the relevant immunogenic prodrugs, haptens, metabolites, and unidentified degradation products or contaminants are often unknown.[34,38,68] For most medications, with the exception of some β-lactam antibiotics, appropriate reagents are not commercially available for use in skin tests, measurement of medication-specific IgE levels, or other *in vitro* tests.[34,68] Customized tests and physician-monitored challenge/provocation tests performed in specialized centers therefore play a central role in assessment of patients with a history of anaphylaxis triggered by a medication.[34,68,120-122]

For assessment of anaphylaxis triggered by vaccines to prevent allergic diseases, skin prick tests should be performed not only with the immunizing agent but also with the relevant excipients in the culprit vaccine, such as gelatin in measles vaccines or egg in some influenza vaccines and in yellow fever vaccine.[48,68]

Assessment of stinging insect–triggered anaphylaxis. Standardized Hymenoptera venoms, such as honeybee, yellow jacket, yellow hornet, white-faced hornet, and paper wasp, are available for skin testing. Skin prick tests, if negative, should be followed by intradermal tests.[52-54] Use of dialyzed venoms in skin tests is reported to improve the identification of venom-sensitized patients.[123] For fire ant-triggered anaphylaxis, whole-body extracts are used as skin test reagents.[54,55] Measurements of venom-specific IgE levels and fire ant whole-body extract–specific

TABLE VII. Laboratory tests: Acute anaphylactic episode

Histamine*
Obtain blood sample within 15 minutes to 1 hour of symptom onset* (use wide-bore needle, keep sample cold (at 4 degrees C), centrifuge it promptly, and freeze plasma promptly).
Twenty-four-hour urine histamine and N-methylhistamine measurements might also be helpful.
Total tryptase* (pro, pro′, and mature forms of α/β-tryptases)
Obtain blood sample within 15 minutes to 3 hours of symptom onset.
Consider comparing the levels measured during the acute episode with a baseline level.†
If higher during the acute episode than in baseline serum, the diagnosis of anaphylaxis is confirmed.‡
If within normal limits during the acute episode, the diagnosis of anaphylaxis cannot be excluded.
Total tryptase level can be measured in postmortem serum.§
Additional laboratory tests‖

Adapted from references 24, 25, 68, 78, 79 and 81.
*Increases of histamine and tryptase levels are not specific for anaphylaxis. For example, histamine levels are increased in patients with scombroid poisoning and tryptase levels are increased in patients with myocardial infarction, trauma, amniotic fluid embolus, and sudden infant death syndrome.
†Obtained 24 hours after resolution of the acute event or on stored serum, if available (levels are stable for ≥1 year if stored at -20 degrees C).
‡If greater than 11.4 ng/mL in both acute and baseline sera, the diagnosis of mastocytosis or clonal mast cell disorder should be considered.
§Blood samples should be obtained from femoral vessels and not the heart; the level needs to be correlated with the clinical history because, as noted above, increased levels are also found in other clinical situations, such as myocardial infarction, trauma, amniotic fluid embolism, and sudden infant death syndrome.
‖When sorting out the differential diagnosis of anaphylaxis, the detailed clinical history and physical examination might suggest the need for additional laboratory tests to confirm or rule out diseases such as mastocytosis, basophilic leukemia, carcinoid (serum serotonin level and urinary 5 hydroxyindoleacetic acid), medullary carcinoma of the thyroid/vasoactive polypeptide–secreting gastrointestinal tumor (substance P and vasointestinal polypeptide), pheochromocytoma (free metanephrine in plasma and urinary vanillylmandelic acid), hereditary angioedema (C4 and C1 esterase inhibitor), or diagnostic imaging to confirm or rule out hydatid cysts. Investigation of the complement cascade (C4a, C5a, and C3a), the contact system (bradykinin, high-molecular-weight kininogen, kallikrein–C1-inhibitor complexes, and factor XIIa–C1-inhibitor complexes), and coagulation pathway (factors V, VIII, and fibrinogen), although usually not performed, might support the clinical diagnosis of anaphylaxis; however, these tests also appear to lack specificity.

TABLE VIII. Confirmation of a potential trigger for an anaphylactic episode

Allergen skin tests
Percutaneous (prick or puncture)*
Intradermal (intracutaneous) for selected allergens such as insect venoms and β-lactam antibiotics†
Allergen-specific serum IgE levels
Quantitative ELISAs‡
Allergen challenge tests§
Most commonly performed with foods or medications
Other challenge tests
Exercise‖
Cold
Heat
Sunlight
Work up of patients with idiopathic anaphylaxis (in whom detailed history of antecedent events/exposures does not yield any clues about triggers and skin test results and allergen-specific IgE measurements are negative)
Search for a previously unrecognized trigger
Measure serum baseline total tryptase levels (normal value, <11.4 ng/mL)
Inspect skin closely for evidence of urticaria pigmentosa
Consider bone marrow biopsy (perform c-Kit mutational analysis in addition to usual stains for identification of spindle-shaped mast cells in clusters)

Adapted from references 24-26, 34, 52, 58, 61, 67, 100-102, and 111.
*Allergens for skin testing should be selected on the basis of the history. Standardized extracts are available only for some Hymenoptera venoms and some inhalant allergens. Patients should discontinue H_1-antihistamines 7 days before skin testing. Many people in the general population are sensitized to allergens (eg, 60% of teens to food and as many as 28.5% of adults to venom).
†Intradermal tests are generally contraindicated in food allergy because of the high likelihood of false-positive results and the possibility of triggering anaphylaxis.
‡Available commercially for foods, insect venoms, and latex but not for most medications or biological agents. Refer to predictive values, where available, for foods such as peanut, tree nuts, fish, milk, and egg.
§Open, single-blind, or double-blind depending on clinical history and allergen. *"First do no harm"*: challenge *only* if assessment (clinical history, skin tests, and/or measurement of allergen-specific IgE levels) indicate that the patient is at low risk for anaphylaxis. Perform *only* under medical supervision in a hospital or other health care facility.
‖Assessment of cotriggers, such as a food, medication, or cold exposure, is needed.

IgE levels are commercially available. Some patients with a history of Hymenoptera sting–triggered anaphylaxis have negative skin test responses to insect venoms but increased specific IgE levels to venoms and *vice versa*.[52,124] Challenge/provocation tests with stinging and biting insects are potentially dangerous and are used only in research.[52-57,68,125]

Positive intradermal tests to stinging insect venoms, increased venom-specific IgE levels, or both occur in up to 28.5% of the general adult population, most of whom do not have systemic symptoms after an insect sting.[52-54,68] It is therefore critically important that the test results be interpreted in the context of the clinical history. Cross-reacting carbohydrate derivatives between venom allergens and plant

or other nonvenom allergens might account for many of these positive test results. In some centers additional tests used to assist in interpretation of positive test results include consideration of total IgE levels as well as venom-specific IgE levels,[125] and measurement of basophil activation markers, such as CD63 or CD203c after incubation with different concentrations of venom.[53,68,125]

Conversely, venom skin tests might be negative and venom-specific IgE levels might be absent or undetectable in patients with a convincing history of insect sting–triggered anaphylaxis. Negative tests might be due to rare IgE- or non–IgE-mediated reactions to a protein or peptide constituent[127] such as melittin in honeybee venom or mastoparan in vespid venom; variability of intradermal testing; anergy in patients tested within a few weeks of the sting; decrease in the immune response to venom over time in patients stung many years before testing; or increased patient vulnerability to anaphylaxis. As noted previously, risk of severe or fatal anaphylaxis increases with older age; concurrent diseases, including CVDs; and concurrent use of medications, such as β-blockers or angiotensin-converting enzyme inhibitors,[52,53,97,103] as well as in patients with mastocytosis, clonal mast cell disorders, or increased baseline tryptase levels.[52,53,72,100-103] If the baseline total tryptase level is greater than 11.4 ng/mL (the new upper limit of normal), meticulous examination for cutaneous mastocytosis is indicated, and if the level is greater than 20 ng/mL, a bone marrow biopsy is indicated, even if cutaneous manifestations are absent.[67] Also, in some patients clinical risk of anaphylaxis is increased by factors such as a recent sting; a previous severe systemic reaction to a sting; a sting on the head, neck, or throat; or the entomology of the stinging insect.[52-54,68,103]

Assessment of anaphylaxis from other triggers. For assessment of anaphylaxis triggered by natural rubber latex, skin prick tests should be performed with commercial latex allergens, where available, or with extracts of rubber products, such as natural rubber latex gloves, where commercial allergens are not available. Consideration should be given to testing with foods that cross-react with latex, such as banana, kiwi, papaya, avocado, potato, and tomato.[58,68] Latex-specific IgE antibodies can also be measured.

For assessment of exercise-triggered anaphylaxis, skin tests should be performed with potential food allergen cotriggers.[61] An exercise intensity threshold can be defined in an exercise challenge test to diagnose food-dependent exercise-induced anaphylaxis.[128]

Assessment of idiopathic anaphylaxis. When a meticulous history of antecedent exposures and events does not yield any clues about potential triggers and when allergen skin tests are negative and specific IgE measurements are absent or undetectable to selected common allergens, patients are said to have idiopathic anaphylaxis. Before making this diagnosis, physicians should consider the possibility of a hidden or previously unrecognized trigger. Sensitization to a novel trigger for which there is no commercially available test allergen can be identified through a history of the event and confirmed by objective tests. These potentially include skin testing the patient and 1 or more controls with crude extracts of the suspected culprit allergen (although there is no quality assurance that such extracts contain the relevant allergenic components) and/or development of customized, sensitive, specific ELISAs and other *in vitro* tests, including gel electrophoresis and IgE immunoblotting, for identification of specific IgE to the suspect allergen.[63,68,69]

The serum total tryptase level should be measured in all patients with idiopathic anaphylaxis.[63-68,78,100-103] This important screening test for mastocytosis reflects the increased burden of mast cells in all forms of this disease.[78]

MANAGEMENT OF PATIENTS AT RISK FOR ANAPHYLAXIS IN COMMUNITY SETTINGS

Long-term preventive measures include optimal management of relevant comorbidities, such as asthma, other chronic respiratory diseases, CVDs, and mastocytosis and clonal mast cell disorders.[63-67,97-102] These measures also include discussion of the relative benefits and risks of concurrent medications (eg, β-blockers, angiotensin-converting enzyme inhibitors, and others that are widely and effectively used in the management of CVDs) with the patient and his or her cardiologist and documentation of the rationale for treatment decisions in the patient's medical record.[97-99,103]

With the exception of venom immunotherapy for patients with insect sting–triggered anaphylaxis, current recommendations for prevention of anaphylaxis and emergency preparedness for treatment of anaphylaxis in the community are based on expert opinion and consensus rather than on randomized, double-blind, placebo-controlled trials. Preventive strategies for anaphylaxis in community settings that involve trigger avoidance and immunomodulation are summarized in Table IX.[1,2,24-26,34,52,54,58,69,93,129-153] Follow-up at regular intervals is an important aspect of long-term risk reduction.

Long-term risk reduction: Prevention of anaphylaxis

Anaphylaxis triggered by food. Written personalized information about avoidance of confirmed relevant food triggers, including lists of common hidden sources of the food or foods and high-risk situations, such as buffet and catered meals and unlabeled desserts, baked goods, and candies, should be provided. Patients should be directed to resources that provide up-to-date, consistent information about avoidance of the specific food or foods (Table IX).[26,129] Food avoidance measures potentially decrease quality of life for those at risk of anaphylaxis and for their caregivers[130,131] because of lifestyle changes that disrupt activities, uncertainty about ambiguities in advisory labeling,[132] and anxiety about the risk of accidental exposures.[26,133] Strict avoidance of many foods potentially leads to nutritional deficiencies.[26] Some patients at risk for anaphylaxis to foods, or their caregivers, turn to complementary and alternative medicine for relief.[115]

Allergen-specific oral immunotherapy is currently a research procedure for prevention of anaphylaxis triggered by food. Clinical trials with foods such as milk, egg, or peanut have been conducted in carefully selected patients in appropriately equipped food allergy research centers by physicians and other health care professionals who have experience in performing food challenges, administering oral immunotherapy, and diagnosing and treating anaphylaxis.[108,112,134-141] A few of the studies have had a double-blind, placebo-controlled design.[137] Adverse effects have been common with some oral immunotherapy dosing regimens, especially on the initial dose escalation day and on subsequent dose build-up days.[141]

In some of these studies, clinical desensitization to a food has been accompanied by long-term, food-specific humoral and cellular changes,[138,140] including decreased titrated skin prick tests, decreased basophil activation, decreased IgE levels, and increased IgG_4, IL-10, IFN-γ, and TNF-α levels.[140] Studies in progress will resolve the issue as to whether oral immunotherapy for food-triggered anaphylaxis leads not only to clinical desensitization but also to true immunologic tolerance in which patients

TABLE IX. Preventive strategies for anaphylaxis in community settings

Allergen-specific trigger avoidance based on history of exposure and confirmation of sensitization (strength of recommendation = C)
Foods,* including additives and contaminants
Medications and biological agents†
Insect stings and bites
Natural rubber latex*
Inhalants
Seminal fluid
Occupational allergens
Other
Nonimmunologic triggers: avoid relevant exposure (strength of recommendation = C)
Exercise-induced anaphylaxis‡
Cold air or water
Heat
Sunlight/UV radiation
Medications, such as opioids
Ethanol
Immunomodulation
Food: Currently, oral immunotherapy is a research procedure supervised by physicians in specialized food allergy centers (strength of recommendation pending).
Insect venoms: allergen-specific immunotherapy (strength of recommendation = A)
Medications†: desensitization (strength of recommendation = B)
Seminal fluid: desensitization (strength of recommendation = C)
Idiopathic anaphylaxis (for frequent episodes only; strength of recommendation = C)
Oral glucocorticoid, such as prednisone; H_1-antihistamine, such as cetirizine (used for prophylaxis)

Adapted from reference 153 and others; see text for details.

*These Web sites consistently provide accurate up-to-date information: the Food Allergy and Anaphylaxis Network (www.foodallergy.org); the American Latex Allergy Association (www.latexallergyresources.org); the American Academy of Allergy, Asthma & Immunology (www.aaaai.org); and the American College of Allergy, Asthma & Immunology (www.acaai.org).

†Avoid the medications suspected of triggering anaphylaxis and substitute a non–cross-reacting medication, preferably from a different therapeutic class. If this is not possible, desensitization should be performed (eg, for β-lactam antibiotics, NSAIDs, and chemotherapy drugs).

‡Avoid relevant cotriggers, such as food, medication, cold air, or cold water.

remain desensitized even if the food is not eaten on a regular basis.[112,134,135]

Future directions in specific immunotherapy to food and other allergens that trigger anaphylaxis might include allergen administration through the sublingual route, "engineered" recombinant protein allergens, a mixture of major recombinant allergens, CpG-oligonucleotide–conjugated allergens, peptides or polymers of major allergens, and other novel approaches.[112]

Immunomodulatory approaches that are not specific for a particular food allergen are also being studied. Food Allergy Herbal Formula-2, a well-characterized mixture of Chinese herbs that prevents food-induced anaphylaxis and leads to long-lasting immunologic tolerance in a murine model, has now entered clinical trials.[142] Subcutaneous injections of anti-IgE antibody potentially provide an increased margin of protection against food and other allergen triggers of anaphylaxis for many, although not all, patients at risk (Table IX).[143]

Medication- or biological agent–triggered anaphylaxis. For anaphylaxis triggered by a medication or a biological agent, avoidance is critically important. An alternative non–cross-reacting agent, preferably from a different therapeutic class but sometimes from the same class, can often be substituted effectively and safely.[34] Where this is not possible, desensitization with the offending agent is indicated.[34,144] Standardized 12-step desensitization protocols in which antigens are introduced in an incremental manner over several hours have been published for some agents, such as β-lactam antibiotics or other antibiotics, aspirin or other NSAIDs, insulin, and chemotherapeutic agents, including taxanes and platins, as well as mAbs.[144] Once achieved, desensitization is maintained through regular administration of the medication. Immunologic tolerance does not occur, and if the medication is discontinued, symptoms can recur when it is restarted.[144] Desensitization should be conducted in an appropriately equipped health care facility staffed by health care professionals who are trained and experienced in using desensitization protocols and in the recognition and treatment of breakthrough symptoms, including those of anaphylaxis.[34,144] The cellular and molecular mechanisms underlying temporary desensitization without immunologic tolerance are not yet fully understood.[144]

In patients with a history of vaccine- or vaccine component–triggered anaphylaxis who have negative skin tests to the vaccine and its components, it is highly unlikely that IgE antibody is present. The vaccine can therefore be administered in the usual manner; however, it is prudent to observe such patients for 1 hour afterward instead of the customary 30 minutes. In patients with a positive history and positive skin tests, a suitable alternative vaccine is sometimes available; for example, egg-free seasonal influenza vaccine and egg-free pandemic A/H1N1 vaccine grown in mammalian cell culture systems are now available in some countries. If a suitable alternative vaccine is not available, the culprit vaccine should be administered in an appropriately equipped and staffed health care facility by using a graded-dose protocol (Table IX).[48]

Stinging insect–triggered anaphylaxis. For anaphylaxis triggered by stinging insects, avoidance of exposure involves several approaches. Yellow jacket, hornet, or wasp nests or fire ant mounds in the vicinity of the patient's home should be professionally exterminated. Awareness of high-risk outdoor work or leisure activities, such as gardening, camping, picnicking, or barbecuing,

is important. When outdoors, appropriate protective clothing, including shoes and socks, should be worn. Personal insect repellents, such as DEET, are not effective in preventing insect stings in contrast to their efficacy in preventing insect bites.[54]

In most patients with Hymenoptera venom–triggered anaphylaxis, a 3- to 5-year course of subcutaneous injections of the relevant standardized insect venom or venoms significantly reduces the risk of anaphylaxis from a subsequent sting and provides long-lasting protection.[52-54,124] This potentially curative treatment is underused.[53] In children a 98% protection rate can be achieved, and the effect lasts for decades after venom injections are discontinued.[52,145] Use of purified extracts potentially reduces large local reactions during venom immunotherapy.[146] Venom immunotherapy can be safely administered to all those at risk, including high-risk patients with mastocytosis or clonal mast cell disorders, although a slow rate of dose escalation is often necessary in such patients.[147,148] Anti-IgE antibody is reported to be useful in controlling reactions to venom immunotherapy in patients with mastocytosis.[149] For prevention of anaphylaxis from fire ant stings[54,55] or from insect bites,[54,57] subcutaneous injections of the relevant whole-body extracts are used.

In adults venom immunotherapy significantly reduces sting-induced cutaneous systemic reactions and is therefore indicated for patients with sting-induced generalized urticaria and no other systemic symptoms.[52,124] It also reduces large local reactions to stings and might be considered for at-risk patients who cannot totally avoid insect exposure, such as beekeepers, and/or those who experience frequent or severe large local reactions.[150] In children, venom immunotherapy is not indicated either for sting-induced generalized urticaria without other systemic symptoms or for large local reactions (Table IX).[145]

Anaphylaxis induced by other triggers. Avoidance of the relevant specific confirmed trigger is the key to prevention of anaphylaxis recurrence, such as avoidance of natural rubber latex[58] or occupational allergens.[1,2,24,25,69] Desensitization provides short-term immunomodulation for patients at risk of anaphylaxis to seminal fluid.[59] In the future, regular subcutaneous injections of anti-IgE antibody might be indicated for patients with anaphylaxis triggered by various allergen triggers. For anaphylaxis induced by some nonimmune triggers, such as cold, heat, sunlight/UV radiation, or ethanol, avoidance of the trigger is the key to prevention of recurrences (Table IX).[25]

Exercise-triggered anaphylaxis. Strategies for prevention of exercise-induced anaphylaxis include strict avoidance of relevant cotriggers, such as food, medication, or ethanol ingestion and cold air or cold water exposure, and awareness of other potential concomitant risk factors, such as acute infection, emotional stress, menses (premenstrual and ovulatory phases), extremes of temperature and humidity, and high pollen counts. Additional precautions include never exercising alone, discontinuing exertion immediately when the first symptom of anaphylaxis is noted, always carrying 1 or more epinephrine autoinjectors, and carrying a cell (mobile) phone for calling 911/emergency medical services during activities such as long-distance running or cross-country skiing. Premedication and warm-up are not effective in preventing exercise-induced anaphylaxis (Table IX).[24,25,61]

Idiopathic anaphylaxis. Immunomodulation with pharmacologic agents is often recommended for patients with frequent episodes of idiopathic anaphylaxis, which is defined as more than 6 per year or more than 2 per 2 months. One example of a prophylaxis regimen involves 60 to 100 mg of prednisone each morning for 1 week, followed by 60 mg on alternate mornings for 3 weeks and then gradual tapering of the dose over 2 months, in addition to an H_1-antihistamine, such as 10 mg of cetirizine daily.[63] Anti-IgE antibody injections have been reported to be helpful in patients with idiopathic anaphylaxis and in anaphylaxis with no apparent trigger that occurs in patients with mastocytosis. (Table IX)[151,152]

Long-term risk reduction: Emergency preparedness for anaphylaxis recurrences in the community

Those at risk for anaphylaxis in the community and their caregivers should be prepared to recognize episodes that occur despite best efforts to avoid the relevant trigger and other preventive measures and to provide prompt life-saving first-aid treatment of such episodes.[2,24-26,34,52,54,69,93,153] Emergency preparedness involves carrying 1 or more epinephrine autoinjectors, having an anaphylaxis emergency action plan, and wearing appropriate medical identification.[1,2,24-26,54,69,153]

Epinephrine (adrenaline): the medication of choice. For treatment of an anaphylaxis recurrence in the community, injection of epinephrine is the first-aid medication of choice, as recommended in all anaphylaxis guidelines. The rationale for this is summarized in Table X.[24,154,156-162] Most guidelines recommend injecting epinephrine from an autoinjector intramuscularly in the midanterolateral aspect of the thigh. The first aid dose of epinephrine is 0.01 mg/kg of a 1 mg/mL (1:1,000) dilution to a maximum dose of 0.5 mg in an adult or 0.3 mg in a child. This dose can be repeated every 5 to 15 minutes, as needed.[154,155,163-165] Patients should not suddenly sit or stand after receiving an epinephrine injection because this can lead to the empty inferior vena cava/empty ventricle syndrome and sudden death.[166]

In patients with anaphylaxis, epinephrine has potent life-saving α_1-adrenergic vasoconstrictor effects on the small arterioles and precapillary sphincters in most body organ systems.[156] It decreases mucosal edema, thereby preventing and relieving upper airway obstruction, and it also prevents and relieves hypotension and shock (Table X).[156-160] In addition, its β_1-adrenergic effects lead to increased force and rate of cardiac contractions, and its β_2 effects lead to increased bronchodilation and decreased release of mediators, such as histamine and tryptase, from mast cells and basophils.[156]

Prompt injection is important. In most countries the highest epinephrine dose currently available in an autoinjector is 0.3 mg. This dose is low compared with the initial adult dose of 1 mg epinephrine used in cardiopulmonary resuscitation and is unlikely to be effective if anaphylaxis has progressed to the point at which cardiopulmonary resuscitation is needed. Delayed injection of epinephrine is associated with fatal anaphylaxis[18-21] and also contributes to the increased likelihood of biphasic anaphylaxis, which is defined as symptom recurrence 1 to 72 hours (usually within 8 hours) after resolution of the initial symptoms despite no further exposure to the trigger.[167-169]

The best way of providing first-aid treatment with epinephrine (adrenaline) for anaphylaxis in the community is by using an autoinjector; however, currently available autoinjectors have a number of limitations. Only 2 fixed epinephrine doses, 0.15 mg and 0.3 mg, are available in autoinjector formulations in most countries (EpiPen, Dey, LP, Napa, Calif; Twinject, Shionogi & Co, Ltd, Osaka, Japan; Anapen, Lincoln Medical, Salisbury, Wiltshire, United Kingdom). The 0.15 mg dose is too high for

TABLE X. Epinephrine (adrenaline): Medication of first choice for anaphylaxis

Strength of recommendation	B-C
Pharmacologic effects when given by injection (oral administration is ineffective because of rapid metabolism in the GI tract)	At α_1-receptor
	↑ Vasoconstriction/↑ vascular resistance in most body organ systems
	↑ Blood pressure
	↓ Mucosal edema (larynx)
	At β_1-receptor
	↑ Heart rate
	↑ Cardiac contraction force
	At β_2-receptor
	↓ Mediator release
	↑ Bronchodilation
	↑ Vasodilation
Practical aspects	↓ Mucosal edema and relieves upper airway obstruction
	↓ Wheezing
	↓ Hives
	↑ Blood pressure and prevents and relieves hypotension and shock
Potential adverse effects (after usual dose of 0.01 mg/kg to a maximum of 0.5 mg [adults] IM)*	Anxiety, pallor, tremor, palpitations, dizziness, and headache; these symptoms indicate that an appropriate pharmacologic dose has been injected.
Potential adverse effects (after overdose, such as IV bolus dose, overly rapid IV infusion, or erroneous administration of a concentrated epinephrine solution 1:1,000 [1 mg/mL] by the IV route)†	Pulmonary edema, hypertension, angina, myocardial infarction, ventricular arrhythmias; note that the latter 3 adverse effects also potentially occur in untreated anaphylaxis when subclinical coronary artery disease is unmasked, because the heart itself is a potential target organ in anaphylaxis.‡
Comment: why the intramuscular route is preferred	Epinephrine has a vasodilator effect in skeletal muscle.‡
	Skeletal muscle is well vascularized.
	After intramuscular injection into the vastus lateralis, absorption is rapid, and epinephrine reaches the central circulation rapidly.
	Rapid absorption is critical in anaphylaxis in which the median time to respiratory or cardiac arrest is 15 minutes (venom) to 30 minutes (food).

Adapted from references 24 and 154-162.

GI, Gastrointestinal; *IM*, intramuscular; *IV*, intravenous.

*The epinephrine dose recommended for initial treatment of anaphylaxis is lower than the dose recommended for initial use in cardiopulmonary resuscitation and is unlikely to be effective after cardiac arrest has occurred. Ideally, epinephrine doses should be stated concentrations (ie, milligrams per milliliter) rather than as ratios; however, both methods are in common use.

†Intravenous infusion of epinephrine presents a high risk of harmful side effects. It should be given only by physicians who are trained and experienced in the dose titration of vasopressors (preferably by using an infusion pump) against continuous hemodynamic monitoring.

‡Epinephrine enhances blood flow in coronary arteries because of increased myocardial contractility and increased duration of diastole. This action and the vasodilator effect in skeletal muscle produced by endogenous epinephrine are well-recognized aspects of the fight-or-flight response.

infants and children weighing less than 15 kg. The 0.3 mg dose is too low for children weighing more than 30 kg and for teens and adults. In the United Kingdom a 0.5 mg epinephrine dose is available in the Anapen. Autoinjectors with 1.43 cm needles might not achieve intramuscular injection in some children and adults, as ascertained by using computed tomographic scans of the thigh to measure the distance from the skin to the surface of the vastus lateralis muscle.[170,171] The force of the injection likely also contributes to intramuscular deposition and rapid absorption of epinephrine.[172]

Health care professionals need to be trained to use epinephrine autoinjectors correctly and safely in order to train and coach those at risk for anaphylaxis and their caregivers in how to use them correctly and safely.[173] Unintentional injections from epinephrine autoinjectors into fingers, thumbs, and hands by patients self-injecting or by caregivers injecting children or others have been reported to poison control centers with increasing frequency in the past decade. These unintentional injections might not only result in injury but also in partial or complete loss of the epinephrine dose for the person having an anaphylactic episode, the so-called "lost dose hazard."[174,175] Epinephrine autoinjectors with improved design, including needle protection features, are being introduced.

Up to 20% of patients who receive an initial first-aid dose of epinephrine for treatment of anaphylaxis in the community are reported to require a second dose, either because of ongoing symptoms or because of biphasic anaphylaxis.[167-169,176-178] Most patients with anaphylaxis respond promptly to epinephrine injections; the potential reasons for apparent lack of response in a minority of patients are summarized in Table XI.[158,166,170,171,175,178-181]

Transient pharmacologic effects of epinephrine, such as pallor, tremor, anxiety, palpitations, headache, and dizziness, that occur within 5 to 10 minutes after injection are usually mild and confirm that a therapeutic epinephrine dose has been given. Serious adverse effects, such as pulmonary edema or hypertension, are usually attributable to epinephrine overdose. Although they can occur after administration by any route, they are most commonly reported after either an intravenous bolus dose, an overly rapid intravenous infusion, or an intravenous injection of a concentrated 1 mg/mL (1:1,000) epinephrine solution instead of the dilute 0.1 mg/mL (1:10,000) epinephrine solution appropriate for intravenous infusion.[24,154]

TABLE XI. Reasons for apparent lack of response to epinephrine

Physician-related factors
Error in diagnosis*
Empty ventricle syndrome†
Patient-related factors
Rapid anaphylaxis progression
Patient taking a medication that interferes with optimal epinephrine effect, such as an α-adrenergic blocker or β-adrenergic blocker
Epinephrine-related factors
Epinephrine autoinjector not available‡
Epinephrine autoinjector not prescribed by physician
Epinephrine autoinjector not affordable (prescription not picked up)
Injected too late
Dose too low on a milligram per kilogram basis
Dose too low because of injection of epinephrine that is past the expiry date§
Injected using incorrect technique, such as not enough force
Injection route not optimal‖
Injection site not optimal
Adverse reaction to sodium metabisulfite preservative in the epinephrine solution (rare)

Adapted from references 158, 166, 170, 171, 175, and 178-181.

*For example, if epinephrine is injected for a disease, such as nonallergic angioedema or food protein–induced enterocolitis, that would not be expected to respond well to it.

†Occurs when the epinephrine injected cannot circulate in the body because the patient is suddenly placed upright and the vena cava (and ventricle) empties.

‡In many countries life-saving epinephrine autoinjectors are not available for those at risk of anaphylaxis. Existing alternatives cannot be depended on to produce high tissue concentrations of epinephrine rapidly. These include having a patient or caregiver draw up epinephrine from an ampule, use of a syringe prefilled with epinephrine, or use of an epinephrine metered-dose inhaler.

§The maximum shelf-life of EpiPen and Twinject autoinjectors is 12 to 18 months. The maximum shelf life of AnaPen autoinjectors (available in the United Kingdom) is 18 to 24 months. The maximum shelf life of a syringe prefilled with epinephrine in a physician's office is 3 to 4 months. *In vitro* degradation (breakdown) products of epinephrine are ineffective in patients with anaphylaxis.

‖Epinephrine through other routes, such as subcutaneous injection or inhalation from a metered-dose inhaler or nebulizer and compressor is not recommended for the treatment of anaphylaxis because it is more difficult to achieve high plasma and tissue concentrations rapidly when these routes are used.

Traditionally, many physicians have been reluctant to inject epinephrine in middle-aged or older patients with anaphylaxis because of concerns regarding cardiac adverse effects. In fact, the heart is a potential target organ in anaphylaxis. In healthy people mast cells are present throughout the myocardium (between myocardial fibers, around blood vessels, and in the coronary artery intima).[72,97] In patients with coronary artery disease, the number and density of cardiac mast cells is increased because mast cells are also present in atherosclerotic plaques, where they contribute to atherogenesis.[97] Histamine, leukotrienes, platelet-activating factor, and other mediators released after mast cell stimulation potentially lead to coronary artery spasm.[97] Patients with anaphylaxis can present with acute coronary syndrome secondary to either vasospasm or acute plaque rupture and thrombus formation. In patients with coronary artery disease, the use of epinephrine requires caution; however, concerns about its potential adverse effects need to be weighed against the cardiac risks of untreated anaphylaxis and the knowledge that epinephrine injection usually enhances blood flow in the coronary arteries because its β_2-adrenergic action leads to increased myocardial contractility and increased duration of diastole compared with systole (Table X).[24,25,97,161,162]

Other medications. More than 40 H_1-antihistamines are available for use,[182] and many of these medications are recommended for use in anaphylaxis; in some anaphylaxis guidelines, dosage regimens are provided for up to 7 different H_1-antihistamines. H_1-antihistamines do not prevent or relieve upper or lower airway obstruction, hypotension or shock.[182,183] After oral administration, their onset of action ranges from 1 to 3 hours.[182] The rapid improvement in symptoms sometimes attributed to oral H_1-antihistamines likely reflects spontaneous resolution of the anaphylactic episode. First-generation, potentially sedating H_1-antihistamines, such as diphenhydramine, chlorpheniramine, and promethazine, have a poor benefit/risk ratio.[182,184] When self-administered in patients with anaphylaxis, these medications potentially impair self-recognition of symptoms. When given to a child, they potentially complicate interpretation of CNS symptoms and signs, such as drowsiness. An H_1-antihistamine might be useful as an adjunctive measure to relieve residual hives that have not disappeared after epinephrine injection (Table XII).[153,183]

β_2-Adrenergic agonists do not have a vasoconstrictor effect and do not decrease mucosal edema, prevent or relieve upper airway obstruction, hypotension or shock. They are potentially useful when administered by nebulization as an adjunctive measure to relieve residual bronchospasm that has not disappeared after epinephrine injection (Table XII).[154]

Glucocorticoids are traditionally given to prevent and relieve biphasic or protracted anaphylaxis (Table XII).[185]

Emergency preparedness in the community: Additional measures. Almost 40% of persons at risk of anaphylaxis in the community reportedly use a written anaphylaxis emergency action plan.[178] Most plans list common symptoms and signs of anaphylaxis and emphasize the importance of using the epinephrine autoinjector promptly and of calling 911 or emergency medical services promptly (download from www.aaaai.org).[69,186] Plans should be personalized for each at-risk patient by listing comorbidities and concurrent medications, describing the epinephrine autoinjector and dose prescribed for the patient, and providing appropriate contact telephone numbers, such as those of family members.[69,186] Plans need to be updated and discussed with the patient, and if relevant, his or her caregivers, on a regular basis. Formal evaluation of the clinical efficacy and cost-effectiveness of these plans is needed.[187]

TABLE XII. Adjunctive medications for the treatment of anaphylaxis

Medication (example)	H_1-antihistamines* (oral, such as cetirizine; IV, such as diphenhydramine)	H_2-antihistamines* (ranitidine)	β_2-Adrenergic agonists* (salbutamol [albuterol])	Glucocorticoids* (oral, such as prednisone; IV, such as methylprednisolone)
Strength of recommendation*	C	C	C	C
Pharmacologic effects	At H_1-receptor	At H_2-receptor ↓ Gastric acid secretion	At β_2-receptor	↓ Late-phase allergic response to allergen
	↓ Itch (skin, mucus membranes)	↓ Vascular permeability	↑ Bronchodilation	
	↓ Flush	↓ Hypotension		
	↓ Hives	↓ Flushing		
	↓ Sneezing	↓ Headache		
	↓ Rhinorrhea	↓ Tachycardia		
		↓ Chronotropic and inotropic activity		
		↓ Mucus production (airway)		
Practical aspects	↓ Itch and hives but not life-saving in anaphylaxis	Small additive effect (10% or so) when used in conjunction with an H_1-antihistamine for ↓ in vascular permeability, ↓ flushing, and ↓ hypotension	↓ Wheeze, cough, and shortness of breath but do not ↓ upper airway obstruction or relieve hypotension and are not life-saving in anaphylaxis	Effects take several hours; used to prevent biphasic or protracted anaphylaxis; however, there is no evidence from high-quality randomized controlled trials that this occurs.
Potential adverse effects (usual doses)	First-generation drugs cause sedation and impair cognitive function.	Ranitidine: unlikely cimetidine: potentially causes hypotension if infused rapidly	Tremor, tachycardia, dizziness, jitteriness	Unlikely to occur during a short 1- to 3-day course
Potential adverse effects (overdose)	Coma, respiratory depression	Unlikely	Headache, hypokalemia	Unlikely
Comment	Many different H_1-antihistamines and different dose regimens are listed as adjunctive medications in anaphylaxis guidelines.	Not mentioned in most anaphylaxis guidelines; an H_2-antihistamine should not be used alone in anaphylaxis; if used, it should be given with an H_1-antihistamine.	Deliver by nebulization and face mask.	Different glucocorticoids and different dose regimens are used; these medications are unlikely to play a role in the initial minutes to hours of an anaphylactic episode.

There are no randomized double-blind, placebo-controlled trials of any of these medications in the treatment of acute anaphylaxis episodes. The route of administration of H_1-antihistamines and glucocorticoids depends on the severity of the anaphylaxis episode. Adapted from reference 153.
*For use in anaphylaxis.

Those at risk for anaphylaxis in the community should wear medical identification jewelry that provides worldwide access to a patient registry service 24 hours a day, 365 days of the year, so that health care professionals treating them can obtain relevant information about their triggers, concomitant diseases, and concurrent medications if needed. An anaphylaxis wallet card listing relevant confirmed triggers, concomitant diseases, and concurrent medications is available at www.aaaai.org.[69,153]

An approach to anaphylaxis education for health care professionals, people at risk of anaphylaxis and their caregivers, and the general public is outlined in Table XIII.[69,153,188,189] The consistent message in anaphylaxis education should be that anaphylaxis is potentially a killer allergy, not a trivial lifestyle disease, and that prompt treatment is life-saving.[69,153]

Anaphylaxis education projects are now becoming a priority in some communities. The main goal of these efforts is to teach people to act promptly, recognize anaphylaxis, use an epinephrine autoinjector correctly and safely, call for help, transfer the patient to a health care facility, and also to recommend follow-up, preferably with an allergy/immunology specialist. Examples of specific education projects are those focusing on anaphylaxis after omalizumab injection in a physician's office,[190] and on follow-up of patients with anaphylaxis who are treated in the emergency department.[191] Many patients discharged from an emergency department after anaphylaxis treatment still do not receive a prescription for self-injectable epinephrine or a referral to a specialist physician.[192] Lack of access to epinephrine autoinjectors for children experiencing anaphylaxis in schools remains a concern.[188,189,193,194]

EMERGENCY MANAGEMENT OF ACUTE ANAPHYLAXIS IN A HEALTH CARE FACILITY

Emergency management of anaphylaxis in a health care facility is reviewed in depth elsewhere.[154,155,163,164] In any physician's

TABLE XIII. Anaphylaxis education

Health care professionals
Who: physicians, nurses, pharmacists, emergency medical technicians, and first responders
What: definition of anaphylaxis (new); shock not necessarily a criterion for diagnosis
Common triggers
Emergency preparedness
Recognition of evolving symptoms and signs; can be difficult in those unable to describe their symptoms, such as infants, or patients with dysphonia, dyspnea, or shock; severity varies among patients and in the same patient from one episode to another
Treatment: promptly and simultaneously inject epinephrine, activate 911 or emergency medical services,* and place patient on the back or in position of comfort with lower extremities elevated
When: at regular intervals
Key messages: Anaphylaxis can kill rapidly (within 15 minutes after an insect sting and within 30 minutes after ingestion of a food trigger). Inject first-aid dose of epinephrine promptly. Especially, do not hesitate if the patient has trouble breathing, throat tightness, or altered level of consciousness.
People at risk for anaphylaxis
Who: those who have experienced anaphylaxis previously and are at risk for recurrences and their families; for teens and young adults, their peers
What: triggers of anaphylaxis, prevention of episodes (trigger specific), emergency preparedness—recognize symptoms and signs, inject epinephrine; activate emergency medical services,* notify family
Hands-on epinephrine autoinjector training and coaching
When: teachable moments in the weeks or months after an anaphylactic episode and then at yearly intervals or more often
Key messages: Death from anaphylaxis can occur within minutes. Promptly inject epinephrine, activate emergency medical services* Place the patient on the back or in a position of comfort with lower extremities elevated.
General public
Who: educators, coaches, camp directors, child care providers, food industry workers, restaurant workers, and transportation workers
What: Anaphylaxis occurs in infants, children, teens, and adults who appear to be in excellent health until exposed to their trigger. Symptoms that mandate immediate treatment are sudden difficulty breathing, throat tightness, and altered level of consciousness.
When: at regular intervals, such as the start of academic year for educators; a highly publicized fatal episode of anaphylaxis increases public awareness.
Key messages: Anaphylaxis is a killer allergy. Promptly inject epinephrine, activate emergency medical services*. Place the patient on the back or in a position of comfort with lower extremities elevated.

Adapted from references 69, 153, 166, 188 and 189.
*Transport of the patient to an emergency department.

TABLE XIV. Reasons for lack of randomized controlled trials in patients with anaphylaxis

Anaphylactic episodes are unpredictable.
Anaphylaxis commonly occurs in community settings (eg, home, restaurant, and school).
Baseline measurements of vital signs and oxygenation are often not available.
Symptoms and signs vary from one person to another and from one episode to another, even in the same person, with regard to time of onset after exposure to trigger (minutes to hours), body organ systems involved, severity, and duration.
Symptoms sometimes resolve spontaneously because of endogenous production of epinephrine, endothelin I, and angiotensin II.
Randomized placebo-controlled trials would be unethical for epinephrine, although randomized placebo-controlled trials of H_1-antihistamines, H_2-antihistamines, and glucocorticoids might be conducted in the future.
Rarely, even with prompt and optimal treatment and monitoring, anaphylaxis can be fatal.

Adapted from reference 200.

office or clinic where allergen skin tests or allergen challenge/provocation tests are performed or allergen-specific immunotherapy, anti-IgE antibody injections or vaccine injections are given, it is important to develop and rehearse an anaphylaxis management plan, train the staff, and ensure availability of essential medications (within expiry date), as well as essential supplies and equipment.[195]

The basic principles of anaphylaxis management in a health care facility include rapid assessment of the patient's airway, breathing, circulation, and orientation/mentation; examination of the skin; and estimation of body weight/mass. Initial treatment involves discontinuing exposure to the trigger, if relevant (eg, discontinuing administration of an intravenous medication or biological agent), and then prompt and simultaneous intramuscular injection of epinephrine in a first-aid dose of 0.01 mg/kg to a maximum adult dose of 0.5 mg, calling for help (either a resuscitation team or 911/emergency medical services, whichever is appropriate), and placing the patient on the back or in a position of comfort with the lower extremities elevated.[154,155,166,195] Administration of supplemental oxygen by face mask at a rate of at least 6 to 8 L/min, airway management, and insertion of 1 or more large-bore (no. 14 or 16) needles or intravenous catheters for infusion of large volumes of fluid, such as 0.5 to 1 L of 0.9% (isotonic) saline in 5 to 10 minutes to an adult, should be performed if needed.[154,155,163,195] Most anaphylaxis guidelines recommend administration of an adjunctive medication such as an H_1-antihistamine, a nebulized β_2-adrenergic agonist, and a glucocorticoid[154,155,163-166] and some also recommend an H_2-antihistamine.[163]

It has also been suggested that epinephrine and other vasopressors should be administered intravenously only by physicians who are trained, experienced, and equipped to administer these potent medications effectively and safely; that is, to titrate the rate of infusion (preferably by using an infusion pump), according to

the patient's hemodynamic response assessed by means of continuous, noninvasive cardiac and blood pressure monitoring and pulse oximetry.[154,155] If it is used, intravenous epinephrine should only be given by slow infusion (not a bolus) of a dilute solution, 0.1 mg/mL (1:10,000) that is appropriate for intravenous use, and not the concentrated 1 mg/mL (1:1,000) dilution that is appropriate for intramuscular injection.[154] Physician confusion between dilute and concentrated epinephrine solutions potentially leads to dosing errors and fatality.[196] Existing studies do not permit a conclusion with regard to whether any one vasopressor is superior to another in preventing mortality in critically ill patients with shock.[197] Even in the hands of intensive care specialists, use of intravenous vasopressors might not improve outcomes and might increase fatality rates.[198,199]

FUTURE DIRECTIONS IN THE PHARMACOLOGIC MANAGEMENT OF ANAPHYLAXIS

Recommendations for the treatment of acute anaphylactic episodes are based on expert opinion rather than on randomized controlled trials in patients experiencing anaphylaxis at the time of the study. The reasons for lack of randomized controlled trials of pharmacologic interventions in anaphylaxis are summarized in Table XIV.[200]

It is important to note that the evidence base for epinephrine injection in the treatment of anaphylaxis is stronger than the evidence base supporting the use of H_1-antihistamines, H_2-antihistamines, or glucocorticoids in anaphylaxis.[160,165,183,185] Recommendations for prompt epinephrine injection are based on fatality studies, epidemiologic studies, observational studies, nonrandomized controlled studies in patients actually experiencing anaphylaxis, randomized controlled studies in patients not experiencing anaphylaxis at the time of the study, *in vitro* studies, and studies in animal models.[157-160,200]

The World Health Organization (www.who.int) and the World Allergy Organization,[159] as well as all anaphylaxis guidelines,[154,155,163-165] are in universal agreement that epinephrine injection is fundamentally important in anaphylaxis management. Placebo-controlled trials of epinephrine are therefore clearly unethical. Recommendations for the maximum initial dose of epinephrine or the route of injection differ among the guidelines, however, and in the future, it might be possible to conduct randomized trials comparing different first-aid epinephrine doses or different routes of injection.[200]

In contrast to the consensus about epinephrine, there is no consensus among published anaphylaxis guidelines with regard to the use of H_1-antihistamines, H_2-antihistamines, or glucocorticoids in the treatment of anaphylaxis. Many different H_1-antihistamines in a variety of dose regimens are recommended.[183] Several different glucocorticoids in a variety of dose regimens are recommended.[185] H_2-antihistamines are not mentioned in most guidelines.[165] In the future, it might therefore be possible to conduct randomized placebo-controlled trials of these medications in acute anaphylaxis episodes.[200]

If randomized controlled trials are conducted, in addition to the intervention being tested, it will be critically important to take rigorous appropriate precautions to ensure that all patients have prompt, optimal, standard-of-care treatment with epinephrine injections, are placed in the recumbent position or a position of comfort with lower extremities elevated; and have appropriate treatment with supplemental oxygen, airway management, and high-volume intravenous fluid resuscitation, as well as continuous noninvasive monitoring of heart rate, blood pressure, and oxygenation.[154,155,163,164,166,190,195]

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Primary immunodeficiencies

Luigi D. Notarangelo, MD *Boston, Mass*

In the last years, advances in molecular genetics and immunology have resulted in the identification of a growing number of genes causing primary immunodeficiencies (PIDs) in human subjects and a better understanding of the pathophysiology of these disorders. Characterization of the molecular mechanisms of PIDs has also facilitated the development of novel diagnostic assays based on analysis of the expression of the protein encoded by the PID-specific gene. Pilot newborn screening programs for the identification of infants with severe combined immunodeficiency have been initiated. Finally, significant advances have been made in the treatment of PIDs based on the use of subcutaneous immunoglobulins, hematopoietic cell transplantation from unrelated donors and cord blood, and gene therapy. In this review we will discuss the pathogenesis, diagnosis, and treatment of PIDs, with special attention to recent advances in the field. (J Allergy Clin Immunol 2010;125:S182-94.)

Key words: *Primary immunodeficiency, T-cell immunodeficiency, antibody deficiency, innate immunity defects, immunoglobulin replacement therapy, hematopoietic cell transplantation, gene therapy*

Primary immunodeficiencies (PIDs) comprise more than 130 different disorders that affect the development, function, or both of the immune system.[1] In most cases PIDs are monogenic disorders that follow a simple mendelian inheritance; however, some PIDs recognize a more complex polygenic origin. Disease penetrance and expression variability and interactions between genetic and environmental factors can also contribute to the phenotypic diversity of PIDs.

With the exception of IgA deficiency (IgAD), all other forms of PID are rare and have an overall prevalence of approximately 1:10,000 live births; however, a much higher rate is observed among populations with high consanguinity rates or among genetically isolated populations.

PIDs are classified according to the component of the immune system that is primarily involved.[1] Defects in adaptive immune responses include antibody deficiency syndromes and combined immunodeficiencies (CIDs). Defects of innate immunity comprise disorders of phagocytes, Toll-like receptor (TLR)–mediated signaling, and complement. All of these forms are characterized by increased susceptibility to recurrent infections, severe infections, or both, with distinctive susceptibility to various types of pathogens depending on the nature of the immune defect. In addition, some forms of PIDs present with immune dysregulation, and others (immunodeficiency syndromes) have a more complex phenotype in which immunodeficiency is only one of multiple components of the disease phenotype. For each of the various categories of PIDs, we will review cause and pathogenesis, clinical and laboratory features, prognosis, and treatment.

PATHOGENESIS AND CLINICAL FEATURES

CIDs

CIDs comprise a heterogeneous group of disorders with impaired development, function, or both of T lymphocytes associated with a defective antibody response.[2] The latter might result from intrinsic defects in B lymphocytes or might reflect inadequate T_H cell activity. In the most severe forms of CID (also known as severe combined immunodeficiency [SCID]), there is a virtual lack of functional peripheral T cells, whereas residual number, function, or both of T lymphocytes are present in other forms of CID.[2] Patients with SCID present early in life with infections of bacterial, viral, or fungal origin (Table I). Pneumonia caused by *Pneumocystis jiroveci* is common; however, interstitial lung disease might also be due to cytomegalovirus (CMV), adenovirus, respiratory syncytial virus, or parainfluenza virus type 3. Many infants with SCID have chronic diarrhea, leading to failure to thrive. Skin rash might reflect graft-versus-host disease caused by maternal T-cell engraftment in infants with SCID[3] or tissue damage caused by infiltration by activated autologous T lymphocytes, as typically seen in Omenn syndrome. In addition, some forms of SCID are associated with distinctive features in other systems.

SCID defects are classified according to the immunologic phenotype and are categorized into (1) SCID with absence of T lymphocytes but presence of B lymphocytes (T^-B^+ SCID) or (2) SCID with absence of both T and B lymphocytes (T^-B^- SCID). Both main groups of SCID include forms with or without natural killer (NK) lymphocytes. Regardless of the immunologic phenotype (T^-B^- or T^-B^+), patients with SCID present with similar clinical features, including early-onset severe respiratory tract infections, chronic diarrhea, and failure to thrive. SCIDs have a prevalence of approximately 1:50,000 live births and are more common in male subjects, reflecting the overrepresentation of X-linked SCID (SCIDX1), the most common form of SCID in human subjects.

The pathogenesis of SCID reflects distinct mechanisms that affect various steps in T-cell development (Fig 1).[2] Impaired survival of lymphocyte precursors is observed in reticular dysgenesis (RD) and in adenosine deaminase (ADA) deficiency. Both forms are inherited as autosomal recessive traits and are characterized by extreme lymphopenia. In addition, patients with RD also have severe neutropenia and sensorineural deafness. RD is a very rare form of SCID and is caused by mutations of the adenylate kinase 2 gene *(AK2)*.[4,5] This mitochondrial enzyme regulates levels of adenosine diphosphate. Adenylate kinase 2 deficiency

From the Division of Immunology and the Manton Center for Orphan Disease Research, Children's Hospital, Harvard Medical School.

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Reprint requests: Luigi D. Notarangelo, MD, Division of Immunology and the Manton Center for Orphan Disease Research, Children's Hospital Boston, Karp Family Research Building, 9th floor, Room 9210, 1 Blackfan Circle, Boston, MA 02115. E-mail: luigi.notarangelo@childrens.harvard.edu.

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Abbreviations used

AD: Autosomal dominant
ADA: Adenosine deaminase
AID: Activation-induced cytidine deaminase
AIRE: Autoimmune regulator
ALPS: Autoimmune lymphoproliferative syndrome
ANC: Absolute neutrophil count
AR: Autosomal recessive
AT: Ataxia-telangiectasia
BCR: B-cell receptor
BTK: Bruton tyrosine kinase
CD40L: CD40 ligand
CGD: Chronic granulomatous disease
CHS: Chediak-Higashi syndrome
CID: Combined immunodeficiency
CMV: Cytomegalovirus
CSR: Class-switch recombination
CVID: Common variable immunodeficiency
DC: Dendritic cell
DGS: DiGeorge syndrome
DHR-123: Dihydrorhodamine-123
DP: Double-positive
FOXP3: Forkhead box protein 3
HCT: Hematopoietic cell transplantation
HIES: Hyper-IgE syndrome
HLH: Hemophagocytic lymphohistiocytosis
ICOS: Inducible T-cell costimulator
IgAD: IgA deficiency
IPEX: Immune dysregulation–polyendocrinopathy–enteropathy–X-linked
IRAK: IL-1 receptor–associated kinase
IVIG: Intravenous immunoglobulin
JAK: Janus kinase
LAD: Leukocyte adhesion deficiency
MBL: Mannose-binding lectin
MMR: Mismatch repair
MSMD: Mendelian susceptibility to mycobacterial disease
NADPH: Reduced nicotinamide adenine dinucleotide phosphate
NHEJ: Nonhomologous end-joining
NK: Natural killer
PID: Primary immunodeficiency
RAG: Recombinase-activating gene
RD: Reticular dysgenesis
SBDS: Schwachman-Bodian-Diamond syndrome
SCID: Severe combined immunodeficiency
SCIDX1: X-linked severe combined immunodeficiency
SCIG: Subcutaneous immunoglobulin
SCN: Severe congenital neutropenia
SHM: Somatic hypermutation
STAT: Signal transducer and activator of transcription
TACI: Transmembrane activator and calcium modulator and cyclophilin ligand interactor
TAP: Transporter of antigenic peptide
TCR: T-cell receptor
THI: Transient hypogammaglobulinemia of infancy
TLR: Toll-like receptor
TREC: T-cell receptor excision circle
Treg: Regulatory T
UNG: Uracil N-glycosidase
WAS: Wiskott-Aldrich syndrome
WASP: Wiskott-Aldrich syndrome protein
XLA: X-linked agammaglobulinemia

results in increased apoptosis of myeloid and lymphoid precursors. ADA is an enzyme of the purine salvage pathway that mediates conversion of adenosine (and deoxyadenosine) to inosine (and deoxyinosine). In the absence of ADA, high intracellular levels of toxic phosphorylated metabolites of adenosine and deoxyadenosine cause apoptosis of lymphoid precursors in the bone marrow and thymus.[6,7] ADA deficiency accounts for 10% to 15% of all forms of SCID. Clinical manifestations of ADA deficiency extend beyond the immune system (deafness, behavioral problems, costochondral abnormalities, and liver toxicity), reflecting the fact that ADA is a housekeeping enzyme. Purine nucleoside phosphorylase is another enzyme of the purine salvage pathway. Purine nucleoside phosphorylase deficiency is rare (1% to 2% of all forms of SCID). In this disease immunologic abnormalities become progressively manifest within a few years after birth and are more pronounced in T than in B lymphocytes.[6] Progressive neurological deterioration and autoimmune hemolytic anemia are typically observed.

Defects of cytokine-mediated signaling are responsible for the majority of SCID in human subjects. SCIDX1 accounts for 40% of all cases of SCID and is caused by mutations of the IL-2 receptor γ gene *(IL2RG)*, which encodes for the common γ chain (γc) shared by cytokine receptors for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21. In particular, IL-7 mediates expansion of early thymocyte progenitors, whereas IL-15 plays a role in NK cell development.[8] Accordingly, patients with SCIDX1 lack both T and NK cells, whereas they have a normal number of circulating B lymphocytes.[9] The γc is physically and functionally coupled to the intracellular tyrosine kinase Janus kinase (JAK) 3, which delivers γc-mediated intracellular signaling. Hence defects of JAK3 result in an autosomal recessive form of SCID with an immunologic phenotype undistinguishable from that of SCIDX1.[10] Mutations of the *IL7R* gene (encoding for the α chain of the IL-7 receptor) abrogate T-lymphocyte development but leave B-cell and NK cell development intact.[11]

Expression of the pre–T-cell receptor (TCR) is a critical landmark during thymocyte development. Similar molecular mechanisms govern expression of the pre-TCR and of the pre–B-cell receptor (BCR) in developing T and B lymphocytes, respectively.[12] In particular, recombinase-activating gene 1 (RAG1) and RAG2 proteins mediate DNA cleavage at the TCR and immunoglobulin heavy and light chain loci, thus initiating V(D)J recombination. Several DNA repair proteins complete this process, allowing assembly of variable (V), diversity (D), and joining (J) elements. Defects of pre-TCR and pre–BCR expression account for a significant fraction of autosomal recessive T^-B^- SCID in human subjects and might reflect mutations in the *RAG1* and *RAG2* genes (which account for 4% to 20% of all cases of SCID) or in genes that encode proteins involved in nonhomologous end-joining (NHEJ) and DNA repair, in particular Artemis, DNA protein-kinase catalytic subunit, Cernunnos/XLF, and DNA ligase IV.[2,12-15] In all of these diseases, development of NK cells proceeds normally, so that NK lymphocytes represent almost all of the circulating lymphocytes, but generation of T and B lymphocytes is severely compromised. However, a leaky phenotype, with residual development of T and B lymphocytes, is typically seen in patients with Cernunnos/XLF deficiency.[16] Genetic defects that affect the NHEJ pathway are characterized also by increased cellular radiosensitivity with extraimmune manifestations (microcephaly, facial dysmorphisms, and defective tooth development).[17]

TABLE I. Type of infections associated with major categories of PIDs

Organism	Antibody deficiencies	CIDs	Phagocytic defects	Complement deficiencies
Viruses	Enteroviruses	All, especially: CMV, respiratory syncytial virus, EBV, parainfluenza type 3	No	No
Bacteria	*Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, Pseudomonas aeruginosa, Staphylococcus aureus, Neisseria meningitidis, Mycoplasma pneumoniae*	As for antibody deficiencies, also: *Salmonella typhi*, *Listeria monocytogenes*, enteric flora	*S aureus, P aeruginosa, Nocardia asteroides, S typhi*	As for antibody deficiencies: especially *N meningitidis* in deficiency of late components
Mycobacteria	No	Nontuberculous, including BCG	Nontuberculous, including BCG	No
Fungi	No	*Candida* species, *Aspergillus* species, *Cryptococcus neoformans, Histoplasmosis capsulatum*	*Candida* species, *Aspergillus* species	No
Protozoa	*Giardia lamblia*	*Pneumocystis jiroveci, Toxoplasma gondii, Cryptosporidium parvum*	No	No

Mutations of the CD3δ, CD3ϵ, and CD3ζ components of the CD3 complex affect signaling through the pre-TCR and hence cause SCID.[18] Few cases of mutations of the tyrosine phosphatase CD45, which mediates signaling in both T and B lymphocytes, have been also reported.[19,20]

Defects in T-cell development that occur beyond the $CD4^+CD8^+$ double-positive cell stage result in CID with residual numbers of T lymphocytes. Mutations of the ζ chain–associated protein of 70 kDa (ZAP-70) tyrosine kinase in human subjects compromise positive selection of $CD8^+$ thymocytes, thus causing severe deficiency of circulating $CD8^+$ T lymphocytes. $CD4^+$ T cells are generated and exported to the periphery; however, their proliferative response to stimulation through the TCR is drastically impaired.[21]

Positive selection of $CD8^+$ thymocytes is dependent on low-affinity recognition of self-peptides in the context of class I MHC molecules. The transporters of antigenic peptides 1 and 2 (TAP1/2) and tapasin shuttle newly synthesized peptides across the endoplasmic reticulum, where they are loaded onto HLA class I molecules and are then directed to the cell membrane. Mutations in the *TAP1*, *TAP2*, or tapasin genes cause selective $CD8^+$ cell deficiency.[22] However, this defect is rarely severe, reflecting residual MHC class I expression. The clinical phenotype of MHC class I deficiency is often marked by midline granulomatous lesions and vasculitis.

Defective expression of MHC class II molecules impairs positive selection of $CD4^+$ lymphocytes. This disease can be caused by mutations in 4 genes that encode for transcriptional activators of MHC class II genes. The disease is more common in certain geographic regions (North Africa). It is characterized by $CD4^+$ cell lymphopenia and has a progressive and severe clinical course.[23]

Recently, novel genetic defects have been identified in patients with other rare forms of CID. Calcium flux is essential to mediate the response of various cell types, including mature lymphocytes, to activatory stimuli. Genetic defects of *STIM1* (a sensor of calcium release from the endoplasmic reticulum) and *ORAI1* (a component of the calcium-release activated channels) cause inability of T lymphocytes to respond to TCR-mediated activation.[24,25] Muscular cells are also affected, causing myopathy.

The signal transducer and activator of transcription (STAT) 5b is a transcription factor that is activated in response to IL-2 and other cytokines and growth factors, including growth hormone. Mutations of *STAT5B* result in a rare form of immunodeficiency with short stature. Because IL-2 plays a critical role in immune homeostasis, STAT5b deficiency is often associated with autoimmune manifestations.[26]

Hypomorphic mutations in genes that are typically associated with SCID can allow residual T-cell development. In these cases impaired cross-talk between thymocytes and thymic epithelial cells might compromise mechanisms of central tolerance, with failure to delete autoreactive T cells and impaired generation of regulatory T (Treg) cells.[27,28] Accordingly, autoimmune or dysreactive manifestations are common, with infiltration of target tissues by activated and oligoclonal T lymphocytes. Omenn syndrome, which is caused by mutations in *RAG1/2* or other genes, is the prototype of these conditions and is characterized by erythroderma, lymphadenopathy, and inflammatory gut disease. Hypomorphic *RAG* mutations have been also associated with a novel phenotype characterized by granuloma formation, EBV-related lymphoma, and survival into late childhood.[29]

Idiopathic CD4 lymphopenia is defined based on a persistently low $CD4^+$ T-cell count ($<0.3\times10^9$/L in adults and $<1.0\times10^9$/L in childhood). It is a diagnosis of exclusion: infections sustained by HIV or other T-cell lymphotropic viruses, immunosuppressive treatment, or underlying autoimmune disease must be ruled out. Most patients with idiopathic CD4 lymphopenia are adults. Clinical features include opportunistic (caused by *P jiroveci*, *Cryptococcus neoformans*, *Candida* species, and mycobacteria) and viral infections.[30] Naive $CD4^+$ T cells are affected more than memory $CD4^+$ lymphocytes. Some degree of hypogammaglobulinemia is common.

CD40 ligand (CD40L) deficiency is inherited as an X-linked trait. CD40L is predominantly expressed by activated $CD4^+$

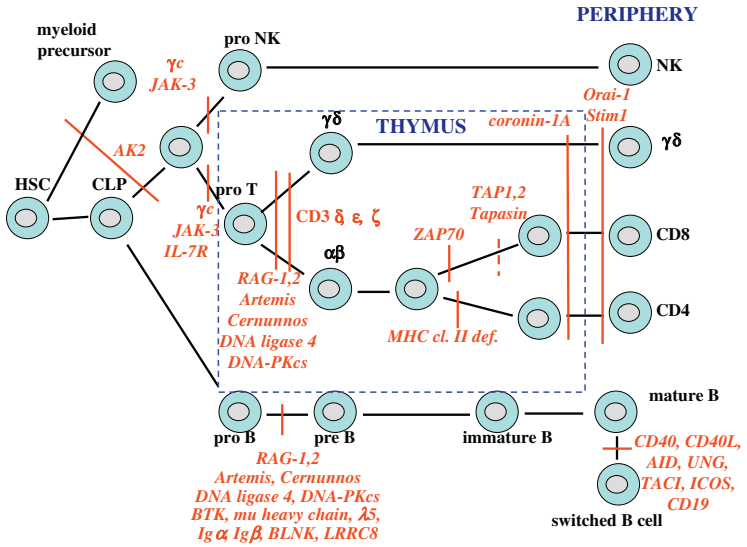

FIG 1. Blocks in T-and B-cell development associated with PIDs.

T lymphocytes and interacts with CD40, which is expressed by B lymphocytes, monocytes, dendritic cells (DCs), and other cell types. CD40L-CD40 interaction is a key signal in driving B-cell activation and, combined with interleukin-mediated signaling, promotes class-switch recombination (CSR). Accordingly, male subjects with CD40L deficiency have a severe defect of all immunoglobulin isotypes other than IgM. In addition, CD40L-CD40 interaction also promotes DC maturation and IL-12 secretion, favoring T-cell priming and production of IFN-γ, a key molecule in the defense against intracellular pathogens. Therefore patients with CD40L deficiency are also prone to opportunistic infections (*P jiroveci* and *Cryptosporidium parvum*), making CD40L deficiency a form of CID.[31] Neutropenia, which is usually associated with a block at the promyelocyte-myelocyte stage of differentiation in the bone marrow, is found in 65% of the patients with CD40L deficiency. A similar phenotype has been reported in patients with CD40 deficiency, a rare PID with autosomal recessive inheritance.[32]

T-cell immunodeficiencies caused by thymic defects

DiGeorge syndrome (DGS) is a developmental defect of the third and fourth pharyngeal pouches and arches, resulting in impaired development of the thymus and parathyroid glands, conotruncal heart abnormalities, facial dysmorphisms, feeding difficulties, and increased frequency of psychiatric disorders in childhood and adulthood.[33] In different series hemizygous deletion of chromosome 22q11 has been observed in 35% to 90% of patients; a minority of patients show deletion of 10p13-14. In most cases (referred to as "partial DGS"), there is mild-to-moderate T-cell deficiency, reflecting residual thymic development. Complete DGS with athymia is rare (1% of all cases) and presents with SCID-like features. Atypical complete DGS includes presentation with development of oligoclonal T cells that undergo extensive *in vivo* activation and infiltrate target organs, mimicking that observed in Omenn syndrome.

Forkhead box N1 (FOXN1) is a transcription factor required for thymic epithelial cells development. Autosomal recessive *FOXN1* deficiency has been reported in a few patients and is characterized by SCID associated with alopecia and nail dystrophy.[34]

Impaired egress of mature thymocytes has been reported in a single patient with mutations in coronin-1A, a regulator of actin cytoskeleton.[35]

Finally, Good syndrome is characterized by the association of hypogammaglobulinemia with thymoma. Opportunistic infections (candidiasis, CMV, and recurrent herpes simplex virus infections) and autoimmune cytopenia (especially red cell aplasia, neutropenia, or both) are common.[36]

Antibody deficiencies

Defective antibody production causes increased susceptibility, mostly to bacterial infections (Table I) that typically involve the upper and lower respiratory tract (otitis, sinusitis, and pneumonia) but might also cause abscesses in the skin or other organs, meningitis, urinary tract infections, and arthritis. Recurrent viral infections are also common. Intestinal *Giardia* species infection can cause protracted diarrhea. Antibody deficiencies might depend on a variety of defects that interfere with B-cell development, maturation, and/or function (Fig 1).[31]

Signaling through the pre-BCR is an essential step in B-cell development. The pre-BCR is composed of immunoglobulin heavy μ chains, surrogate light chains (V-preB and λ5), and the signal-transducing subunits Igα (CD79a) and Igβ (CD79b). This complex recruits a number of intracytoplasmic proteins, among which are the adaptor molecule B cell linker protein (BLNK) and Bruton tyrosine kinase (BTK). Defects in BTK account for X-linked agammaglobulinemia (XLA), the most common form (85%) of early-onset agammaglobulinemia in human subjects. Mutations in the immunoglobulin heavy μ chain gene *(IGHM)* are the second most common cause (5%), whereas only a few patients have been identified with defects in λ5, Igα, Igβ, and BLNK.[31,37] In all of these cases, there is a block at the pro-B to pre-B stage of differentiation in the bone marrow, resulting in virtual absence (<1%) of circulating B lymphocytes. However, the defect is often incomplete in patients with XLA, and few B cells might be identified in peripheral blood. A chromosomal translocation involving the leucine-rich repeat-containing protein 8 gene *(LRRC8)* has been reported in 1 patient in whom congenital agammaglobulinemia with developmental arrest at the pro–B-cell

stage was associated with facial dysmorphisms.[38] In addition to bacterial infections, patients with agammaglobulinemia are uniquely susceptible to enteroviral infections (which might cause meningoencephalitis or severe dermatomyositis) and mycoplasma (arthritis). Fortunately, both these complications are rare in patients treated appropriately with replacement immunoglobulins.

Maturation of the antibody response is marked by 2 key processes: CSR and somatic hypermutation (SHM).[39] During CSR, the μ chain is replaced by other immunoglobulin heavy chains, resulting in the production of IgG, IgA, and IgE, which have distinct physicochemical and biologic properties. SHM is the process by which point mutations are introduced in the variable region of the immunoglobulin genes, leading to increased binding affinity for antigen (affinity maturation). Although CSR and SHM are independent and distinct processes, they both occur in the germinal centers and are triggered by similar signals, such as CD40L-CD40 interaction and TLR-mediated signaling. CSR involves active transcription through the heavy chain constant-regions loci, with formation of DNA/RNA hybrids that leave one strand of DNA accessible to changes and cleavage. Induction of CSR promotes transcription of the activation-induced cytidine deaminase gene *(AICDA)*, which encodes a DNA-editing enzyme (also known as activation-induced cytidine deaminase [AID]) that replaces deoxycytidine residues with deoxyuracil. The resulting mismatch in the DNA is recognized by the enzyme uracil N-glycosylase (UNG), which removes the deoxyuracil residues, leaving abasic sites that are resolved by means of DNA repair mechanisms. Similar events occur during SHM; however, the mechanisms of DNA repair between these processes are distinct.[39] In particular, DNA repair during CSR involves proteins of the NHEJ pathway, as well as the Ataxia-Telangiectasia Mutated (ATM), Meiotic Recombination 11 (MRE11) and Nijmegen Breakage Syndrome 1 (NBS1) proteins. Furthermore, the DNA mismatch repair (MMR) system also participates in CSR. In contrast, SHM involves error-prone DNA polymerases and the MMR pathway. Mutations in the *AICDA* and *UNG* genes account for B-cell intrinsic defects of CSR, resulting in absent or very low levels of serum IgG, IgA, and IgE, whereas IgM levels are often increased (thus resulting in a hyper-IgM phenotype).[39] The vast majority of AID and all of the few cases of UNG deficiency reported thus far are inherited as autosomal recessive traits; however, few cases of mutations in the C-terminal region of AID have autosomal dominant inheritance.[31,39] SHM is differently affected by AID versus UNG deficiency; in particular, SHM is abolished in the former (with the exception of mutations that affect the C-terminus of the AID molecule), whereas it is preserved but biased (with lack of mutations at A:T residues) in UNG deficiency.[39]Impaired CSR with reduced levels of IgG, IgA, and IgE is also observed in patients with ataxia-telangiectasia (caused by mutations of the *ATM* gene), ataxia-telangiectasia–like syndrome (*MRE11* mutations), Nijmegen breakage syndrome (*NBS1* mutation), and ligase IV syndrome in keeping with the role that these proteins play in CSR.[39,40] Also, mutations of Post-Meiotic Segregation increased *2 (PMS2)*, which is involved in MMR, cause impaired CSR, which is associated with high susceptibility to malignancies and café au lait spots.[41] In spite of these advances, a significant proportion (about 15%) of CSR defects currently grouped under the definition of hyper-IgM syndrome remain genetically undefined.[39]

Common variable immunodeficiency disorders (CVIDs) are the most common form of clinically significant PIDs and present mainly in adults, although they can also be observed in children. CVIDs are defined by reduced levels of 1 or more isotypes and impaired antibody production in response to immunization antigens or natural infections.[42] CVIDs are a diagnosis of exclusion of all known causes of poor antibody production or low serum immunoglobulin levels. In addition to recurrent infections of the respiratory tract (sinusitis, otitis, bronchitis, and pneumonia) caused by common bacteria (eg, nontypeable *Haemophilus influenzae* and *Streptococcus pneumoniae*), some patients with a CVID are highly prone to autoimmmune manifestations (cytopenias and inflammatory bowel disease), granulomatous lesions, lymphoid hyperplasia, and tumors (especially lymphomas).[42-44] There is now clear evidence that CVIDs include a group of clinically and genetically heterogeneous conditions. CVIDs are mostly sporadic; however, autosomal dominant and autosomal recessive forms are also possible. Although most patients with a CVID have a normal number of B lymphocytes, some (12%) will turn out to have differentiation defects. Among those who do have B cells, some have reduced switched memory ($CD27^+IgD^-$) B lymphocytes and a low rate of SHM[45]; this might turn out to be due to a T- or B-cell failure. Most cases of CVIDs remain genetically undefined and might well turn out to be polygenic. Several studies of familial cases of CVIDs have shown association with the MHC region; however, the underlying gene defect has not been clearly identified. Mutations of the *T*umor *N*ecrosis *F*actor *R*eceptor *S*oluble *F*actor *13B (TNFRSF13B)* gene, which encodes the transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI), a member of the TNF receptor family, have been identified in 15% of the patients, most often in heterozygosity,[31,42,46] and have been found to be present in the general population as well. TACI is expressed by B lymphocytes and interacts with 2 ligands: the B-cell activating factor (BAFF) and a proliferation–inducing ligand (APRIL). In particular, APRIL/TACI interaction promotes B-cell activation and CSR. Disruption of the *tnfrsf13b* gene in mice leads to lymphoid proliferation, and lymphoid hyperplasia and granulomatous lesions are often seen in patients with a CVID. Nine patients have been identified with mutations in the inducible T-cell costimulator gene *(ICOS)*.[47] ICOS is expressed by activated T cells and interacts with ICOS ligand expressed by B lymphocytes. This interaction promotes B-cell activation and antibody production. Finally, a few cases of CVIDs are due to mutations of the *CD19* gene.[48] The CD19 protein forms a complex with CD21, CD81, and CD225 and decreases the threshold of BCR-mediated activation. CD19 deficiency does not affect B-cell development, as shown by a normal number of circulating $CD20^+$ lymphocytes in CD19-deficient patients.

IgAD is the most common PID, with approximately 1:700 affected individuals worldwide. Both partial and complete forms of IgAD are known. The pathophysiology of IgAD remains poorly understood, although association with MHC alleles and higher frequency within families with a CVID have been reported. Approximately two thirds of adults with IgAD are asymptomatic, but the remaining might experience recurrent infections, autoimmunity, or allergy.[49] Adult patients with IgAD and a history of infections often have associated defects of IgG subclasses, especially IgG2. However, children are more likely to have delayed maturation of immunoglobulin synthesis and will not progress to significant immunodeficiency. IgG subclass

deficiency might also occur without abnormalities in other isotypes. The pathophysiology of IgG subclass deficiency remains unknown in most cases; interstitial deletions on both alleles of the immunoglobulin heavy chain constant region have been reported in a minority of patients, most of whom have been infection free.

Specific antibody deficiency is characterized by impaired antibody production (especially to carbohydrate antigens) without abnormalities in total immunoglobulin levels or in B-cell numbers. The pathophysiology of this disorder, which is associated with recurrent upper and lower respiratory tract infections,[50] remains unclear.

Patients with transient hypogammaglobulinemia of infancy (THI) have low immunoglobulin levels that spontaneously return to normal, usually within 2 years of age, although this is very variable.[51,52] Although many subjects with THI remain asymptomatic, this condition is associated with a higher rate of recurrent infections, especially upper respiratory tract infections of viral origin. The pathophysiology of THI is unknown. In a prospective study of infants who presented with hypogammaglobulinemia, a low number of memory B cells and inability to produce IgG *in vitro* were associated with persistence of hypogammaglobulinemia and increased risk of infection beyond 2 years of age.[51]

Immunodeficiency with immune dysregulation

Some forms of immunodeficiency are characterized by significant autoimmune manifestations, reflecting disturbance in immune homeostasis.[53,54] Central immune tolerance is achieved through deletion of autoreactive T-cell clones in the thymus. The autoimmune regulator (AIRE) protein is a transcription factor expressed by mature medullary thymic epithelial cells. AIRE drives expression of tissue-restricted antigens that are presented by medullary thymic epithelial cells and thymic DCs to nascent T lymphocytes, thereby permitting deletion of T-cell clones that recognize self-antigens with high affinity. Mutations of the *AIRE* gene disrupt this protein, causing autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy syndrome.[55] Hypoparathyroidism and adrenal insufficiency are prominent autoimmune manifestations of this autosomal recessive disorder. Treg cells mediate suppression of immune responses to self-antigens in the periphery. Generation of Treg cells in the thymus is controlled by the transcription factor forkhead box protein 3 (FOXP3). Mutations of the *FOXP3* gene cause immune dysregulation–polyendocrinopathy–enteropathy–X-linked (IPEX) syndrome, with severe and early-onset autoimmune enteropathy, insulin-dependent diabetes, and eczema.[56] In typical cases the disease evolves rapidly, unless treated with hematopoietic cell transplantation (HCT). IL-2 plays an important role in immune homeostasis and upregulates expression of FOXP3 by $CD4^{+}CD25^{hi}$ Treg cells. Mutations of the *IL2RA* gene, which encodes for the α chain of the IL-2 receptor, cause immunodeficiency with IPEX-like features.[57] Peripheral immune homeostasis is also based on apoptosis of autoreactive lymphocytes in the periphery. Interaction between Fas ligand, expressed by activated lymphocytes and Fas (CD95) triggers intracellular signaling pathways that ultimately result in activation of caspases and cell death. Mutations of Fas are the predominant cause of autoimmune lymphoproliferative syndrome (ALPS), with lymphadenopathy, hepatosplenomegaly, and autoimmune cytopenia.[58] There is an increased risk of malignancies (especially B-cell lymphomas), which occur in 10% of the patients with Fas mutations.[59] ALPS is most often inherited as an autosomal dominant trait and is caused by dominant-negative mutations that interfere with the signal-transducing activity of Fas trimeric complexes. Somatic mutations of the Fas gene have been reported in a few cases. A rare variant of Fas is caused by Fas ligand mutations. In a few patients, mutations of caspase-8 and caspase-10 have been identified also.[60,61] IL-2 starvation induces apoptosis through a mechanism that depends on the proto-oncogene Neuroblastoma RAS viral oncogene homolog (NRAS). Mutations of this gene have been identified in a single family with ALPS.[62] A significant fraction of patients with ALPS remain genetically undefined.

Immunodeficiency with impaired cell-mediated cytotoxicity

The cytotoxic activity of T and NK lymphocytes depends on the expression of cytolytic proteins that are assembled into granules and transported through microtubules to the lytic synapse that is formed on contact with target cells. Some forms of immunodeficiency are characterized by impairment of the mechanisms of transport, docking, or release of the lytic granules. These disorders are frequently associated with defective intracellular transport of melanin, resulting in immunodeficiency with pigmentary dilution disorders.[63] The Chediak-Higashi syndrome (CHS) is an autosomal recessive disease caused by mutations of the Lysosomal Trafficking regulator *(LYST)* gene. In addition to impaired cytotoxicity, the phenotype of CHS includes the presence of giant lysosomes in leukocytes, gray-silvery hair, and peripheral neuropathy that reflects primary involvement of the nervous system.[64] Cytotoxicity defects characterize also the autosomal recessive forms of hemophagocytic lymphohistiocytosis (HLH) because of deficiency of perforin (which forms the cytolytic pores on contact with target cells), Munc13-4 (involved in intracellular transport of lytic granules), and syntaxin 11 (presumably involved in permitting fusion of the cell membrane between cytotoxic lymphocytes and target cells). In these diseases the inability to extinguish inflammatory reactions results in sustained and excessive production of T_H1 cytokines and IFN-γ in particular. This inflammatory reaction characterizes the life-threatening "accelerated phase" of the disease with hemophagocytosis. A similar phenotype has been occasionally observed also in Hermansky-Pudlak syndrome type 2, a condition with oculocutaneous albinism, severe neutropenia, and tendency to bleeding, which is caused by mutations of the β component of the adaptor-related protein complex 3 (AP3) involved in sorting of granules to the endosomal pathway.[65]

The X-linked lymphoproliferative disease, mainly caused by EBV infection, might be due to mutation of the *SH2D1A* gene, which encodes an adaptor protein involved in intracellular signaling in T and NK lymphocytes,[66] or of the *BIRC4* gene, which encodes for the X-linked inhibitor of apoptosis.[67] More recently, an autosomal recessive form of the disease has been identified that is caused by mutations of *ITK*, an intracellular tyrosine kinase expressed in T lymphocytes.[68] All of these 3 forms of lymphoproliferative disease share a lack of NK T cells, suggesting a possible role of these rare populations of lymphocytes in controlling EBV infection.

Defects of innate immunity: Phagocytes, TLRs, leukocyte signaling pathways, and complement

Phagocytic cell defects. Phagocytes play a key role in the defense against bacteria and fungi; accordingly, patients with defects of phagocytic cell number, function, or both experience recurrent and severe infections of fungal (especially *Candida* and *Aspergillus* species) and bacterial origin. Respiratory tract and cutaneous infections predominate, but deep-seeded abscesses are also common. Recurrent oral stomatitis is present in most cases.

Severe congenital neutropenia (SCN) is defined as a neutrophil count that is persistently less than 0.5×10^9 cells/L. A variety of genetic defects might cause SCN in human subjects.[69] The most common form of SCN is due to mutation of the *ELA2* gene, which encodes neutrophil elastase. This disease might be sporadic or autosomal dominant and is associated with a block at the promyelocyte-myelocyte stage in the bone marrow. Some *ELA2* mutations cause cyclic neutropenia, with oscillations in neutrophil count, which reach a nadir approximately every 21 days, resulting in periodicity of the infections. *ELA2* mutations carry an increased risk of myelodysplasia and myeloid leukemia associated with somatic mutations in the granulocyte colony-stimulating factor receptor *(GCSFR)* gene. Among autosomal recessive forms of SCN, HAX1 deficiency (identified also in the original SCN pedigree reported by Kostmann et al) causes increased apoptosis of myeloid cells. Some *HAX1* mutations cause also increased neuronal cell death, leading to a severe neurological phenotype.[70] Increased apoptosis caused by intracellular unbalance of glucose levels is also observed in myeloid cells from patients with mutations in the glucose-6-phosphatase catalytic subunit 3 gene *(G6PCS3)* or in patients with glycogenosis 1b caused by defects of the *G6PT1* gene, which encodes a G6P transporter. Heart and urogenital defects and prominence of superficial veins are part of the *G6PCS3* deficiency phenotype.[71] More rarely, autosomal recessive SCN is due to p14 deficiency; this form is associated with partial oculocutaneous albinism and short stature. Mutation of the Growth Factor Independent 1 *(GFI1)* gene, encoding a myeloid transcription factor, causes a rare form of autosomal dominant SCN; T and B lymphopenia is also observed. A rare X-linked recessive form of SCN with increased risk of myelodysplasia is caused by activating mutations of the Wiskott-Aldrich syndrome protein gene *(WASP)*. Finally, X-linked neutropenia, heart defects, and growth retardation are features of Barth syndrome, which is caused by mutation of the Tafazzin gene *(TAZ)* with mitochondrial defects.

Chronic granulomatous disease (CGD) represents the prototype of defects of phagocyte function and is caused by defects in the reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex.[72] In 75% of cases, CGD is inherited as an X-linked trait caused by mutations in the X-linked *CYBB* gene, which encodes the $gp91^{phox}$ component of NADPH oxidase. Autosomal recessive forms caused by defects of the $p22^{phox}$, $p47^{phox}$, and $p67^{phox}$ components are also known. The $gp91^{phox}$ and $p22^{phox}$ proteins are located in the phagosome membrane, where cytosolic $p47^{phox}$ and $p67^{phox}$ are translocated after phagocytic cell activation. Assembly of the NADPH complex is regulated by 2 glutathione triphosphatases, Rac2 and Rap1, and defects of Rac2 have been identified in 1 patient with CGD-like disease. After phagocytosis, induction of the NADPH oxidase complex results in production of microbicidal compounds (superoxide radicals and hydrogen peroxide) and activation of lytic enzymes (cathepsin G, elastase, and myeloperoxidase), resulting in intracellular killing of bacteria and fungi. Patients with CGD experience recurrent and often severe infections (skin, liver and perirectal abscesses, pneumonia, and lymphadenitis). *Staphylococcus aureus*, *Serratia marcescens*, *Burkholderia cepacia*, and *Nocardia* species are responsible for most of the bacterial infections, whereas *Candida* and *Aspergillus* species cause most of the fungal infections. Patients with CGD are also at higher risk for mycobacterial infections. The sustained inflammatory response observed in patients with CGD is also responsible for the granulomatous manifestations of the disease that recur, especially in hollow organs, even in the absence of infections, causing gastric outlet obstruction, noninfective colitis, and hydronephrosis.[72]

Leukocyte adhesion deficiency (LAD) includes a series of syndromes characterized by impaired trafficking of leukocytes.[73] LAD1 is due to mutations of the *ITGB2* gene, which encodes the β_2 integrin component (also known as CD18) shared by the lymphocyte function–associated antigen 1, complement receptor 3, and complement receptor 4 molecules. These β_2 integrins mediate stable adhesion between leukocytes and endothelial cells, permitting transendothelial passage of leukocytes that can thus reach the sites of infection/inflammation. LAD2 is caused by mutations in a GDP-fucose transporter. In this disease defective fucosylation of proteins results in a lack of expression of Sialyl-Lewis-X, the ligand for E-selectin. This defect causes impaired rolling of leukocytes along the endothelium.[73] Finally, LAD3 is caused by mutations of kindlin-3, which is involved in inside-outside integrin signaling.[74] All 3 forms of LAD are inherited as an autosomal recessive trait, and they all present early in life with very severe infections and lack of pus formation. Impaired wound healing (even after minor trauma) and severe periodontitis are typically present. In addition, patients with LAD2 have short stature, facial dysmorphisms, mental retardation, and Bombay blood-type phenotype, reflecting the generalized defect of fucose metabolism. LAD3 is associated with increased bleeding caused by platelet functional defects.

Defects of TLRs. TLRs are a series of molecules that are expressed at the cell surface or endosomal membrane and mediate recognition of pathogen-associated molecular patterns, such as LPS, glycolipids, and single- or double-stranded RNA. The classical pathway of TLR activation involves the adaptor molecules MyD88 and the intracellular kinases IL-1 receptor–associated kinase (IRAK) 4 and IRAK-1 and ultimately results in the induction of nuclear factor κB and production of inflammatory cytokines (IL-1, IL-6, TNF-α, and IL-12). TLR-3, TLR-7, TLR-8, and TLR-9 can activate an alternative pathway that involves the adaptor molecules TRIF and the UNC-93B protein, resulting in the induction of type 1 interferons (IFN-α/β). Surprisingly, mutations in genes involved in TLR-mediated signaling result in a selective susceptibility to pathogens in human subjects. In particular, *IRAK4* and *MYD88* gene mutations (both inherited as autosomal recessive traits) have a similar phenotype, with severe and invasive pyogenic infections early in life, often without significant inflammatory response.[75,76] Infections tend to become less frequent later in life. Heterozygous mutations in *TLR3* and biallelic mutations in *UNC93B* have been identified in patients with selective susceptibility to herpes simplex encephalitis, with reduced production of type 1 IFN.[77,78]

Defects of the IL-12/IFN-γ signaling pathway. The immune response against mycobacteria is based on secretion of

IL-12 by macrophages. IL-12 binds to a specific receptor expressed by T and NK lymphocytes and induces secretion of IFN-γ that triggers macrophage microbicide on binding to the IFN-γ receptor. Defects of the IL-12/IFN-γ axis account for mendelian susceptibility to mycobacterial disease (MSMD).[79] Mutations might affect the IL-12 p40 subunit, the IL-12 receptor β1 chain, both chains of the IFN-γ receptor (encoded by the *IFNGR1* and *IFNGR2* genes), and the *STAT1* gene, which encodes a transcription factor downstream of the IFN-γ receptor. Different mutations in these genes account for autosomal dominant and autosomal recessive variants of MSMD. In addition, mutations in the *IKBKG* gene, encoding the IKK-γ (also known as NF-κB essential modulator, NEMO) regulatory component of the nuclear factor κB signaling pathway, also cause increased susceptibility to mycobacterial disease associated with CID, impaired CSR, and ectodermal dystrophy.[80] NEMO deficiency is inherited as an X-linked trait. All forms of MSMD are characterized by increased susceptibility to environmental mycobacteria (*Mycobacterium avium*, *Mycobacterium kansasii*, and *Mycobacterium fortuitum*) and to BCG vaccine strain. *Salmonella*, *Listeria*, and *Histoplasma* species infections can also be observed, especially in patients with *IL12RB1* mutations. Complete STAT1 deficiency causes susceptibility to severe viral infections in addition to MSMD.[81]

Complement defects. A variety of inherited defects of complement components have been reported.[82] Deficiency of the early components of the classical pathway of complement (C1q, C1r, C1s, C4, C2, and C3) causes autoimmune manifestations resembling systemic lupus erythematosus. C2 and C3 deficiencies also lead to increased risk of infections caused by capsulated bacteria. Defects of late components (C5-C9) are associated with recurrent and invasive neisserial infections. A similar phenotype is observed in patients with defects of factor D or of properdin, 2 components of the alternative pathway of complement activation. Deficiencies of the regulatory components Factor H and Factor I cause membranoproliferative glomerulonephritis and recurrent atypical hemolytic-uremic syndrome. The latter can also be caused by deficiency of membrane cofactor protein, a C3b/C4b-binding molecule of the complement system with cofactor activity for the Factor I–dependent cleavage of C3b and C4b. Deficiency of the mannose-binding lectin (MBL), a component of the MBL-dependent pathway of complement, has been associated with increased risk of recurrent bacterial infections, especially during the first years of life. However, it is more plausible that MBL deficiency plays a contributory role in patients who have additional risk factors. Mutations of the MBL-associated serine-protease-2 gene *(MASP2)* have been also linked to increased occurrence of infections. Finally, deficiency of the C1 esterase inhibitor, a regulatory component of the classical pathway of complement activation, does not cause immunodeficiency but hereditary angioedema, with recurrent episodes of edema that might involve the mucosa of the larynx and the gut, as well as the face and the extremities. These manifestations reflect uncontrolled release of bradykinin caused by lack of inhibition by C1 esterase inhibitor of the kallikrein-kinin system.

Immunodeficiency syndromes

The term immunodeficiency syndromes applies to several disorders in which other clinical features are present in addition to immunodeficiency. The Wiskott-Aldrich syndrome (WAS) is an X-linked disease characterized by eczema, immunodeficiency (with increased susceptibility to infections, autoimmunity, and lymphoid malignancies), and congenital small-sized thrombocytopenia.[83] However, only one third of the patients present all of the elements of this triad. WAS is cased by mutations in the *WASP* gene, which encodes a regulator of actin cytoskeleton the expression of which is restricted to hematopoietic cells. Hypomorphic mutations of the *WASP* gene, especially in exons 1 and 2, are often associated with a milder variant of the disease isolated X-linked thrombocytopenia.[83] In contrast, transactivating mutations of *WASP* cause X-linked neutropenia and myelodysplasia (see above).

Several immunodeficiencies are caused by defects in mechanisms that sense, repair, or both DNA breaks. Some of these defects are associated with SCID (see above). Ataxia-telangiectasia is an autosomal recessive disease caused by mutations of the ataxia-telangiectasia mutated gene *(ATM)*.[84] Patients with ataxia-telangiectasia have ataxia, ocular telangiectasia, increased risk of infections, and tumors. The immunodeficiency is marked by a progressive decrease of T-lymphocyte counts and function and hypogammaglobulinemia. Typically, levels of α-fetoprotein are increased. A similar phenotype, although without the increase in α-fetoprotein levels, is observed in patients with mutations in the *MRE11* gene, which is involved in DNA repair. The Nijmegen syndrome associates immunologic findings with microcephaly, "bird-like" facies, short stature, and increased occurrence of malignancies and is due to mutations in the Nibrin gene *(NBS1)*.[85] Ligase IV (LIG4) syndrome is due to defects of the *LIG4*gene, which encodes a factor involved in the DNA repair phase of the NHEJ process. Patients with LIG4 syndrome present with microcephaly, facial dysmorphism and increased susceptibility to tumors, and a variable degree of immunodeficiency that ranges from SCID/Omenn syndrome to hypogammaglobulinemia with impaired CSR to moderate or even very modest defects of T- and B-cell immunity.[86] Immunodeficiency–centromeric instability–facial anomalies syndrome is most often due to mutations of the DNA methyltransferase 3B gene *(DNMT3B)*. From an immunologic standpoint, immunodeficiency–centromeric instability–facial anomalies syndrome is characterized by recurrent bacterial and opportunistic infections, hypogammaglobulinemia, and a reduced number of T and B lymphocytes.[87]

The hyper-IgE syndrome (HIES) is characterized by eczema, increased occurrence of cutaneous and pulmonary infections sustained by *S aureus* (with formation of pneumatoceles) and *Candida* species, and markedly increased IgE levels.[88] Life-threatening superinfection of pneumatoceles by *Aspergillus* and *Scedosporium* species is common. Sporadic autosomal dominant and autosomal recessive forms are known. The autosomal dominant HIES associates defective shedding of primary teeth, scoliosis, higher risk of bone fractures, joint hyperextensibility, characteristic facial appearance, and vascular abnormalities with aneurysms and is due to dominant-negative heterozygous mutations of the *STAT3* gene.[89] STAT3 is a transcription factor that is activated in response to activation of the JAK-STAT signaling pathway through cytokine and growth factor receptors that contain the gp130 protein. Biologic responses to IL-6 and IL-10 are decreased, and development of T_H17 cells is impaired, resulting in poor secretion of IL-17, IL-21, and IL-22. The phenotype of autosomal recessive HIES is different. These patients might also have viral disease but do not present with skeletal or dental abnormalities. Vasculitis and autoimmunity are common in autosomal

recessive HIES. In one case of autosomal recessive HIES, mutations of the tyrosine kinase 2 gene *(TYK2)*, encoding one of the members of the JAK family upstream of STAT3, were demonstrated.[90] However, most cases of autosomal recessive HIES remain genetically undefined.

Veno-occlusive disease with immunodeficiency syndrome is a CID associated with early-onset severe liver disease and profound hypogammaglobulinemia and is caused by a mutation of the gene encoding the nuclear body protein Sp110.[91]

Immuno-osseous dysplasias include cartilage hair hypoplasia, Schimke disease, and Shwachman-Bodian-Diamond syndrome (SBDS). Cartilage hair hypoplasia is caused by mutations of the RNAse mitochondrial ribonucleoprotein gene *(RMRP)*. It is characterized by short-limbed dwarfism, sparse hair, and frequent occurrence of anemia or other forms of bone marrow failure associated with a variable degree of immunodeficiency, ranging from SCID to virtually normal immune function.[92] Schimke disease (caused by mutations of *SMARCAL1*)[93] associates short stature and progressive renal disease. SBDS is characterized by exocrine pancreatic insufficiency, bone marrow failure, and metaphyseal chondrodysplasia and is caused by a mutation of a gene involved in ribosome biogenesis.[94] Neutropenia is a prominent feature of SBDS and might cause severe infections.

Warts-hypogammaglobulinemia-infections-myelokathexis syndrome is an autosomal dominant disease caused by heterozygous mutations of the *CXCR4* chemokine receptor, which cause sustained and inappropriate signaling mediated by the ligand CXCL12. CXCL12-CXCR4 interaction is important in the governance of leukocyte trafficking. In patients with Warts-hypogammaglobulinemia-infections-myelokathexis, there is retention of mature neutrophils in the bone marrow (myelokathexis), resulting in severe neutropenia. Trafficking of B lymphocytes is also affected, causing B-cell lymphopenia and a variable degree of hypogammaglobulinemia. Patients are highly prone to papillomavirus infections (warts).[95]

DIAGNOSTIC APPROACH TO PIDS

The main forms of PID (CIDs, antibody deficiencies, and defects of innate immunity) are characterized by different susceptibilities to pathogens (Table I). Accordingly, medical history (with particular regard to type, location, age at onset, and severity of infections) might provide important insights into the possible underlying mechanisms of immunodeficiency. Additional aspects of past medical history might also help. Certainly a history of HIV infection is very important in the differential diagnosis of SCID. Also, a history of seizures during neonatal age should prompt one to consider DGS.

Family history is also important in the approach to PIDs because of the monogenic nature of most forms of these disorders. However, most patients with PIDs do not have a positive family history because they represent *de novo* mutations or the first occurrence of an autosomal recessive disease.

Physical examination can also provide important hints. Patients with agammaglobulinemia show absence of tonsils and other lymphoid tissues. Partial albinism characterizes pigmentary dilution disorders, ataxia and ocular telangiectasias are observed in ataxia-telangiectasia, microcephaly is common in PIDs associated with defects in DNA repair, petechiae and other bleeding manifestations associated with eczema are highly suggestive of WAS, and patients with immuno-osseous disorders have short stature. Generalized erythroderma is typical of Omenn syndrome but can also occur in IPEX, in SCID with maternal T-cell engraftment, in atypical complete DGS, and occasionally in NEMO deficiency.

Clinical immunologic laboratory tests are very important to validate the suspicion of PID. Lymphopenia, and marked reduction of T-lymphocyte counts in particular, is a hallmark of SCID, but HIV infection must be excluded in all cases. It is very important to compare lymphocyte counts with those of age-matched healthy control subjects. The presence of maternal T-cell engraftment or of residual autologous T cells in patients with CID might result in relatively preserved (and even normal) T-lymphocyte counts and hence confound the picture; however, in these cases most circulating T lymphocytes have an activated/memory ($CD45R0^+$) phenotype, and there is a virtual lack of naive ($CD45RA^+$) T lymphocytes. T-cell receptor excision circles (TRECs), consisting of circularized signal joints, are a byproduct of V(D)J recombination and are exported to the periphery by newly generated T lymphocytes that leave the thymus. Levels of TRECs in circulating lymphocytes are particularly high in newborns and infants (reflecting active thymic function) and progressively decrease with age. No TRECs are detected in infants with SCID; assessment of TREC levels by means of PCR has been proposed for newborn screening of SCID,[96] and a pilot study has been recently started in Wisconsin and Massachusetts.[97] In addition to the severe T-cell lymphopenia, *in vitro* response to mitogens is absent in patients with SCID; however, the number and proliferative responses of circulating T lymphocytes are often variable in patients with other forms of CID.

Evaluation of patients with putative antibody deficiency should include enumeration of B lymphocytes, measurement of total immunoglobulins, and assessment of specific antibodies to both protein and polysaccharide antigens. B lymphocytes (identified based on CD19 or CD20 expression) are absent in patients with congenital agammaglobulinemia, some adult patients with CVIDs, and patients with thymoma. Differential diagnosis includes some forms of SCID and myelodysplasia. Immunoglobulin serum levels should be compared with values of age-matched control subjects. It is important to remember that during the first months of life, IgGs are predominantly of maternal origin. Therefore apparently normal IgG serum levels can be detected during the first 2 to 3 months of life, even in patients with impaired ability to produce antibodies. There is large variability in the ability to produce IgA, and some individual attain normal levels only after the first few years of life. Demonstration of very low levels of serum IgG and IgA, with normal to increased serum IgM levels, is suggestive of CSR defects caused by either intrinsic B-cell problems (AID or UNG deficiency) or impaired cross-talk between T and B lymphocytes (CD40L or CD40 deficiency or NEMO defect). The term hyper-IgM syndrome, which is commonly used to identify these disorders, is in fact misleading because the majority of patients with these disorders have normal IgM serum levels.

Determination of serum levels of IgG subclasses has limited value and should not be used as a screening assay. More important information is provided by the assessment of antibody titers. In particular, antibodies to tetanus toxoid and diphtheria toxoid represent robust assays to measure the antibody response to protein (T-dependent) antigens. It is important to remember that use of conjugated vaccines to pneumococcus, *H influenzae*, or meningococcus elicits T-dependent responses,

even if the antibodies are ultimately directed against polysaccharide antigens. Therefore pneumococcal polysaccharide vaccine should be used to test the antibody response to polysaccharide (T-independent) antigens. If basal antibody titers appear nonprotective, a boosting immunization should be performed, followed by repeat measurement of specific antibodies 4 weeks later. A 4-fold increase of specific antibodies is considered indicative of robust antibody production. Isohemagglutinins are antibodies directed against the polysaccharide moieties of AB0 blood group antigens and represent "natural" anti-polysaccharide antibodies. However, isoagglutinin titers are often low in the first 2 years of life, thus limiting the value of this test in infancy.

The occurrence of severe bacterial and fungal infections since early life, especially if associated with a history of gingivostomatitis, should prompt consideration of disorders of neutrophil numbers, functions, or both. The absolute neutrophil count (ANC) is markedly reduced in all forms of SCN. If infections recur approximately every 3 weeks, the possibility of cyclic neutropenia should be entertained. In this case ANCs should be evaluated once a week for 6 consecutive weeks to identify possible decreases in the neutrophil count. Diagnosis of CGD is most commonly based on evaluation of dihydrorhodamine-123 (DHR-123) oxidation, as assessed by means of flow cytometry. This assay is quantitative and objective and also permits identification of carriers of X-linked CGD, who have 2 populations of neutrophils, only one of which is capable of mediating DHR-123 oxidation. Patients with autosomal recessive CGD often have very modest but detectable levels of activity, as detected by using this assay. Diagnosis of LAD1 is straightforward and is based on flow cytometric evaluation of CD18 expression on the surface of leukocytes. Partial defects (2% to 10% of normal density of CD18 molecules at the cell surface) are associated with a moderate form of the disease that permits more prolonged survival.

Investigation of patients with putative TLR-signaling defects can be facilitated by using a screening assay to measure IL-6 and TNF-α production on stimulation of whole blood with TLR agonists or by the failure of affected neutrophils to shed CD62 ligand following stimulation *in vitro*.[98]

Measurement of hemolytic activity of the classical (CH50) and alternative (AP50) pathways of complement, as well as of C3 and C4 levels, might guide in the diagnosis of complement deficiencies. However, as for other forms of PIDs, gene mutations might allow production of nonfunctional proteins, so that ultimately the diagnosis of deficiency of single complement components can rely on appropriate functional assays. The importance of measuring MBL levels is less well defined because of the uncertainties on the pathogenic role of this deficiency, if isolated.

Flow cytometric assays targeted to disease-associated proteins might help define the diagnosis,[99] as discussed above for assessment of CD18 expression in patients with LAD1. Impaired expression of CD40L is typically observed following *in vitro* activation of $CD4^+$ T cells in patients with CD40L deficiency. Patients with IPEX usually lack circulating Treg cells, which are defined as $CD4^+CD25^{bright}$ cells that express intracellular FOXP3. However, a minority of patients with IPEX show residual expression of nonfunctional FOXP3 protein. Caution should be used in the enumeration of Treg cells because FOXP3 can also be expressed by activated T lymphocytes. Protein-specific flow cytometric assays can also be used in the diagnosis of WAS (lack of WASP protein), SCIDX1 (absence of γc), XLA (lack of BTK protein in monocytes), and HLH caused by perforin deficiency. However, interpretation of these assays should take into account that some mutations are permissive for residual protein expression.

Increase of $TCR\alpha\beta^+CD4^-CD8^-$ (double-negative) T cells is suggestive of ALPS; increased levels of Fas ligand and IL-10 represent additional biomarkers of this disease.[100]

Functional assays are also important. In addition to *in vitro* proliferative response to mitogens and antigens for the diagnosis of CID, specific antibody responses in patients with hypogammaglobulinemia, and DHR-123 oxidation in the diagnosis of CGD, other clinically relevant functional assays include the demonstration of markedly reduced NK cytotoxicity (measured against K562 erythroleukemic target cells) in patients with familial forms of HLH and impaired Fas-mediated apoptosis in patients with ALPS.

In the last years, genetic tests have become more widely available. Identification of specific mutations is important not only to confirm the diagnosis but also to guide genetic counseling and to facilitate carrier detection and prenatal diagnosis. However, it should be noted that not all DNA changes are necessarily disease causing; some might represent polymorphisms, rare variants, or disease-contributing variations. These non–disease-causing DNA changes contribute to the heterogeneity of clinical and immunologic phenotypes associated with PIDs, and overall, they make genotype-phenotype correlation less stringent. Analysis of mRNA and protein expression and functional studies are often warranted to define in better detail the possible pathogenicity of specific gene mutations. However, it is important to recognize that in some cases (eg, patients with SCID), the urgency of the condition might require that patients be treated aggressively, even if definition of the genetic defect is still under way.

TREATMENT

Infants with a suspicion of SCID or other CIDs require prompt intervention with use of cotrimoxazole to prevent *Pneumocystis* infection, prophylactic use of antifungal drugs, immunoglobulin replacement therapy, and aggressive treatment of any infectious episodes. Nutritional support is often necessary. Immune suppression helps in controlling the inflammatory reactions associated with Omenn syndrome. Only irradiated and filtered blood products should be used in patients with SCID because of the high risk of otherwise fatal graft-versus-host disease and of transmitting infections (CMV in particular). Use of live attenuated viral vaccines must be avoided to prevent uncontrolled vaccine-associated infections. Ultimately, permanent cure of SCID depends on HCT. When performed from an HLA-identical sibling, HCT can allow greater than 90% long-term survival and very robust and long-lasting immune reconstitutition.[101] HCT from HLA-mismatched related donors provides excellent results when performed in the first 3.5 months of life[102]; however, the outcome is less satisfactory in older patients. Encouraging results have been reported with HCT from matched unrelated donors.[103] It is important to distinguish typical forms of SCID (T^-B^- or T^-B^+) from the most severe forms of CID. Although HCT is required in both, the presence of residual autologous T cells in patients with CID has important implications, both because it might cause symptoms associated with immune dysregulation and

because chemotherapy is usually needed to eliminate autologous T cells before HCT. Nonetheless, some forms of CID are characterized by worse outcome after HCT. In particular, reduced survival and a higher rate of complications after HCT have been reported in patients with radiation-sensitive SCID, Omenn syndrome, MHC class II deficiency, and ADA deficiency who did not have HLA-matched siblings.

Gene therapy has been shown to be effective for patients with ADA deficiency and with SCIDX1, leading to survival with immune reconstitution.[104,105] However, 5 of the 20 infants with SCIDX1 treated at 2 centers have experienced clonal proliferation caused by insertional mutagenesis, calling for the development of novel and safer vectors. Enzyme replacement therapy with weekly intramuscular injections of pegylated bovine ADA is available for patients with ADA deficiency.[106] Thymic transplantation from unrelated donors has been shown to restore T-lymphocyte development in patients with complete DGS.[107] Alternatively, unmanipulated bone marrow transplantation from HLA-identical siblings can also allow T-cell reconstitution caused by expansion of mature T lymphocytes contained in the graft.[108]

Treatment of antibody deficiency is based on immunoglobulin replacement therapy. This can be performed with intravenous or subcutaneous preparations (intravenous immunoglobulin [IVIG] and subcutaneous immunoglobulin [SCIG]). Both are effective in reducing the incidence of infections. The usual dose of IVIG is 400 mg/kg per 21 days, but higher doses might be needed in patients with bronchiectasis or enterovial meningoencephalitis. SCIG is administered at the dose of 100 mg/kg/wk; potential advantages of SCIG include a lower rate of adverse reactions and more stable IgG trough levels.[109] On the other hand, IVIG might be more useful when there is a need to administer higher doses (as in the treatment of patients with bronchiectasis or with associated autoimmune complications). Although no studies have been performed to support the use of continuous antibiotic prophylaxis in patients with antibody deficiency, they might be beneficial in patients with bronchiectasis and recurrent sinusitis.

Defects of neutrophils require regular antibiotic and antifungal prophylaxis. Cotrimoxazole and itraconazole are efficacious in the treatment of CGD, and injection of IFN-γ might further reduce the incidence of severe infections. Overall survival in patients with CGD is now around 90%.[72] HCT is the treatment of choice for LAD and can be proposed for patients with CGD who have HLA-matched family donors. Use of HCT from Matched Unrelated Donors or cord blood in patients with CGD remains controversial, although recent reports have shown that it can correct severe inflammatory complications.[110] Gene therapy has been attempted in a few patients with CGD, although because of the lack of selective advantage for gene-corrected cells, only a very small fraction of cells in the periphery carry the transgene if no conditioning regimen is used. In a recent trial with nonmyeloablative conditioning, clonal expansion caused by insertional mutagenesis has been reported.[111] Regular subcutaneous administration of recombinant granulocyte colony-stimulating factor can increase ANCs in patients with SCN. Management of HIES is based on hygiene and regular prophylaxis of staphylococcal and fungal infections.[88]

Patients with WAS should be treated with infusion of immunoglobulins, antibiotic prophylaxis, and appropriate measures to prevent severe bleeding episodes (eg, use of helmets).[83] However, the ultimate therapy of WAS is represented by means of HCT. Excellent results have been obtained with HCT from HLA-identical related donors. Results of MUD-HCT are also good, but mixed chimerism is associated with an increased rate of autoimmune complications.[112] Demonstration of lack of WASP protein expression might help identify high-risk patients who should be treated with HCT; in contrast, the approach to patients with XLT is more controversial.

HCT should be used without delay in patients with familial forms of HLH and in those with X-linked lymphoproliferative disease. Immunosuppressive treatment is required to treat the accelerated phase of the disease both in patients with X-linked lymphoproliferative disease and in those with PIDs with defective cytotoxicity associated with pigmentary dilution. Although HCT can cure the hematologic and immunologic manifestations of the disease, it does not prevent progressive neurological deterioration in patients with CHS.

Finally, although all patients with immunodeficiency associated with autoimmunity benefit from immunosuppressive treatment, alternative strategies can be considered, depending on the severity of the underlying defect. In particular, HCT is needed in patients with IPEX, whereas the significant splenomegaly of ALPS might require splenectomy.

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Secondary immunodeficiencies, including HIV infection

Javier Chinen, MD, PhD, and William T. Shearer, MD, PhD *Houston, Tex*

Extrinsic factors can adversely affect immune responses, producing states of secondary immunodeficiency and consequent increased risk of infections. These immunodeficiencies, which can be encountered in routine clinical practice, arise from a number of conditions, such as treatment with glucocorticoids and immunomodulatory drugs, surgery and trauma, extreme environmental conditions, and chronic infections, such as those caused by HIV. The most common cause of immunodeficiency is malnutrition, affecting many communities around the world with restricted access to food resources. Protein-calorie deficiency and micronutrient deficiencies have been shown to alter immune responses; of note, recent progress has been made in the influence of vitamin D deficiency in causing failure of immune activation. Other categories of disease that might present with secondary immunodeficiency include metabolic diseases and genetic multisystemic syndromes. The immune defects observed in secondary immunodeficiency are usually heterogeneous in their clinical presentation, and their prognosis depends on the severity of the immune defect. Management of the primary condition often results in improvement of the immunodeficiency; however, this is sometimes not possible, and the risk of infections can be reduced with prompt antimicrobial treatment and prophylaxis. (J Allergy Clin Immunol 2010;125:S195-203.)

Key words: *Secondary immunodeficiency, immunosuppression, lymphopenia, AIDS*

Secondary immunodeficiencies are far more common than primary immunodeficiencies, which are, by definition, caused by genetic defects affecting cells of the immune system.[1] Secondary immunodeficiencies result from a variety of factors that can affect a host with an intrinsically normal immune system, including infectious agents, drugs, metabolic diseases, and environmental conditions. These deficiencies of immunity are clinically manifested by an increased frequency or unusual complications of common infections and occasionally by the occurrence of opportunistic infections (Fig 1). The secondary immunodeficiencies have a wide spectrum of presentation, depending on the magnitude of the offending external condition and on the host susceptibility. For example, the immunodeficiency induced by the use of corticosteroids and other immunosuppressive drugs depends on the dose used[2,3] and, to a lesser degree, on concomitant disease processes of the host, such as the presence of sepsis. AIDS, resulting from infection by HIV, is the best known secondary immunodeficiency largely because of its prevalence and its high mortality rate if not treated. However, the most common immunodeficiency worldwide results from severe malnutrition, affecting both innate and adaptive immunity.[4] The restoration of immunity in secondary immunodeficiencies is generally achieved with the management of the primary condition or the removal of the offending agent. We summarize reports of immune defects occurring in a variety of clinical scenarios (Table I), with special emphasis on HIV infection. We selected diseases and conditions based on their frequent presentation in general medical practice and their relevance for allergists and immunologists. We do not discuss immunomodulating mAbs and fusion proteins, which are covered in Chapter 28 of this primer.[5]

Abbreviations used
GvHD: Graft-versus-host disease
HAART: Highly active antiretroviral therapy
IRIS: Immune reconstitution inflammatory syndrome

EXTREMES OF AGE: NEWBORN PERIOD AND ADVANCED AGE

Newborn period

Neonates have an increased susceptibility to common and opportunistic infections and sepsis compared with older children.[6] There is an inverse association of infection susceptibility and the age of prematurity. In early life there are fewer marginal-zone B cells in lymphoid tissue and a decreased expression of CD21 on B cells, thus limiting the ability of B cells to develop specific responses.[7] Although they can develop humoral responses to some antigens after exposure *in utero*, impaired immunity in newborns can be attributed to the relative lack of maturity of secondary lymphoid organs, including the lymphoid tissue associated to mucosa in the gastrointestinal and respiratory tracts. This immaturity is related to the absence of memory cell development because of the relative isolation provided by the maternal environment. In addition, premature infants are more vulnerable to infections because of the absence of maternal IgG transfer before 32 weeks of gestational age. Other significant recent observations described at this early age are related to innate immunity mechanisms, such as a decreased neutrophil storage pool, as defined by the ability of neutrophilia to develop in response to an infection; decreased *in vitro* neutrophil functions (ie, phagocytosis, oxidative burst, chemotaxis, and adhesion); capacity to develop a neutrophil extracellular trap[8]; decreased natural killer cell activity; decreased Toll-like receptor signaling; decreased production of cytokines; and reduced complement components.

From the Department of Pediatrics, Allergy and Immunology Section, Baylor College of Medicine.

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Reprint requests: Javier Chinen, MD, PhD, Department of Pediatrics, Allergy and Immunology Section, Baylor College of Medicine, Texas Children's Hospital, 1102 Bates St FC 330.01, Houston, TX 77030. E-mail: jxchinen@texaschildrenshospital.org.

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Advanced age

Among the elderly, some subjects experience malignancies and an excessive number of infections caused by viruses and bacteria, reflecting a decrease in the immune defenses, particularly in the cellular compartment. Decreased delayed-type hypersensitivity skin reactions and decreased lymphocyte proliferative responses to mitogens can be demonstrated in this patient population. This relative impairment of the immune response has been linked to the development of T-cell oligoclonality together with a limited capacity of the thymus to generate naive T cells and therefore reduced responses to new antigens. Oligoclonal expansion of $CD8^+$ T cells begins in the seventh decade of life, which results in the skewing of the T-cell repertoire and an increased number of differentiated memory $CD8^+$ T cells.[9] Advanced age is similarly associated with a restricted B-cell diversity repertoire and a limited response to vaccines; however, there is also an increased number of total memory B cells and increased total IgG levels. The innate immunity might be compromised in the elderly, with increased breakdown of skin and mucosal barriers and slow healing processes caused by metabolic and endocrinologic changes associated with aging. A diminished production of hematopoietic growth factors has been postulated to occur in the elderly, resulting in decreased ability to upregulate the production and function of macrophages and neutrophils.[10] Some subjects are at higher risk of infections when these immunologic defects are combined with other environmental factors, such as malnutrition or the concomitant presence of chronic inflammation caused by autoimmunity or persistent infections.[11] Progress in understanding the aging-associated immune defect is of importance to optimize protective immunity against preventable infectious diseases.[12]

MALNUTRITION

Worldwide, protein-calorie malnutrition is the most common cause of immunodeficiency.[13] Malnutrition can result from limited access to food sources and chronic diseases that induce cachexia, such as neoplastic diseases. Diarrhea caused by infections and respiratory tract infections are common. T-cell production and function decrease in proportion to the severity of hypoproteinemia; however, specific antibody titers and immune responses to vaccines can be detected in a malnourished subject for a relatively prolonged period. Eventually, these immune responses decrease if malnutrition persists. The deficiency of micronutrients (eg, zinc and ascorbic acid) contributes to increased susceptibility to infections through the weakening of barrier mucosa, therefore facilitating a pathogen's invasiveness.[14,15] Other essential molecules have been shown to have specific roles in the immune system; for example, vitamin D appears to be necessary in the macrophage activity against intracellular pathogens, remarkably *Mycobacterium tuberculosis* (Fig 2).[16] Correction of the nutritional deficiencies often results in the resolution of these immunologic defects.

METABOLIC DISEASES: DIABETES MELLITUS AND UREMIA

Many disease processes originating from dysfunctional metabolic pathways significantly affect the cells involved in the immune response. Diabetes mellitus and uremia resulting from kidney or liver disease are 2 common metabolic disorders with known deleterious effects on immunity. Optimal control of the metabolic abnormality usually leads to improved immune function. The defective immune functions reported in patients with diabetes mellitus include defective phagocytosis and macrophage chemotaxis *in vitro*, T-cell anergy demonstrated by delayed hypersensitivity skin tests, and poor lymphoproliferative response to mitogens caused by chronic exposure to hyperglycemia.[17] Impaired glucose metabolism, insufficient blood supply, and denervation are other factors that contribute to the increased susceptibility to infection in patients with diabetes, who present most commonly with skin sores, bacterial and fungal respiratory tract infections, and systemic viral diseases.

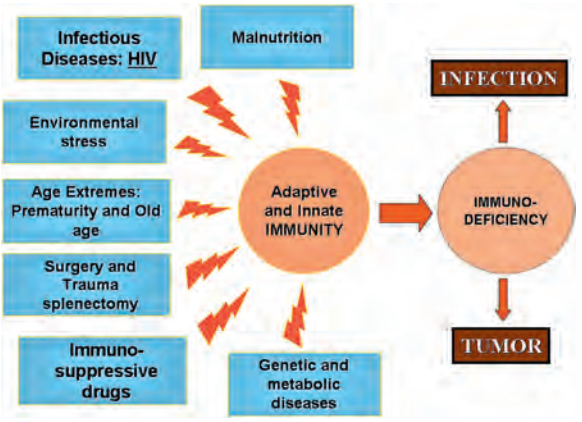

FIG 1. Extrinsic factors leading to defects of immune function.

Uremic patients experience increased incidence and severity of infections compared with the general population. Even when disparities in age, sex, race, and diabetes mellitus were taken into account, mortality rates in patients undergoing dialysis attributed to sepsis were higher by a factor of 100 to 300.[18] The need for dialysis procedures and use of vascular devices are independent risk factors for invasive infections. Multiple defects of the innate and adaptive immunity have been described to have a role in the increased frequency of infections, summarized as immune hyporesponsiveness and a state of chronic activation. The diminished capacity to generate memory antibody responses, regardless of repeated vaccination, and defective phagocyte chemotaxis and microbicidal activity *in vitro* are examples of the immune defects present in uremic patients.[19,20]

INHERITED DEFECTS OTHER THAN PRIMARY IMMUNODEFICIENCIES

Diseases caused by genetic defects might not primarily affect the immune system, but they can present with impaired immunity to infections resulting from metabolic and cellular dysfunction, such as poor expression of adhesion molecules or defects in the DNA repair machinery. The molecular mechanisms leading to immunologic defects remain not well defined. Genetic syndromes are relatively rare, and usually only a subset of patients present with an immune defect of clinical severity that increases their risk of infections or malignancies. The disease processes caused by chromosomal number abnormalities are the most common within the genetic disorders. As an example, patients with Down syndrome or trisomy of chromosome 21 present with increased incidence of infections, although they are usually not severe, including skin abscesses, periodontitis, and upper respiratory

TABLE I. Selected causes of secondary immunodeficiencies

Condition	Effect on immune function
Extremes of age	
Newborn period	Immature lymphoid organs Absent memory immunity Low maternal IgG levels in premature infants Decreased neutrophil storage pool Decreased neutrophil function Decreased natural killer activity
Advanced age	Decreased antigen-specific cellular immunity T-cell oligoclonality Restricted B-cell repertoire
Malnutrition	Decreased cellular immune response Weakened mucosal barriers
Metabolic diseases	
Diabetes mellitus	Decreased mitogen-induced lymphoproliferation Defective phagocytosis Decreased chemotaxis
Chronic uremia	Decreased cellular immune response Decreased generation of memory antibody responses Decreased chemotaxis
Genetic syndromes: trisomy 21	Defective phagocytosis Defective chemotaxis Variable defects of antigen-specific immune responses
Anti-inflammatory, immunomodulatory, and immunosuppressive drug therapy: corticosteroids, calcineurin inhibitors, cytotoxic agents	Lymphopenia Decreased cellular immune response and anergy Decreased proinflammatory cytokines Decreased phagocytosis Decreased chemotaxis Neutropenia (cytotoxic agents) Weakened mucosal barriers (cytotoxic agents)
Surgery and trauma	Disruption of epithelial and mucosal barriers T-cell anergy caused by nonspecific immune activation
Environmental conditions UV light, radiation, hypoxia, space Flight	Increased lymphocyte apoptosis Increased secretion of tolerogenic cytokines Cytopenias Decreased cellular immunity and anergy Stress-induced nonspecific immune activation
Infectious diseases: HIV infection	T-cell lymphopenia Decreased cellular immune response and anergy Defective antigen-specific antibody responses

tract infections. T- and B-cell number and function are variably affected.[21] Neutrophils isolated from patients with Down syndrome have shown defects in chemotaxis and phagocytosis *in vitro*. Most recent studies have focused on the overexpression of the gene Down syndrome critical region 1 *(DSCR1)* and its role in contributing to phagocyte dysfunction.[22] Patients with Turner syndrome (complete or partial absence of the second X chromosome) also have an increased number of respiratory tract infections, and hypogammaglobulinemia can be identified, although this immune defect is not consistently demonstrated in these patients. The gene defects involved in the decrease of immunoglobulin production are not known.

In other genetic diseases, such as cystic fibrosis, caused by deleterious mutations in the cystic fibrosis transmembrane conductance regulator, the increased susceptibility to sinusitis and pneumonia is explained by defective mechanisms of innate immunity.[23] Patients with cystic fibrosis have an impaired airway mucous clearance caused by the thickness of the mucous secretions, which favors the development of respiratory infections caused by *Pseudomonas* species. It is recommended that patients receive prompt antibiotic therapy when infection is suspected, and antibiotic prophylaxis should be prescribed to those patients with recurrent infections to reduce the number of infectious episodes.

ANTI-INFLAMMATORY, IMMUNOMODULATORY, AND IMMUNOSUPPRESSIVE DRUG THERAPY

The use of drugs to ameliorate undesirable immune responses is common in clinical practice as a consequence of the increasing prevalence of inflammatory conditions. These diseases include the categories of autoimmune disorders, allergic disorders, transplant rejection, and graft-versus-host disease (GvHD). Broadly, we can study these drugs by dividing them into biologic, physical, and chemical categories. The chemical agents are the most available clinically and have in common their ability to inhibit lymphocyte proliferation and their lack of specificity for the immune response causing the particular illness of interest.

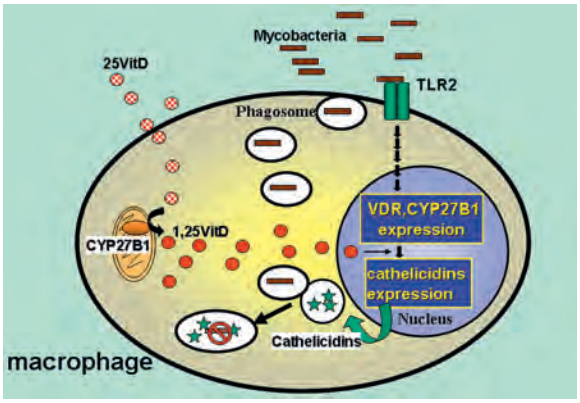

FIG 2. Role of vitamin D *(VitD)* in macrophage activation. Toll-like receptor 2 *(TLR2)* activation increases expression of CYP21B1, a mitochondrial enzyme that converts vitamin D into its active form, 1,25OH vitamin D, and vitamin D nuclear receptor *(VDR)* expression, which when bound to 1,25OH vitamin D promotes cathelicidin synthesis. Cathelicidins are intracellular bactericidal proteins.

Biologic immunosuppressive drugs have been developed to increase the immune specificity by targeting specific components of the immune response, such as cytokines or a particular lymphocyte subset. Physical agents (ie, UV light and ionizing radiation) can also be used to ablate immune responses.

In addition, there are drugs that might have an immunosuppressive effect that is not clearly related to the pharmacologic activity of the molecule. Its occurrence is not predictable and varies within different patient populations. Well-known examples of this drug mechanism are the development of hypogammaglobulinemia in patients receiving antiepileptic drugs and the leukopenia seen in patients taking trimethoprim-sulfamethoxazole.

Based on their structure and mechanism of action, most molecules with immunosuppressive activity can be grouped into corticosteroids, calcineurin inhibitors, and cytotoxic drugs. The adverse side effect of these drugs is that they tend to weaken the cellular immune response, rendering patients more susceptible to fungal and viral infections (acute, chronic, and reactivated).

Corticosteroids

The corticosteroids include both glucocorticoid and mineralocorticoid molecules. Only the glucocorticoids have significant anti-inflammatory activity. Glucocorticoids are well known for their variety of applications in both general and subspecialty medicine to reduce tissue damage caused by an excessive inflammatory response. The range of potency of the different molecules of this group and their routes of administration is diverse, each designed to different applications. For example, betamethasone is 25 times more potent than cortisol and can be used in topical, oral, and injectable preparations. Glucocorticoids bind a cytosolic receptor, which then translocates to the nucleus to act as a transcription factor affecting the expression of a number of genes, resulting in an anti-inflammatory effect (Fig 3).[24] The bound complex-glucocorticoid receptor modulates signal transduction pathways, resulting in the activation of the transcription factors nuclear factor κB, nuclear factor of activated T cells, and activator protein 1. It has been suggested that glucocorticoids might also cause an effect on cell function by interacting with the cell membranes, which could explain observed clinical benefits when used as "pulse therapy," with doses higher than required for receptor saturation. The overall results are decreased cytokine production (IL-1, IL-6, and TNF-α) and impaired leukocyte chemotaxis, cell adhesion, phagocytosis, and lymphocyte anergy. Lymphopenia occurs as a result of the proapoptotic activity and inhibition of IL-2–mediated proliferative responses. When used at large doses, antibody responses and delayed-type hypersensitivity responses are reversibly suppressed. This wide range of immune defects renders the patient susceptible to viral, bacterial, and fungal infections, according to the degree of immunosuppression and the administration route. Examples of these are oral candidiasis, a frequent complication of the use of inhaled steroids, and herpes zoster disease, which often presents with chronic use of systemic corticosteroids.

Calcineurin inhibitors

Calcineurin inhibitors bind cytoplasmic proteins from the immunophilin family and inhibit their interaction with calcineurin, which is essential for the activation of IL-2 transcription and T-cell function (Fig 4). The advantage of these drugs over corticosteroids and cytotoxic drugs is to spare macrophage and neutrophil functions, reducing the spectrum of susceptibilities to infections. However, these drugs cause respiratory tract and skin infections, usually of viral cause, to occur with increased frequency. The most common adverse effects of calcineurin inhibitors are hypertension and renal dysfunction; less common but more serious is the increased frequency of lymphoproliferative disorders and skin neoplasias. The first drug in this category was cyclosporine, which has been extensively used to prevent organ transplant rejection,[25] GvHD, and corticosteroid-resistant autoimmune disorders. Other agents with a similar mechanism of action and immune selectivity are tacrolimus and pimecrolimus. The latter is the most recent member of this group, and it was developed for topical use in the treatment of severe atopic dermatitis. An agent with a similar name, sirolimus or rapamycin, also binds an immunophilin but does not inhibit calcineurin. Instead, sirolimus inhibits the IL-2–induced response by inhibiting the mammalian target of rapamycin, a protein essential for cell activation and proliferation.

Cytotoxic agents

Cytotoxic agents were conceived to control neoplastic cell growth and ablate the bone marrow for transplantation. They have progressively found their niche in the immunosuppressive drugs category because of the selectivity conferred by the proliferative nature of the immune response, and their application has extended to autoimmune and inflammatory disorders, including GvHD and the prevention of graft rejection.[26] The most common drugs used for these applications are the alkylating agent cyclophosphamide and the antimetabolites methotrexate, mycophenolate, azathioprine, and 6-mercaptopurine. Other drugs with predominant use in autoimmune disorders are sulfasalazine, hydroxychloroquine, and leflunomide.[26] These compounds interfere with the synthesis of DNA, arresting the cell cycle and inducing apoptosis. Generally, they inhibit both T- and B-cell proliferation and therefore any new immune responses. In addition, depending on the dose used, they inhibit cellular and antibody responses resulting from previous sensitizations. The major limitation of the use of these agents is their toxicity to other hematopoietic and nonhematopoietic cells, with development of cytopenias, gastrointestinal

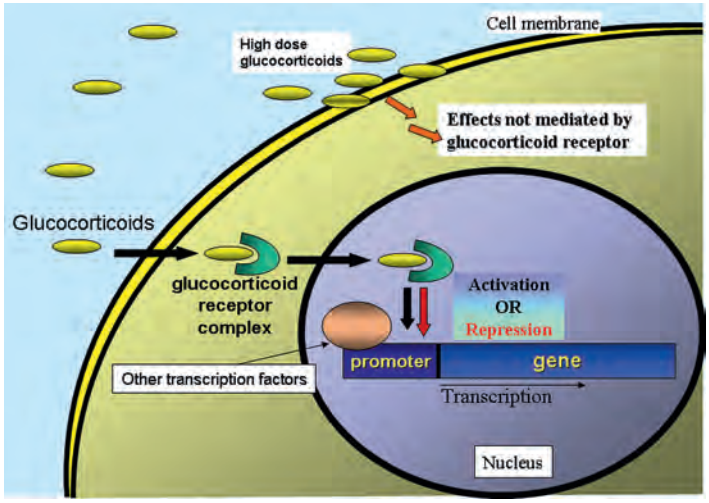

FIG 3. Molecular mechanism of action of glucocorticoids. A cytosolic receptor binds glucocorticoids and translocates them to the nucleus, where they either activate anti-inflammatory genes or inhibit proinflammatory genes. At high doses, corticosteroids can also affect cell function by non–receptor-dependent mechanisms.

mucosa, and skin deterioration. These cytopenias contribute to the state of secondary immunodeficiency and susceptibility to infections.

SURGERY AND TRAUMA

Surgery and trauma cause disruption of epithelial barriers and cell destruction that triggers an inflammatory response to promote healing and local microbicidal activity.[27,28] Microorganisms contain surface pathogen–derived molecules that activate pattern-recognition receptors expressed on antigen-presenting cells and other immune cells to induce cytokine and chemokine release and recruitment of the adaptive immune system.[29] Massive tissue injury further increases activation of proinflammatory mechanisms in response to the presence of toxic byproducts of cell death.[30] In this inflammatory response Toll-like receptors play a central role in activating immune cells, resulting in the release of inflammatory cytokines, such as IL-6 and TNF-α. If this response is severe, trauma patients might experience the adult inflammatory respiratory syndrome in the lung or the systemic inflammatory response syndrome when there is multiorgan failure. The inflammatory response observed in patients with severe trauma develops gradually: loss of epithelial barriers, vasodilatation and increased vascular permeability, cellular activation and increased adhesion to endothelia, and a neuroendocrine stress response. At the same time, injured patients are relatively immunosuppressed because of nonspecific cell activation leading to an anergic immune state and because of increased levels of cortisol induced by stress in addition to the loss of containment provided by epithelial barriers. This process occurs within the context of a delicate balance of inflammatory and counterinflammatory mechanisms.[31]

Patients who have undergone splenectomy deserve special consideration because they are particularly susceptible to infections by encapsulated bacteria, such as *Streptococcus pneumoniae*. The mortality for sepsis in splenectomized patients is between 50% and 70%, emphasizing the need to avoid splenectomy when possible. Patients who are scheduled for elective splenectomy should receive antipneumococcal, anti–*Haemophilus*

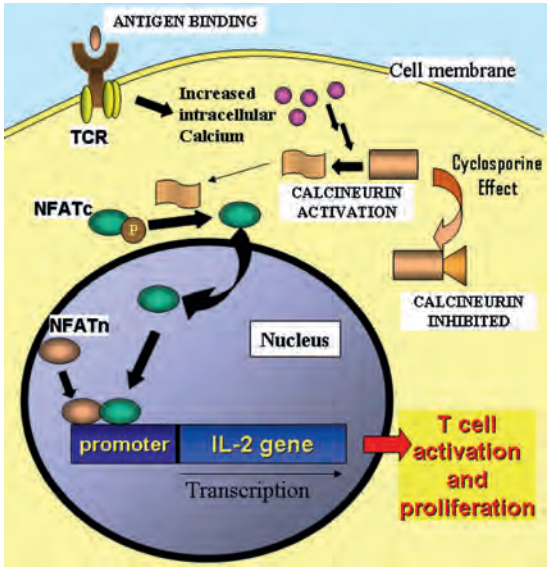

FIG 4. Effect of cyclosporine on T cells. Inhibition of calcineurin activity by cyclosporine results in decreased activation of IL-2 transcription. *TCR*, T-cell receptor; *NFAT*, nuclear factor of activated T cells; *NFATc*, cytoplasmic monomer; *NFATn*, nuclear monomer.

influenzae, and antimeningococcal immunizations at least 2 weeks before surgical intervention.[32]

ENVIRONMENTAL CONDITIONS: UV LIGHT, IONIZING RADIATION, HIGH ALTITUDE, CHRONIC HYPOXIA, AND SPACE FLIGHTS

There is increased awareness of potential adverse effects caused by chronic exposure to inhospitable environmental conditions, such as extreme cold or high altitude. It has been recommended to avoid exposure to sunlight because of increased risk of malignancies; however, beneficial effects of sunlight have also been observed, particularly in patients with skin inflammatory conditions, such as psoriasis.[33] The biologic effect

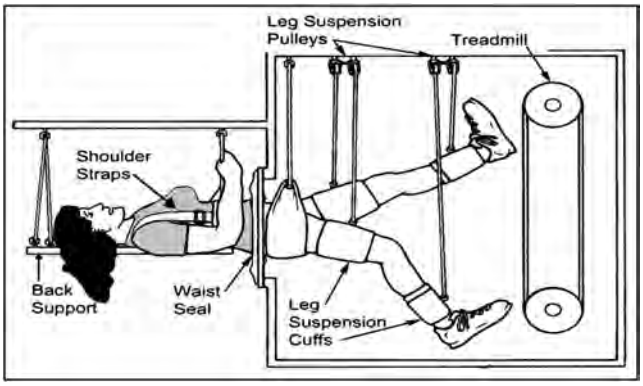

FIG 5. Human model to test the effects of microgravity. Volunteers are maintained in bedrest position for 60 days to mimic the affects of microgravity in space. Exercise is used as a countermeasure.

of sunlight in inflammation is mediated by UV light, which induces T-cell apoptosis, nonspecific release of tolerogenic cytokines from antigen-presenting cells in the epidermis, and differentiation of regulatory T cells; hence UV light is used in the treatment of eczema and the skin manifestations of autoimmune disorders.

The immunosuppressive effect of ionizing radiation affects all blood cell lineages by depleting the bone marrow and inducing cytopenias, whereas the humoral response and phagocytosis are considered radioresistant.[34] However, continuous exposure to radiation eventually weakens all immune functions. Animal experiments of space radiation similar to that human subjects would experience during long-duration space flights have demonstrated a weakness of T cell–mediated immunity and reactivation of latent viral infections.[35] Other adverse conditions, such as chronic hypoxia at high-altitude locations and long-duration space flights, might affect immunity by causing physical and mental stress. Confinement, isolation, and sleep-cycle alterations induce chronic stress, which disturbs the corticoadrenal regulation and increases cortisol levels. In human subjects space flight–equivalent models, including acute sleep deprivation, have been shown to increase blood levels of inflammatory cytokines and suppression of IL-10 secretion.[36] Prolonged bedrest (ie, 60 days) with head-down tilt, a model of microgravity in space, has produced a significant increase of serum TNF-α soluble receptor levels in female volunteers (Fig 5).[37] Interestingly, vigorous exercise served as an effective countermeasure in negating this effect.

INFECTIOUS DISEASES

Transient periods of immunosuppression have been associated with viral infections since the 1900s, when it was observed that tuberculin skin test results became negative in patients with measles during the acute phase of the infection. Some infectious agents or their toxins and metabolites might be present in excess amounts to activate the immune system, leading to a nonresponsive state, such as the T-cell anergy observed after toxic shock syndrome induced by staphylococcal superantigen. Tissue destruction caused by microbial-induced damage or inflammatory reaction to a particular infection facilitates access for other microbes to develop secondary infections. Infections with measles virus, CMV, and influenza virus can induce lymphopenia and also T-cell anergy; however, these are transient and usually less severe than the immunodeficiency seen in AIDS. One additional mechanism of immune compromise is infection of the bone marrow by viral and bacterial organisms producing neutropenia or pancytopenia, particularly in immunocompromised hosts.[38]

HIV INFECTION: AIDS

Background

Without antiretroviral drug treatment, HIV infection almost always progresses to the advanced stage of the disease called AIDS that is characterized by profound lymphopenia and susceptibility to infections with opportunistic pathogens. HIV is transmitted sexually, for the most part, but it is also transmitted parenterally among intravenous drug users and vertically from mothers to their infants.[39] Initially recognized during the early 1980s in a handful of cases, it is currently estimated that more than 30 million persons are infected with HIV worldwide. Two thirds of these subjects are living in the sub-Saharan region of Africa, and approximately half of them are women and children (Fig 6).[40] The HIV epidemics in North America and Europe have shown decreasing trends in the last decade, thanks to massive education campaigns and the use of potent anti-HIV drugs. However, more than 56,000 new cases of HIV infection were reported in the United States in the last HIV infection survey by the Centers for Disease Control and Prevention, and approximately half of these were in subjects younger than 25 years.[41] There is an increasing number of reports of viral multidrug resistance and clinical complications caused by the chronic use of antiretroviral drugs.[42]

Virology

HIV is a double-stranded, enveloped RNA retrovirus from the group lentiviruses, with a tropism for human $CD4^+$ expressing cells, including T cells and macrophages.[41] Two HIV types have been identified, HIV-1 and HIV-2, and both cause human disease. HIV-2 is more prevalent in West Africa and might take more time from infection to the development of immunodeficiency than HIV-1. The HIV genome contains 3 structural genes (*gag*, *pol*, and *env*) and 6 regulatory genes (*tat*, *rev*, *nef*, *vif*, *vpr*, and *vpu*). Gag protein is split by the HIV protease into the proteins named capsid (p24), matrix, nucleocapsid, p6, and p2, all of which form the viral particle and stabilize the viral genome. Pol protein is also split to produce 3 enzymes: integrase, reverse transcriptase, and the protease that cleaves the viral proteins. After the viral genomic RNA is converted into DNA by the reverse transcriptase, the integrase facilitates the incorporation of the viral DNA into the host genome and uses the host cell's replication mechanisms to produce more virions. The Env protein is also cleaved to produce 2 envelope proteins named gp120 and gp41, which are involved in the binding to CD4 and the chemokine receptors CXCR4 and CCR5 on the cell surface. Tat protein increases the transcription of HIV genes by 100-fold, whereas Rev protein allows the expression of the different HIV genes by regulating mRNA splicing.

The roles of the other regulatory genes have only been clarified in the last few years. Nef protein downregulates CD4 and MHC class I surface expression on the membranes of infected cells, probably facilitating escape from immune surveillance. Vif is a protein that induces the degradation of APOBEC3 G, a cytosine deaminase that causes mutations during viral transcription. Vpr

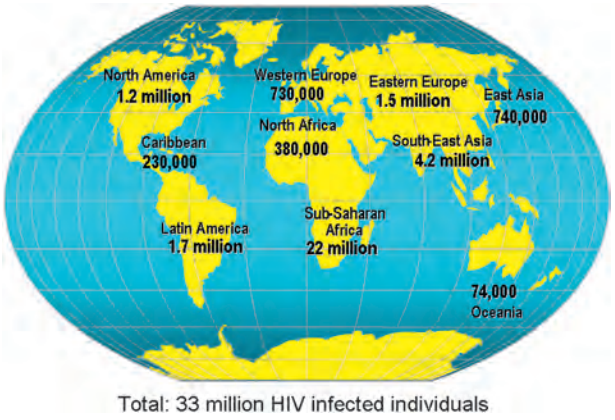

FIG 6. Worldwide prevalence of HIV infection. Adapted from the United Nations Programme on HIV/AIDS.[40]

and Vpu proteins seem to facilitate the intracellular transport of viral proteins for viral particle formation.

Immunopathogenesis

HIV infection begins with the binding of the HIV gp120 protein to the CD4 molecule and the chemokine receptor CCR5 on target cells. Infected cells migrate to the lymph nodes, where initial replication and infection of nearby $CD4^+$ T cells occur.[43] During acute HIV infection, the gut-associated lymphoid tissue is severely depleted, with predominant loss of memory $CD4^+$ T cells and with high viremia and immune activation.[44,45] HIV induces T-cell lymphopenia through several mechanisms: HIV-induced apoptosis, viral cytopathic effect, apoptosis caused by nonspecific immune activation, and cytotoxicity to HIV-infected cells. An additional form of cell death named autophagy, in which organelles are sequestered and directed toward lysosomal pathways, has been shown to be induced by HIV Env protein in uninfected T cells.[46] The acute phase of HIV infection occurs 1 to 6 weeks after infection, with nonspecific symptoms, such as fever, fatigue, myalgia, and headaches. The period of clinical latency that follows is characterized by a virtual absence of signs or symptoms until symptomatic disease occurs and can last as long as 10 years. Levels of several cytokines are increased and contribute to determine the degree of control of HIV viremia. Higher viral loads at the initial stage predict shorter clinical latency. Without anti-HIV drug treatment, $CD4^+$ T-cell counts progressively decrease, and the host usually succumbs to infections with opportunistic organisms that take place because of the immune deficiency. Investigators have been able to demonstrate the production of specific anti-HIV $CD4^+$ T cells and $CD8^+$ T cells, as well as neutralizing anti-HIV antibodies; however, these immune responses are eventually overcome by viral escape strategies. At this stage, patients present with fever, weight loss, diarrhea, lymphadenopathy, and fungal and viral skin infections, indicating compromise of the immune system. When the peripheral $CD4^+$ T-cell count is less than 200 cells/mL, the patient can present with any of a number of infections that define AIDS, such as *Pseudomonas jiroveci*–induced pneumonia, histoplasmosis, toxoplasmosis, and coccidioidomycosis.[47] If the patient does not receive antiretroviral treatment, repeated infections that are difficult to manage lead to the patient's death. A small proportion of HIV-infected patients remain asymptomatic and do not have AIDS. These patients are called long-term nonprogressors and have been the focus of multiple studies to understand the basis of their protection. Those who maintain low levels of HIV (ie, <50 RNA copies/mL) without treatment are called elite controllers.[48] This immunity appears to be explained by different viral and host factors. The best known of these factors is the inherited defect in the gene encoding the CCR5 receptor, a T-cell surface molecule that is necessary for HIV cell entry. *CCR5* gene mutations have been found with significant prevalence in persons of Northern European ancestry. Other factors identified in long-term nonprogressors include a low number of activated $CD8^+$ T cells,[49] the presence of particular HLA haplotypes, and viral mutations that result in low virulence. The diagnosis of HIV infection is made by using a sensitive ELISA to detect antibodies against the HIV protein p24. A positive HIV ELISA result is confirmed by using the more specific Western blot, which detects antibodies to several HIV proteins, or the detection of HIV DNA sequences by PCR. Rapid diagnostic tests to rule out HIV infection use serum, saliva, or urine with similar sensitivity and specificity to the ELISA and can be performed in the office or at home. Infants and children up to 18 months of age born to HIV-infected mothers should be evaluated with an HIV DNA PCR test because the presence of passively acquired maternal antibodies in the serum of the child can result in a positive HIV ELISA test result, even if the child is not infected with HIV. Other useful laboratory tests are genotyping and phenotyping assays. Genotyping identifies HIV mutations that confer viral resistance to antiretroviral drugs. Phenotyping measures the inhibitory action of anti-HIV drugs on the isolated HIV strain, which is similar to a bacterial susceptibility assay. These assays define anti-HIV drug susceptibility profiles of viral strains isolated from infected patients and help in the design of the combination of drugs with the most probability to have a therapeutic effect in a particular patient.

Treatment

In adults specific anti-HIV therapy is recommended when the patient has an AIDS-defining illness, the $CD4^+$ T-cell count is less than 350 cells/mm^3, or the HIV viral load is greater than 100,000 copies/mL. Caution should be exercised in other clinical situations because of the development of viral resistance to the antiretroviral agents and significant drug-induced adverse effects, including allergic and metabolic syndromes.[50,51] In children treatment is considered for any HIV-infected infant because disease progresses faster than in older children. For children older than 12 months, the criteria are similar to those in adults: presence of an AIDS-defining illness, $CD4^+$ T-cell count of less than 15% of PBMCs, or viral load greater than 100,000 copies/mL.[52] Anti-HIV drug classes are defined according to their mechanism of action: nucleoside reverse transcriptase inhibitor, nonnucleoside reverse transcriptase inhibitor, protease inhibitor, and cell fusion inhibitor. In the last 2 years, CCR5 inhibitors and integrase inhibitors have been added to the arsenal of anti-HIV medications.[53,54] Combinations of 3 synergistic anti-HIV drugs from 2 different classes are known as highly active antiretroviral therapy (HAART). HAART protocols have been effective in reducing viremia and restoring normal T-cell counts, with drastic reduction of mortality and number of infections; however, they do not eradicate HIV and need to be administered continuously for life. As an adjuvant treatment to improve baseline immunity, the

administrations of IL-7 and IL-2 have been independently tested to increase $CD4^+$ T-cell counts, with promising results.[55,56]

Immunologic reactions associated with anti-HIV treatment

The immune reconstitution inflammatory syndrome (IRIS) is a severe inflammatory response to existing opportunistic infections that can be observed in 15% to 25% of patients with AIDS 2 to 3 weeks after starting HAART treatment.[57] The management of IRIS consists of corticosteroid therapy and simultaneous treatment of the opportunistic infections; however, IRIS might not occur if these infections are recognized and treated before starting the HAART therapy. A similar clinical observation is the increased incidence of asthma in HIV-infected patients receiving HAART, up to 3 times the rate of HIV-negative control subjects.[58]

Drug-allergic reactions have an increased prevalence in this patient population. Urticarial or maculopapular rashes, which occasionally present as the Steven-Johnson syndrome, occur in as many as 60% of patients with HIV receiving trimethoprim-sulfamethoxazole and in 17% of those receiving the antiretroviral nevirapine.[59] Abacavir is a nucleoside reverse transcriptase inhibitor that causes a multiorgan hypersensitivity syndrome characterized by fever, rash, diarrhea, myalgia, and arthralgia in as many as 14% of patients who take this drug. This has a strong association with the presence of HLA B5701. This syndrome presents within the first weeks of treatment and can be fatal; however, it usually resolves after 72 hours of discontinuing the drug.

HIV vaccine

The failure of current antiretroviral therapy to eliminate the HIV virus emphasizes the need of preventive measures to control the HIV pandemic. Research for an effective anti-HIV vaccine has yielded several lessons; perhaps the most important is the need to demonstrate the development of specific cellular immunity and humoral responses and include mucosal protective immunity.[60] The first vaccine candidates were based on strategies that had worked for other infectious diseases, such as inactivated virus and HIV proteins conjugated to adjuvants. These were able to induce only weak neutralizing antibody activity and did not provide significant protection against HIV infection in clinical trials. Live attenuated simian immunodeficiency virus strains have been demonstrated to protect macaques from simian immunodeficiency virus challenge; however, there are safety concerns related to the extraordinary capacity of HIV for recombination, which might lead to wild-type revertant strains. A novel approach using an adenovirus-based vaccine expressing HIV proteins elicited strong anti-HIV immunity; however, it was unable to demonstrate a protective effect over placebo in a phase I/II clinical trial with more than 3,000 subjects.[61]

Prevention measures

Considerable resources have been placed on educational campaigns to control the HIV epidemics. Preventive interventions that have been useful are using condoms, providing intravenous drug users with free sterile needles, screening blood products, and administering antiretroviral agents to HIV-infected pregnant women and their infants. Avoidance of breast-feeding has been recommended on the basis of the increased risk of transmitting the virus through breast milk; however, this might be revised in communities with poor resources, where it has been demonstrated that breast-feeding up to 1 month in combination with antiretroviral therapy does not increase early transmission and provides immune and nutritional support to the newborn.[62] Other preventative interventions are male circumcision, with a reduction of the risk of HIV infection in heterosexual males by 50% to 60%,[63] and topical anti-HIV microbicidals as an alternative to the use of condoms.[64] The control of this deadly disease will only result from a combined effort of researchers and physicians developing and using anti-HIV drugs effectively and educators working in the promotion of safe behavioral practices in communities at risk.

CONCLUSION

There is an increased awareness of the variety of factors that can affect the immune response. When evaluating a patient with increased frequency or severity of infections suggesting immunodeficiency, physicians should consider that secondary immunodeficiencies are far more common than primary immune defects of genetic cause. A detailed clinical history might uncover the condition affecting the immune system, such as infection, malnutrition, age extremes, concomitant metabolic or neoplastic diseases, use of immunosuppressive drugs, surgery and trauma, and exposure to harsh environmental conditions. Because of its prevalence and clinical progression, HIV infection should be considered and ruled out. The specific immune defects and clinical presentation in other secondary immunodeficiencies are usually heterogeneous, affecting both the innate and the adaptive immunity. The immune impairment improves with the resolution of the primary condition.

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Immunologic rheumatic disorders

Amy Joseph, MD,[a,b] Richard Brasington, MD,[a] Leslie Kahl, MD,[a] Prabha Ranganathan, MD,[a] Tammy P. Cheng, MD,[a] and John Atkinson, MD[a] *St Louis, Mo*

We provide the basics for clinicians who might be called on to consider the diagnosis of diseases such as systemic lupus erythematosus (SLE) or rheumatoid arthritis (RA) in their practice. We will emphasize clinical recognition and first-line laboratory testing. Only characteristics of the classic rheumatic inflammatory diseases (ie, RA, seronegative spondyloarthropathy, SLE, antiphospholipid syndrome, Sjögren syndrome, scleroderma, and polymyositis/dermatomyositis) will be covered. In the past decade, treatment for RA and seronegative spondyloarthropathy has substantially improved. Their treatment has been revolutionized by the use of methotrexate and, more recently, TNF inhibitors, T-cell costimulation modulators, and B-cell depletion. The goal of RA treatment today is to induce a complete remission as early as possible in the disease process, with the mantra being "elimination of synovitis equals elimination of joint destruction." The hope is that if the major mediators of Sjögren syndrome, SLE, or scleroderma can be identified and then blocked, as in the example of TNF inhibitors in patients with RA, more specific treatments will become available. Thus RA has become an excellent model of this evolving paradigm. Through the identification of major mediators in its pathogenesis, novel and highly efficacious therapeutic agents have been developed. (J Allergy Clin Immunol 2010;125:S204-15.)

Key words: *Rheumatoid arthritis, seronegative spondyloarthritis, juvenile rheumatoid arthritis, systemic lupus erythematosus, antiphospholipid syndrome, Sjögren syndrome, scleroderma polymyositis, dermatomyositis, and inclusion-body myositis*

Abbreviations used

ACA:	Anticentromere antibody
ACLA:	Anticardiolipin antibody
ANA:	Antinuclear antibody
Anti-CCP:	Anti–cyclic citrullinated peptide
APA:	Antiphospholipid antibody
APLS:	Antiphospholipid syndrome
aPTT:	Activated partial thromboplastin time
AS:	Ankylosing spondylitis
β2GP1:	β_2-glycoprotein 1
DM:	Dermatomyositis
DMARD:	Disease-modifying antirheumatic drug
dRVVT:	Dilute Russell viper venom time
IBD:	Inflammatory bowel disease
IBM:	Inclusion-body myositis
IVIG:	Intravenous immunoglobulin
JIA:	Juvenile idiopathic arthritis
JRA:	Juvenile rheumatoid arthritis
MRI:	Magnetic resonance imaging
NSAID:	Nonsteroidal anti-inflammatory drug
PM:	Polymyositis
PsA:	Psoriatic arthritis
RA:	Rheumatoid arthritis
RF:	Rheumatoid factor
Scl:	Scleroderma
SLE:	Systemic lupus erythematosus
SNSA:	Seronegative spondyloarthropathy
SS:	Sjögren syndrome
USpA:	Undifferentiated spondyloarthropathy
VDRL:	Venereal disease research laboratory

In this chapter we will provide the basics for clinicians who might be called on in their practice to consider the diagnosis of diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). We will emphasize clinical recognition and first-line laboratory testing. Characteristics of the classic rheumatic inflammatory diseases (ie, RA and its variants, seronegative spondyloarthritis [SNSA], SLE, antiphospholipid syndrome [APLS], Sjögren syndrome [SS], scleroderma [Scl], and polymyositis [PM]/dermatomyositis [DM]) will be covered.

To begin, some general principles relative to these disease entities will be outlined to place them in the overall context of autoimmune and chronic inflammatory disorders. First, although often described as autoimmune diseases and displaying immunologic features, the cause and pathophysiology of these diseases are poorly understood. Infections, toxins, and drugs have been implicated as well, but there is no consensus about causation. The standard textbook explanation is that an interplay among genetic, hormonal, environmental, and immunologic factors produces these illnesses.

Second, these diseases are probably more accurately described as syndromes. Does each category represent one entity with a single cause? More likely, we are dealing with clinical syndromes with similar phenotypes, resulting from many distinct insults.

Third, female subjects have these syndromes more commonly than male subjects and often do so relatively early in adult life. The responsible hormonal or reproductive predisposing factors remain unknown.

Fourth, these diseases feature autoantibodies, and the antigenic reactivity profile of each is helpful in establishing the diagnosis. In most cases, though, we are lacking direct proof that the

From [a]Department of Internal Medicine, Division of Rheumatology, Washington University School of Medicine, St Louis, and [b]St Louis VA Medical Center, St Louis.

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Reprint requests: John Atkinson, MD, Medicine/Rheumatology, Washington University School of Medicine, 660 South Euclid Ave, Campus Box 8045, St Louis, MO 63110. E-mail: jatkinso@dom.wustl.edu.

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autoantibodies are pathogenic rather than bystander phenomena. It is also unknown, for example, whether an autoantibody to human native DNA develops in response to autologous, viral, or bacterial RNA/DNA or even to other materials (phospholipids) that structurally resemble DNA. Moreover, approximately 20% of patients with RA have negative rheumatoid factor (RF) and anti–cyclic citrullinated peptide (anti-CCP) antibody test results. Only 40% to 50% of patients with SLE have anti-DNA antibodies, although 99% have positive antinuclear antibody (ANA) test results. A substantial fraction, approximately one third, of patients with SS, Scl, and PM/DM are also serologically negative. Either there are as yet undiscovered antibodies, or autoantibodies are not mandatory players in the pathogenesis. Furthermore, positive but usually low-to-moderate titers of ANAs and RFs are commonly detected in most chronic inflammatory diseases.

Fifth, for unclear reasons, these diseases tend to pick an organ system (RA, synovial joints; SS, exocrine glands; Scl, skin; and PM/DM, muscle) to damage. The autoantibody profile, although facilitating disease classification, does not tell us why the skin is a major target in patients with Scl or the synovial-based joints are major targets in patients with RA. Multiorgan involvement is particularly characteristic of SLE, probably because of immune complex deposition in vascular structures.

Sixth, except for Scl, these diseases generally respond to anti-inflammatory and immunosuppressive drugs. Such a response, of course, does not mean that the immune system was at fault initially but does imply a role for immunologically mediated inflammation in producing tissue damage. A persistent stimulus or an overactive inflammatory response mediated by the innate or adaptive arms of the immune system could be the responsible process.

In the past decade, RA is the entity for which treatment has substantially improved. The management of RA has been revolutionized by the regular use of methotrexate and, more recently, use of this agent in combination with a TNF inhibitor, a T-cell costimulation modulator, or B-cell depletion therapy. The goal of RA treatment today is to induce a complete remission as early as possible with the objective of eliminating the synovitis to prevent joint destruction. The hope is that if the major mediators of SS, SLE, or Scl can be identified and then blocked, as per the example of TNF inhibitors in patients with RA, more specific and efficacious treatments await us. Thus RA has become an excellent model for this evolving paradigm. Through the identification of key players in pathogenesis, novel and effective therapeutic agents have been developed, and more are anticipated.

RA

General information

RA is a symmetric inflammatory polyarthritis that affects approximately 1% of the population and accounts for significant morbidity and mortality. RA occurs worldwide, increases in incidence with age, and affects women about 3 times more often than men. Although the cause of RA is unknown, we have learned much about the inflammatory process that leads to joint destruction and how we might selectively target this process.

Clinical features

The clinical presentation and course of RA are variable. Patients most often have an insidious onset of symmetric joint pain, swelling, and morning stiffness that worsens over several weeks. Generalized malaise and fatigue accompany active inflammation. Progressive joint damage from suboptimally controlled RA leads to deformities and increasing disability.

Physical findings in patients with RA include symmetric joint inflammation early in the course of the disease and later manifestations of joint destruction with chronic disease. Classically, RA causes synovitis in the metacarpophalangeal, proximal interphalangeal, and wrist joints in a symmetric distribution. Clinically, this is manifested as swelling, warmth, tenderness, and loss of range of motion and grip strength in the hands. Range of motion can be restricted in deeper joints, in which demonstration of other signs is not possible. RA commonly affects the knees, shoulders, ankles, and feet, as well as the hips and cervical spine.

Extra-articular manifestations of RA include subcutaneous rheumatoid nodules, vasculitic skin ulceration, sicca symptoms of dry eyes and dry mouth, pulmonary nodules and pulmonary interstitial fibrosis, mononeuritis multiplex, and Felty syndrome (triad of RA, neutropenia, and splenomegaly). However, with the discovery of more effective treatments, extra-articular manifestations of RA are less common than in previous decades.

Immunologic features and disease pathogenesis

The underlying cause of RA remains incompletely understood. Data from the past decade support an immune-mediated process leading to joint inflammation and destruction. Genetic studies have demonstrated links to major MHC class II molecules. In particular, patients with the shared epitope, which is found in the hypervariable region of the HLA-DR β chain, are more likely to have RA than those without it. Environmental factors, such as cigarette smoking, have been identified as risk factors for RA. Other, yet to be identified genetic and environmental factors are likely also involved. The purported scenario is that a genetically susceptible subject is exposed to particular environmental, hormonal, or infectious factors. Proinflammatory cytokines, including IL-1, IL-6, and TNF-α, have been linked to RA and thus provide new therapeutic targets.[1]

The pathogenesis of joint destruction in patients with RA includes synovial immune complex deposition, neutrophil infiltration, angiogenesis, and T-cell activation.[2] Blocking T-cell costimulation by antigen-presenting cells is a recently developed strategy for treating RA that provides evidence for the importance of T-cell activation in pathogenesis. Additionally, leukocytes, including macrophages, are subsequently activated and propagate the cytokine-rich inflammatory environment. The synovial membrane enlarges to form the pannus, which begins to invade the cartilage and bone. Finally, proliferation of the pannus leads to more profound cartilage destruction, subchondral bone erosions, and periarticular ligamentous laxity. Cytokine-stimulated osteoclast activity leads to erosions and periarticular osteoporosis.

The search for specific immunologic mechanisms has led to the discovery of autoantibodies in patients with RA. The role of these autoantibodies and their respective autoantigens in pathogenesis is unknown. The long-recognized RF, a polyclonal IgM directed against the Fc portion of IgG, is found in about two thirds of patients with RA. It binds to IgG-containing immune complexes and augments immunoinflammatory responses. RF is present in all subjects but in higher amounts in patients with RA. The key autoantibody convincingly associated with RA is against citrullinated peptides. In the past decade, measurement of the anti-CCP antibody has become recognized as a highly specific and fairly sensitive diagnostic test for RA. It occurs in approximately 60%

TABLE I. American College of Rheumatology criteria for the classification of rheumatoid arthritis

1. Morning stiffness lasting >1 hour
2. Swelling of ≥3 joints observed by a physician
3. Symmetric distribution of joint involvement
4. Involvement of the wrists, metacarpophalangeal joints, and proximal interphalangeal joints, sparing the distal interphalangeal joints
5. Positive RF test result
6. Rheumatoid nodules on extensor tendon surfaces
7. Radiographic changes (periarticular osteopenia and erosions)

For the diagnosis of RA, a patient should have at least 4 of the 7 criteria. Criteria 1 through 4 must be present for at least 6 weeks.

of patients with RA and is more specific than RF for the diagnosis of RA. RF and anti-CCP antibodies can be identified in patients years before they have clinical disease, suggesting that the disease process begins long before it is clinically apparent. The mechanisms that facilitate the transition to clinical disease are unknown but are likely varied and multiple.

Diagnosis

The American College of Rheumatology classification criteria for the diagnosis of RA (Table I) were designed for inclusion of patients in clinical studies and not for routine clinical diagnosis. In fact, an important limitation of these criteria is that they were derived from hospitalized patients with established RA and have not been validated for the diagnosis of "early RA." However, for the clinician unfamiliar with RA, they are beneficial as guidelines for the evaluation of patients with suspected RA. In practice, a patient with symmetric inflammatory polyarthritis of the small joints of the hands with a positive RF test result, CCP antibody test result, or both likely has RA. In fact, if both RF and CCP antibody test results are positive, the diagnosis of RA is almost certain. A positive RF test result, anti-CCP antibody test result, or both in a patients with RA portends more aggressive clinical disease and radiographic damage. Nonspecific indicators of inflammation, such as erythrocyte sedimentation rate and C-reactive protein level, might be increased and might also correlate with the severity of clinical disease.[2]

Recent studies support the value of early recognition and aggressive treatment of RA to limit long-term disease sequelae. If there is a clinical suspicion of RA,[3] prompt referral to a rheumatologist and immediate initiation of therapy to prevent damage is recommended.

Treatment

The goals of treatment in patients with RA are to control inflammation, prevent progressive joint destruction, preserve and improve performance of activities of daily living, and alleviate pain. Medical treatment includes the use of nonsteroidal anti-inflammatory drugs (NSAIDs), disease-modifying antirheumatic drugs (DMARDs), and corticosteroids. Nonpharmacologic treatment, including patient education, physical therapy, occupational therapy, orthotics, and surgery, is also important in the management of RA.

Initiation of DMARD therapy within 3 months of diagnosis is currently recommended. DMARDs suppress immune-mediated inflammation by decreasing the activity of target cells (eg, lymphocytes) or specifically targeting cytokine pathways. Commonly used DMARDs include methotrexate, leflunomide, sulfasalazine, and hydroxychloroquine. When maximal dosing of a DMARD provides suboptimal disease control, addition of other DMARDs often improves effectiveness. Routine monitoring for RA disease activity and drug toxicity is mandatory.

"Biologic DMARDs" targeting TNF-α include the fusion protein etanercept; the mAbs infliximab, adalimumab, and golimumab; and the pegylated TNF receptor fusion protein certolizumab pegol. Other currently available biologic DMARDs include abatacept (a cytotoxic T-lymphocyte antigen 4 fusion protein that blocks T-cell costimulation by antigen-presenting cells), rituximab (an mAb to CD20 that depletes B cells), and the IL-1 receptor antagonist anakinra. Tocilizumab is an mAb to the IL-6 receptor recently approved for the treatment of RA.

Corticosteroids in low doses (≤10 mg of prednisone) are effective for promptly reducing the symptoms of RA, but care should be taken to use the lowest effective dose. Corticosteroids are appropriate in patients with significant limitations in their activities of daily living, particularly early in the course of disease while awaiting the efficacy of the generally slower-acting DMARDs.

NSAIDs, including the selective COX-2 inhibitors, are commonly added to treatment regimens for pain relief and to decrease inflammation. However, they should be used with caution because of their potential to increase cardiovascular risk, which is increased anyway in patients with RA.

JUVENILE IDIOPATHIC ARTHRITIS

Clinical features

Juvenile idiopathic arthritis (JIA; formerly juvenile rheumatoid arthritis [JRA]) represents a family of inflammatory articular disorders that occur before the age of 16 years. Six distinct presentations have been described in patients with JIA. There are 2 subtypes of the polyarthritis (≥5 joints, previously called polyarticular-onset JRA): those with positive RF test results, who generally have more severe disease, and those with negative RF test results. Patients with 4 or fewer joints involved are said to have oligoarthritis (previously called pauciarticular-onset JRA). Young girls with oligoarthritis and a positive ANA test result are at increased risk for chronic iritis. Systemic disease (previously called systemic-onset JRA or Still disease) is characterized by intermittent fever, hepatosplenomegaly, lymphadenopathy, leukocytosis, pleuropericarditis, and the classic "Still rash," a faint, evanescent, salmon-colored eruption that tends to occur during periods of fever. The Koebner phenomenon refers to the fact that this rash can be elicited by stroking the skin. Adult Still disease is a similar syndrome occurring in adults. Differential diagnosis includes SLE, spondyloarthropathy, infectious arthritis, Henoch-Schönlein purpura, vasculitic syndromes, inflammatory bowel disease (IBD), leukemia, sickle cell anemia, and hemophilia.

TABLE II. Characteristics of the seronegative spondyloarthropathies

Disease	Pattern of arthritis	Cutaneous involvement	Other features
AS	Axial: sacroiliitis, continuous involvement of lumbar through cervical spine		
PsA	1. Distal interphalangeal arthritis	Psoriasis, nail pitting associated with distal interphalangeal arthritis	Onset of arthritis before, simultaneous with, or after psoriasis; cervical spine involvement without lumbar spine involvement
	2. Peripheral oligoarthritis		
	3. Symmetric polyarthritis		
	4. Arthritis mutilans		
	5. Spondylitis and sacroiliitis		
Reactive arthritis	Asymmetric oligoarthritis, lower extremity > upper extremity	Keratoderma blennorrhagica, circinate balanitis, psoriasis-like nail changes	Onset days to 6 wk after bacterial gastroenteritis or genitourinary *Chlamydia* species infection
IBD-associated spondyloarthritis	Axial: spondylitis, sacroiliitis Peripheral:		Equal prevalence in men and women
	1. oligoarthritis		
	2. polyarthritis		
USpA	Spondylitis, sacroiliitis, and/or peripheral arthritis		

Enthesitis-related arthritis is characterized by arthritis, enthesitis, and at least 2 of the following: sacroiliac joint tenderness, inflammatory pain of the spine, HLA-B27 positivity, uveitis, or family history of painful uveitis, spondyloarthropathy, or inflammatory bowel disease (IBD). Psoriatic arthritis (PsA) includes children with arthritis who either have psoriasis or a family history of psoriasis.

Immunologic features

RF (see RA) test results are positive in 5% to 10% of older patients with polyarticular symmetric disease. ANA (see SLE) test results tend to be positive in young girls with oligoarthritis and chronic inflammatory eye disease.

Treatment

The medications used for the treatment of JIA include many of the same ones used for the treatment of RA: NSAIDs, prednisone, sulfasalazine, hydroxychloroquine, methotrexate, TNF inhibitors, anakinra, and abatacept. A pediatric rheumatologist should be consulted. The chronic iridocyclitis in patients with JIA is usually asymptomatic until visual loss occurs from glaucoma because of a chronic increase of intraocular pressure. Children with JIA should undergo an eye examination every 3 to 12 months by an ophthalmologist.

SERONEGATIVE SPONDYLOARTHROPATHIES

General information

The seronegative spondyloarthropathies (SNSAs) are a group of inflammatory arthropathies affecting axial and peripheral joints sometimes associated with extra-articular features (Table II). They include ankylosing spondylitis (AS), PsA, reactive arthritis, and IBD-associated arthritis. Patients with undifferentiated spondyloarthropathy (USpA) have features of the SNSAs but do not fulfill the diagnostic criteria for any of them.

Clinical features

The spondyloarthropathies are more common in men than in women. Onset is generally before the fifth decade. Patients present with inflammatory symptoms affecting the spine, the peripheral joints, and/or the entheses (tendon or ligament insertions). Inflammatory back pain has gradual onset, is associated with morning stiffness, and is improved by activity. Sacroiliitis is common and often presents with buttock pain. Peripheral arthritis is typically asymmetric and affects 3 or fewer joints of the lower extremities. Dactylitis or "sausage digit" reflects inflammation of the soft tissue of an entire digit and is frequently seen in patients with PsA and reactive arthritis.

Inflammatory eye disease is commonly seen in the SNSAs, including conjunctivitis, which is generally benign, and anterior uveitis (iritis), which can threaten vision and should be evaluated by an ophthalmologist. A majority of patients with SNSAs have bowel inflammation, usually asymptomatic,[4] and 30% of patients with IBD have SNSAs.[5] Among patients with psoriasis, a recent cross-sectional study revealed a 19% prevalence of PsA.[6]

Levels of acute-phase reactants are often increased in patients with SNSAs. HLA-B27 levels are positive in about 90% of white subjects with AS and in 70% with USpA. Early sacroiliitis is visible on magnetic resonance imaging (MRI), but plain radiographic evidence of sacroiliitis can take years to develop. Sacroiliitis is usually symmetric in patients with AS but can be asymmetric in patients with PsA or USpA. Plain radiographs of the spine show bridging syndesmophytes in patients with advanced AS, with the "bamboo spine" appearance reflecting complete spine fusion. Syndesmophytes in patients with PsA are often nonmarginal and asymmetric in contrast to those seen in patients with AS.

Immunologic features

HLA-B27 is associated with the SNSAs, but only a small percentage of subjects with HLA-B27 have SNSAs, suggesting interplay between genetic and environmental factors. HLA-B27

transgenic rats have SNSA-like disease spontaneously, suggesting a critical role for HLA-B27 in pathogenesis, although the nature of that role is unknown.[7] Interestingly, the B27 transgenic rats did not have SNSA-like disease when raised in a germ-free environment.[8] HLA-B27 on the surface of antigen-presenting cells preferentially binds certain antigens. One hypothesis is that arthritogenic peptides bind HLA-B27 and cross-react with autoantigens.

IBD-associated arthritis might provide a model for pathogenesis of the SNSAs. Arthritis is thought to result when gut inflammation increases intestinal permeability in a susceptible host, allowing absorption of bacterial or other material that increases secretory IgA levels and activates lymphocytes and macrophages. Exactly how this initiates musculoskeletal inflammation is unknown. A similar breach of normal barriers to foreign proteins likely occurs in the gut or the genitourinary tract in patients with reactive arthritis and possibly in the skin in patients with PsA. The asymptomatic bowel inflammation seen in patients with AS might be analogous to IBD-associated increased intestinal permeability.

Diagnosis

Early diagnosis is beneficial because of the availability of effective therapy for the SNSAs. A definitive diagnosis of AS can be made on the basis of inflammatory back pain lasting more than 3 months in the setting of radiographic sacroiliitis. MRI is more likely than a plain radiograph to reveal early sacroiliac inflammation. A patient with inflammatory spine pain or peripheral synovitis who also has one of the following likely has an SNSA and should be referred to a rheumatologist: psoriasis, IBD, acute infection (gastroenteritis, urethritis, or cervicitis) preceding the arthritis, enthesopathy, radiographic sacroiliitis, or a family history of a SNSA.[9]

Treatment

NSAIDs frequently reduce symptoms in patients with the SNSAs. Peripheral joint involvement in patients with the SNSAs often responds to the oral DMARDs used to treat RA, particularly sulfasalazine. The anti-TNF medications etanercept, infliximab, adalimumab, and golimumab have demonstrated considerable efficacy for axial disease. Another TNF inhibitor, certolizumab, is approved for use in IBD and can benefit IBD-associated arthritis. Current data suggest that the TNF inhibitors do not slow the spinal radiographic changes of the SNSAs.[10,11] Local glucocorticoid injections in inflamed joints or entheses, except for the Achilles tendon insertion, can be effective.[12]

Despite the possible role of infections in the pathogenesis of the SNSAs, antibiotics are ineffective in the treatment of these conditions, except in treating the gastrointestinal or genitourinary tract infections that lead to the development of reactive arthritis.

Physical therapy is beneficial in maintaining function and reducing pain. Because of the risk of restrictive lung disease resulting from thoracic spine disease, smoking cessation is important. Joint replacement is beneficial when more conservative measures fail for severe pain or impaired function.

SLE

General information

SLE is a multisystemic "autoimmune" disease affecting the joints, skin, heart, lungs, central nervous system, kidneys, and hematopoietic system. In 99% of patients, it is associated with a positive ANA test result by means of indirect immunofluorescence with the Hep-2 epithelial tumor cell line as substrate. Young women of childbearing age are most commonly affected; drug-induced disease also occurs but is rare today. The most common mistake made in establishing the diagnosis of SLE is inappropriate emphasis on the positive ANA test result in the absence of clinical features of this disease. The ANA test is sensitive but not specific for SLE.

Clinical features

SLE has protean manifestations. The clinical skills of a physician might be tested when assessing whether a patient has this diagnosis.[13] The American College of Rheumatology has articulated 11 clinical classification criteria, of which 4 must be satisfied for the diagnosis. However, these criteria were established for the purpose of including patients in clinical trials and not for making the initial diagnosis in clinical practice. Although these criteria can serve as a general guide for recognizing the clinical features of SLE, "counting criteria" is no substitute for clinical judgment in making the diagnosis of SLE (Table III). Alopecia, Raynaud phenomenon, and systemic complaints, such as fever and fatigue, are other common manifestations of SLE. APLS (see below) is frequently encountered in patients with SLE, particularly in the setting of thromboembolic disease and neuropsychiatric events.

Immunologic features

The immunopathology of SLE has been the subject of many sophisticated and intricate hypotheses, which unfortunately have been impossible to prove or disprove. SLE is characterized by immune complex deposition, of which the best example is glomerulonephritis. The other central concept in the immunopathology of SLE is the presence of autoantibodies; although their presence is indisputable, they might not always be pathogenic but rather epiphenomena. The autoantibodies associated with SLE are perhaps the most confusing and misunderstood characteristics of this disease.[14] The ANA test by means of indirect immunofluorescence is so sensitive that a negative test result effectively rules out the diagnosis of SLE. (Note: The ELISA ANA testing now available is not recommended for screening because 10% to 20% of patients with SLE might have negative results with this currently unsatisfactory method.) The indirect immunofluorescence technique often produces low-to-moderate titers of ANA in patients with chronic inflammatory conditions and sometimes in healthy subjects; thus reduced specificity is the price paid for the high sensitivity of this test. The higher the titer, the more likely SLE or a related syndrome is present. Therefore the ANA test is most useful in testing the clinical hypothesis that a patient might have SLE in that a negative test result excludes the diagnosis, whereas a positive test result means that a diagnosis of SLE is possible but not established. If the ANA test is used as a screen for autoimmune disease, a positive ANA test result has little or no immediate significance, especially in the absence of clinical evidence of SLE.

Antibodies to double-stranded DNA, by contrast, have lower sensitivity but high specificity: a high titer of anti-DNA antibodies makes the diagnosis of SLE very likely, although a negative anti-DNA test result does not exclude the diagnosis because 50% of patients with SLE never have anti-DNA

TABLE III. American College of Rheumatology classification criteria for SLE*

1. Malar rash: fixed malar erythema, flat or raised, sparing nasolabial folds
2. Discoid rash: erythematous raised patches with keratotic scaling and follicular plugging
3. Photosensitivity: skin rash as an unusual reaction to sunlight
4. Oral or nasopharyngeal ulcers
5. Arthritis: nonerosive
6. Serositis: pleuritis or pericarditis
7. Renal disorder: proteinuria (>0.5 g/d or >3+) or cellular casts
8. Neurologic disorder: seizures or psychosis
9. Hematologic disorder: hemolytic anemia or leukopenia (<4,000/mL), lymphopenia (<1,500/mL), or thrombocytopenia (<100,000/mL)
10. Immunologic disorder: anti–double-stranded DNA, anti-Smith, false-positive test result for syphilis, or positive lupus erythematosus (LE) cell preparation (a test rarely performed)
11. Abnormal titer of ANA by means of immunofluorescence or an equivalent assay at any point in time and in the absence of drugs known to be associated with "drug-induced lupus" syndrome

*Note that these are classification criteria and not diagnostic criteria. The diagnosis of SLE is based on the physician's overall evaluation. SLE often presents with a positive ANA test result and single organ system involvement. Diagnosis by counting criteria is not how one makes an initial diagnosis of SLE.

antibodies. There are 2 common techniques for detecting anti-DNA antibodies. The *Crithidia luciliae* technique is an indirect immunofluorescence test analogous to the ANA test. The *Crithidia* organism contains a kinetoplast that is comprised of pure double-stranded DNA, a convenient target for anti-DNA antibodies when the organisms are fixed to a microscope slide. The other technique is an ELISA, which is a quantitative assay with the potential to allow processing of large numbers of samples rapidly in an automated fashion.

Another highly specific assay for the presence of SLE is anti-Smith, which reacts with one of the soluble extractable nuclear antigens. It occurs in approximately 25% of patients with SLE. Anti-ribonucleoprotein can be seen in some cases of SLE, especially in the setting of myositis, but is classically associated with mixed connective tissue disease when present in high titers. Anti-Ro (SSA) is observed in approximately 25% of patients with SLE, especially those with subacute cutaneous lupus erythematosus, an annular erythematous rash that heals without central scarring, in contrast to discoid lupus erythematosus rash, which does produce scarring. Anti-Ro and anti-La are serologic markers for the neonatal lupus syndrome, which is characterized by congenital heart block, thrombocytopenia, and an annular rash. This is an excellent example of pathogenic antibodies in the mother that cross the placenta and induce disease in the newborn. Ironically, about 50% of the mothers of babies with neonatal lupus do not clinically have SLE yet carry the autoantibodies. Nevertheless, neonatal lupus only occurs in 15% to 20% of the children born to mothers with SLE who have positive anti-Ro/La test results, and of those, approximately 15% have skin disease and 1% to 2% have congenital heart block.

Serum complement levels C3 and C4 might be decreased in patients with active disease because of complement consumption by immune complexes, especially in the setting of active glomerulonephritis. Levels usually increase with clinical improvement, although they do not always normalize. The total hemolytic complement assay is not particularly sensitive for detecting complement consumption but is valuable in detecting an isolated complement component deficiency (C1q, C1r, C1s, C4, or C2), which usually presents with an SLE-like syndrome (albeit rare, occurring in 0.5% to 1% of patients with SLE).

More recently, an IFN-α signature, meaning activation of many genes by increased levels of this cytokine, has been recognized in a substantial fraction (approximately 50%) of patients with SLE. Treatments designed to interfere with this process are awaited. Lastly, whole-genome screens have identified many additional targets for therapeutic intervention.

Treatment

A common misconception is that all patients with SLE require therapy with systemic corticosteroids, when in fact, milder manifestations of the disease can be treated with less potent medications. Rash can be effectively treated with topical corticosteroids, but care must be taken to avoid use of fluorinated corticosteroids on the face because of the potential for the development of subcutaneous atrophy. Hydroxychloroquine can be effective for control of skin disease, as well as for arthritis. NSAIDs and low daily doses of prednisone (10-20 mg) can bring relief from arthritis and milder cases of pleurisy and pericarditis; more severe cases require high daily doses of corticosteroids (40-60 mg of prednisone). Kidney disease that threatens renal function is typically treated with high-dose corticosteroids and intermittent intravenous cyclophosphamide or oral mycophenolate mofetil. Treatment decisions must be individualized for the particular clinical situation.

APLS

General

APLS can occur as part of SLE or as an idiopathic isolated entity. Ten percent to 30% of patients with SLE have antiphospholipid antibodies (APAs), and of those, 30% to 50% have APLS. Conversely, about 50% of patients with APLS have SLE. Serologic hallmarks of this prothrombotic disorder have been recognized for a long time as the biological false-positive test result for syphilis and the lupus anticoagulant. It was not, however, until the early 1980s that a clinical syndrome consisting of venous and arterial thrombotic events and recurrent miscarriages was widely recognized as being related to these tests. Thus we have the paradox of a clinical syndrome featuring excessive clotting diagnosed by the prolongation of a coagulation test that might be expected to lead to excessive bleeding.

Clinical features

These patients are usually young and middle-aged women, with or without lupus, who present with a prothrombotic medical

history. The second major feature is a history of multiple miscarriages, especially in the second and third trimesters. A patient might have only venous or arterial thromboses, miscarriages, or a combination thereof. The most common problem is deep venous thrombosis, whereas on the arterial side, strokes and transient ischemic attacks are prominent. However, any vessel can be involved, leading to infarctions of the gut, heart, adrenals, and extremities. Miscarriages most likely relate to thrombosis of placental vessels, leading to infarction, placental insufficiency, and a small fetus. Livedo reticularis, which is observed in about one third of patients, is readily apparent on physical examination. Patients with APLS also commonly have thrombocytopenia, which is sometimes severe, but usually in the 75,000/mm^3 to 150,000/mm^3 range.[15] The diagnosis should be strongly considered in young women with no explanation for their thromboses or if the thromboses are in unusual sites. Catastrophic APLS is rare. It occurs when there are acute, multiple, diffuse, simultaneous vascular infarcts, often with a lethal outcome.

Cause and pathogenesis

How APAs induce thrombosis is unknown. Inhibition of protein C activation, activation of platelets, alterations in prostaglandin synthesis, and stimulation of endothelial cells have been proposed. There is *in vitro* evidence to support each possibility. In a murine model animals injected with APAs from patients display fetal wastage caused by placental destruction by antibodies and complement. Patient-derived APAs also facilitate thrombus formation in other murine organs.

Laboratory tests

Four tests for APAs are lupus anticoagulant, anticardiolipin antibodies (ACLAs), antibody to β_2-glycoprotein 1 (β2GP1), and the false-positive venereal disease research laboratory (VDRL) tests. In the lupus anticoagulant test the activated partial thromboplastin time (aPTT) is prolonged but is not corrected by mixing with normal plasma (to rule out a factor deficiency); it is, however, normalized by addition of phospholipids (to compensate for the phospholipids tied up by the APA). The failure of the prolonged aPTT to correct with the addition of normal plasma combined with correction of the prolonged aPTT by the addition of phospholipid defines the phenomenon referred to as "lupus anticoagulant."

Another test for this is the dilute Russell viper venom time (dRVVT). This is analogous to the aPTT, except that dilute Russell viper venom replaces activated thromboplastin as the stimulus for the intrinsic coagulation pathway. The advantages of the dRVVT are that it is more sensitive for the detection of smaller amounts of APA than the aPTT (the dRVVT might be prolonged even with the aPTT is not) and that it can be performed in the setting of therapeutic anticoagulation. ACLAs are detected by using an ELISA, which can measure IgG, IgM, and IgA ACLAs. Medium-to-high titers of IgG are most diagnostic.

Another ELISA detects antibodies to β2GP1, a plasma protein that binds negatively charged phospholipids; the ACLAs in patients with APLS are often directed to β2GP1. It might function as an anticoagulant through its binding to phospholipids, and therefore interference with its function by antibodies could induce thrombosis.

Finally, the "false-positive VDRL" (so-called because the fluorescent treponemal antibody [FTA] test result is negative for syphilis) reflects the presence of antibodies that react with cardiolipin.

Treatment

Among patients with asymptomatic APA, the risk of thrombosis is 0% to 3.8% annually.[16] Aspirin has not been shown to be effective in preventing first thrombosis. Other risk factors for thrombosis, including smoking and oral contraceptive use, should be addressed. Once a thrombosis has occurred, recurrence is common, and patients are usually treated indefinitely with oral anticoagulants. Although some patient subsets respond solely to aspirin, most require prolonged warfarin therapy in which the international normalized ratio goal is 2.0 to 3.0, although there is controversy as to how high the target international normalized ratio should be. Treatment is usually successful in preventing further episodes. Regimens using heparin, aspirin, intravenous immunoglobulin (IVIG), and prednisone are used as monotherapy and in various combinations to treat recurrent pregnancy wastage. Patients who have recurrent vascular thrombosis despite adequate oral anticoagulation are also treated in this manner. Catastrophic APLS is managed with a combination of anticoagulation, high-dose corticosteroids, plasma exchange, IVIG, and, in some instances, immunosuppression with cyclophosphamide or rituximab.[3] It has a very high mortality rate.

SS

Background

SS is a chronic autoimmune disease affecting the exocrine glands, particularly the lacrimal and salivary glands (autoimmune exocrinopathy). SS affects approximately 0.3% to 0.6% of the population worldwide and is one of the most common autoimmune diseases. With a female/male ratio of 9:1, SS exhibits one of the highest female/male ratios among the autoimmune diseases and has a peak incidence in the fourth and fifth decades. SS can occur alone, when it is called primary SS, or in association with another defined autoimmune disease (eg, SLE, RA, or Scl), when it is termed secondary SS.[17]

Clinical features

The most common symptoms are dry eyes (xerophthalmia) and dry mouth (xerostomia), together called sicca symptoms. Patients present with symptoms of ocular discomfort and visual disturbance caused by tear film instability and inflammation of the ocular surface.[18] Patients complain of difficulty eating dry foods. Decreased salivary flow leads to frequent dental caries and accelerated periodontal disease. The most prominent sign of SS is swelling of the parotid gland, which can be unilateral or bilateral and is often recurrent. The most common cutaneous finding in patients with SS is dry skin; less commonly, palpable purpura, urticaria, and annular lesions occur. Arthralgias are common. Pulmonary manifestations of SS include dryness of the airways, hyperreactive airway disease, interstitial lung disease, lymphoproliferative diseases, pulmonary hypertension, and amyloidosis.[19] Nonspecific interstitial pneumonia, a type of interstitial lung disease, is the most common pulmonary pathology seen in patients with primary SS.[20]

Dysphagia, esophageal webs and/or dysmotility, chronic atrophic gastritis, and rarely ischemic colitis caused by vasculitis are gastrointestinal features of SS. Neurologic complications in patients with SS are varied and include meningitis, myelopathy, cranial neuropathy, sensorimotor polyneuropathy, and mononeuritis multiplex. The syndrome of pure sensory neuropathy is characteristic of primary SS.[21] Patients who have SS are at risk for lymphomas, usually low-grade, non-Hodgkin, mucosal associated lymphoid tumor–associated lymphomas.[22,23] Certain patient characteristics are predictive of lymphoma, including low C4 levels, especially at the time of presentation[24]; recurrent glandular swelling; palpable purpura; and increased IgM levels.[24,25] Such patients require close observation and monitoring for malignant changes.[25] A recent study in Sweden and a meta-analysis found the relative risk for lymphoma in patients with primary SS to be between 16 and 18 compared with the general population.[26,27] Infants of mothers with SS with a high titer of antibodies to SSA/Ro have an increased risk of congenital heart block.

Pathogenesis

Recent evidence suggests that glandular epithelial cells play a central role in the pathogenesis of SS.[28] The glandular epithelial cells found in patients with SS are immunologically activated, expressing MHC class I and II molecules and B7 costimulatory molecules. These epithelial cells release proinflammatory cytokines and chemokines that attract lymphocytes into the affected glands, producing the characteristic periductal, focal, lymphocytic infiltrate with T_H cells, B cells, and plasma cells observed in SS. The initial event that stimulates the activation of these glandular epithelial cells is unknown, but persistent viral infections might play a role.[29] Chronic HIV and hepatitis C infection can lead to a glandular pathology similar to that found in patients with idiopathic SS, and patients infected with such viruses often have sicca symptoms and glandular swelling.

Diagnosis

Antibodies to Ro/SSA and La/SSB are the best-characterized serologic features of SS. Anti-La antibodies are almost invariably accompanied by anti-Ro antibodies because of the physical association of these molecules in Ro/La ribonucleoprotein particles, but anti-Ro antibodies frequently occur in the absence of anti-La antibodies. Anti-Ro antibodies are present in approximately 70% and anti-La antibodies in 40% of patients with primary SS. Anti–thyroid microsomal and anti–gastric parietal cell antibodies are seen in a third of patients with primary and secondary SS. Other antibodies reported in patients with SS include perinuclear antineutrophil cytoplasmic antibodies and antibodies directed against carbonic anhydrase, proteasomal subunits, α-fodrin, and the muscarinic M3 receptor.[30] Positive test results for ANA and RF are present in 60% to 80%, and polyclonal hypergammaglobulinemia is demonstrable in 50% of patients with primary SS.

Diagnosis of primary SS according to the current American European Consensus Group criteria requires that at least 4 of the following 6 criteria be present: subjective xerophthalmia, subjective xerostomia, objective tests of xerophthalmia, objective evidence of salivary gland dysfunction, presence of either anti-Ro/SSA or anti-La/SSB antibodies, and histopathologic criteria for SS on minor salivary gland biopsy. One of the 4 criteria must be either positive by means of serology or positive by means of histopathology.[31] Xerophthalmia is often demonstrated by using the Schirmer test, in which a standardized strip of filter paper is placed for 5 minutes between the eyeball and the lateral part of the inferior eyelid. The test result is positive when the wetting is 5 mm or less in 5 minutes. Saliva production tests, the results of which are considered positive when 1.5 mL or less of whole saliva is collected in 15 minutes, although highly specific for SS, are rarely done in clinical practice.

Treatment

The mainstay of treatment of dry eye disease in patients with SS is lubrication with a variety of artificial tear supplements. Recently, topical cyclosporine 0.05% emulsion has been approved for and shown to be effective in patients with decreased tear production caused by inflammation, including those with SS. In patients with inadequate response to artificial tears, punctal occlusion performed by an ophthalmologist might help retain the instilled artificial tears. Attention to dental hygiene and use of topical fluorides might retard dental caries and periodontal disease. Orally administered secretagogues improve dry mouth symptoms better than dry eye symptoms, and their use is mainly limited by their cholinergic side effects of sweating and diarrhea. Pilocarpine, the first approved secretagogue for SS, was shown in a prospective, randomized clinical trial to be effective in improving symptoms of dry mouth, with a smaller effect on symptoms of dry eye.[32] Cevimeline, another secretagogue, might be better tolerated than pilocarpine and is effective in treating symptoms of both dry eyes and mouth.[33]

Arthralgias might respond to nonsteroidal anti-inflammatory agents or antimalarial agents. Systemic steroids and cytotoxics are mainly used for treatment of extraglandular complications, such as vasculitis, pulmonary disease, and neurologic involvement. Two randomized controlled trials, one with infliximab and another with etanercept, showed a lack of efficacy of these TNF antagonists in patients with primary SS.[24,25] Recently, rituximab, an anti-CD20 mAb, has shown efficacy in the treatment of primary SS in 3 open-label studies and 1 randomized controlled trial, indicating that B-cell modulation might be a promising new treatment strategy for this disease.[26,34,35]

SCL

General information

Scl (also called systemic sclerosis) is a systemic disorder characterized by excess collagen deposition in the skin and viscera, along with vascular abnormalities, including vasospasm and microvascular occlusion. There are 2 subsets of Scl with differing clinical presentations and prognosis: limited and diffuse Scl. A localized form of disease can occur, and there are several Scl-like disorders that must be distinguished from Scl (Table IV). Scl has a peak age at onset of 35 to 65 years and a female/male ratio of approximately 3:1.

Clinical features

The hallmark of both limited and diffuse Scl is thickening of the skin. In limited disease this is gradual in onset and, by definition, is limited to the hands, face, forearms, and feet (Table V). In diffuse disease both the pace and extent of skin

TABLE IV. Classification of Scl

Systemic sclerosis
Diffuse Scl
Limited Scl
Localized Scl
Morphea Scl
Linear Scl
Scl-like syndromes
Graft-versus-host disease
Diabetic cheiroarthropathy
Eosinophilic fasciitis
Scleredema
POEMS syndrome
Carcinoid
Drug induced
Bleomycin
Tryptophan
Occupational and environmental
Silica
Solvents
Trichlorethylene
Perchlorethylene
Vinyl chloride
Toxic oil
Nephrogenic systemic fibrosis

POEMS, Polyneuropathy, organomegaly, endocrinopathy/edema, M-protein, and skin abnormalities.

TABLE V. Comparison of limited and diffuse Scl

Manifestation	Limited	Diffuse
Site of skin involvement	Distal and face only	Distal and proximal
Pace of skin involvement	Slow	Rapid
Telangiectasias	+++	+ (late)
Calcinosis	+++	+ (late)
Tendon friction rubs	0	+++ (early)
Pulmonary hypertension	++	(+/−)
"Renal crisis"	0	++
ACA	+++	0
Survival (10 y)	>70%	<50%

thickening are more extreme, with involvement of the entire trunk and upper and lower extremities. Calcinosis and telangiectasias occur in both forms of the disease but later in diffuse disease. Musculoskeletal features of this disease include acro-osteolysis, myositis, and severe flexion contractures from skin and tendon involvement, particularly in diffuse disease.

Raynaud phenomenon is nearly universal among patients with Scl. In limited disease it can precede skin changes by months to years, but in diffuse disease the onset of both symptoms is usually nearly simultaneous. Raynaud phenomenon occurs in 10% to 20% of the general population, most of whom have no underlying or associated disorder. The differential diagnosis includes Scl and other connective tissue diseases; exposure to percussive or vibratory equipment; drugs, including ergots and sympathomimetics; and cryoglobulinemia.

Esophageal dysmotility is the most common gastrointestinal manifestation and can present with dysphagia, reflux, or stricture. Decreased small-intestine motility can also occur, resulting in bacterial overgrowth, diarrhea, and malabsorption. Large-intestine involvement presents as constipation or pseudo-obstruction.

Pulmonary parenchymal fibrosis is common but often asymptomatic and occurs in both forms of the disease. Approximately 10% to 30% of patients experience pulmonary hypertension, more commonly in those with limited disease. Cardiac involvement includes arrhythmias, microvascular ischemia, and congestive heart failure. Patients with diffuse Scl are at risk for "renal crisis," with hypertension, microangiopathy, and renal insufficiency without nephritis. Cardiopulmonary disease is the most common cause of death in both forms of Scl.

Immunologic features

The pathogenesis of Scl is unknown, but immune mechanisms appear to play a role. Polyclonal hypergammaglobulinemia is common. Approximately 80% of patients have ANA in a centromere or nucleolar pattern. Anticentromere antibody (ACA) is strongly associated with limited disease; anti–Scl-70 is more common with diffuse disease. Antibody to RNA polymerase III is strongly associated with renal crisis. There is an increased frequency of other autoimmune disorders, including hypothyroidism, primary biliary cirrhosis, and SS.

Microvascular occlusion is another hallmark of this disease. Endothelial cell activation results in increased levels of IL-1, which upregulates adhesion molecule expression. Platelet activation causes release of connective tissue growth factor, fibroblast-activating factor, platelet-derived growth factor, and TGF-β. TGF-β stimulates endothelin and fibroblast synthesis. Fibroblasts proliferate abnormally and also secrete increased amounts of collagen and fibronectin. Levels of tissue inhibitor of metalloproteinase 1, an inhibitor of procollagenase, are increased, resulting in decreased remodeling of tissue collagen matrix. The net result is an overproduction of collagen, endothelial fibrosis, and relative tissue ischemia.

Diagnosis

The diagnosis of Scl is made on clinical grounds, with Raynaud phenomenon and skin thickening as diagnostic clues. ACA or anti–Scl-70 antibody testing can be used to confirm the clinical suspicion. Skin biopsy is rarely needed. Occasionally, the diagnosis of "scleroderma sine scleroderma" is made in the setting of Raynaud phenomenon and severe esophageal dysmotility without skin thickening, particularly if ACA or anti–SCL-70 is present. Baseline and periodic pulmonary function tests and echocardiograms are useful in monitoring disease and guiding treatment.

Treatment

Treatment of patients with Scl is symptomatic and problem oriented. Management of Raynaud phenomenon includes avoiding cold exposure, nicotine, caffeine, and sympathomimetics. Raynaud phenomenon might respond partially to vasodilators, including calcium-channel blockers. Losartan and sildenafil might also be useful. Ischemic digital ulcers can be managed with cervical sympathetic blockade; prostacyclin infusion; phosphodiesterase-5 inhibitors, such as sildenafil; and meticulous local skin care. Arthritis and serositis generally respond to NSAIDs, which must be used cautiously. Corticosteroids are reserved for resistant symptoms. Use of moderate- to high-dose steroids might increase the risk of hypertensive crisis. This complication is treated with angiotensin-converting enzyme inhibitors. Proton pump inhibitors are used for esophageal symptoms and should be prescribed to all patients because of

TABLE VI. Myositis-specific autoantibodies

Autoantibody	Associated clinical manifestations	Response to therapy	Frequency in myositis
Antisynthetase (anti–Jo-1 is most common)	Antisynthetase syndrome: myositis, arthritis, fever, interstitial lung disease, "mechanic's hands," Raynaud phenomenon	Moderate	20% to 30%
Anti-SRP	Myalgias and severe weakness with cardiac involvement	Poor	<5%
Anti–Mi-2	Classic dermatomyositis	Good	5% to 10%

SRP, Signal recognition particle.

the high frequency of reflux and aspiration. Promotility agents can also be added. There is no definitive treatment for the skin thickening. Occupational therapy is of value in maintaining hand function. Pulmonary hypertension might respond to prostacyclin analogs, phosphodiesterase 5 inhibitors, or the endothelin 1 receptor blocker bosentan.[36] Patients with inflammatory interstitial lung disease might derive modest benefit from treatment with cyclophosphamide.[37]

POLYMYOSITIS, DERMATOMYOSITIS, AND INCLUSION BODY MYOSITIS

General information

PM, DM, and inclusion-body myositis (IBM) are the major distinct forms of idiopathic inflammatory myopathies.[38] These diseases are characterized by inflammation and weakness of skeletal muscle. The inflammatory myopathies are rare, with an incidence of approximately 1 in 100,000. Other categories of inflammatory myopathies include DM of childhood, PM and DM associated with neoplasia, and PM or DM associated with connective-tissue diseases, such as Scl, SLE, and mixed connective tissue disease.

Clinical features

PM and DM affect women more often than men, usually after the second decade of life. These inflammatory myopathies cause muscle weakness that is proximal and symmetric and progresses over weeks to months. Patients typically have difficulty climbing stairs, rising from a chair, or lifting objects above their heads. Neck flexor weakness or dysphagia can occur, but facial or ocular muscle involvement is rare. Systemic manifestations (fatigue, anorexia, and fever) are common. Up to 50% of patients have extramuscular involvement, usually cardiac (conduction defects and cardiomyopathy) or pulmonary (alveolitis and respiratory muscle weakness).

DM has skin findings that distinguish it from other myopathies. A lilac discoloration of the eyelids (heliotrope rash), often with periorbital edema, and erythematous papules over the knuckles (Gottron sign), elbows, knees, and ankles are characteristic. The DM lesions of the elbows and knees can resemble psoriasis. A macular rash of the face or upper anterior chest (V sign), upper back, or shoulders (shawl sign) is also seen in DM and is frequently photosensitive. Dilated nailfold capillary loops at the base of the fingernails can be visualized with a handheld ophthalmoscope set at +40 diopters. The skin of the fingers might be rough and cracked, with dark, "dirty" lines ("mechanic's hands"). Skin lesions can exist without evidence of muscle weakness (amyopathic DM).

The antisynthetase syndrome of DM or PM is characterized by some or all of the following manifestations in the setting of antisynthetase antibody positivity: acute onset of disease, constitutional symptoms, interstitial lung disease, inflammatory arthritis, mechanic's hands, rash, and Raynaud phenomenon. Myositis can be mild or even absent in some patients with this syndrome.

As opposed to PM and DM, IBM is more common after age 50 years and affects men twice as often as women. IBM commonly presents with weakness and atrophy that might be distal and asymmetric and progresses over months to years. This type of myopathy does not respond to treatment as well as PM, but stabilization or modest improvement of muscle weakness can sometimes be achieved.

The association between myopathies and cancer is stronger with DM than with PM; multiple studies have demonstrated an increased malignancy incidence in both patients with DM and those with PM. An appropriate workup includes a thorough history and physical examination, including pelvic examinations for women and age-appropriate malignancy screenings for the breast, colon, and prostate. Whereas most experts agree that an exhaustive search for occult malignancy is not indicated, one group reported 11 malignancies in 24 patients who underwent computed tomographic scans of the chest, abdomen, and pelvis after the diagnosis of DM or PM.[39] This finding highlighted the concept that primary DM and PM might be clinically difficult to distinguish from malignancy-associated PM/DM and that a thorough evaluation is warranted in the appropriate setting. Furthermore, malignancy risks continued to be increased 3 years after the PM/DM diagnosis, necessitating ongoing close monitoring during this period.

DM is by far the most common inflammatory myopathy in children. Up to 40% of children with DM have calcinosis of the skin and muscle.

Immunologic features

Muscle biopsy specimens from patients with PM reveal $CD8^+$ T-cell infiltrates in the endomysium. In contrast, muscles affected by DM show $CD4^+$ T-cell and B-cell infiltrates in the perimysium and perivascular area. DM appears to be a vasculopathy with immune complex deposition and the complement membrane attack complex in vessel walls.

Recently, dendritic cells have been identified by means of immunohistochemistry in muscle biopsy specimens of inflammatory myopathies.[40] These cells are the professional antigen-

presenting cells and might play a central role in recruiting T and B cells and promoting their activation. Furthermore, cytokine profiling of inflammatory myositic lesions revealed an interferon signature, which is consistent with the hypothesis that dendritic cell activation is one major pathogenic step in inflammatory myositis. Lastly, autoantibodies, such as myositis-specific antibodies, might be an epiphenomenon associated with dendritic cell activation.

Nevertheless, myositis-specific autoantibodies define subgroups of patients with more uniform clinical features and prognosis (Table VI).[41,42] Although these autoantibodies are specifically increased in patients with inflammatory myopathies, they target ubiquitous antigens. These autoantibodies can be found in patients before the development of weakness, and their titers can vary with disease activity. However, there is no direct evidence to support a pathogenic role. The most prevalent myositis-specific antibodies are targeted to aminoacyl-tRNA synthetases. The most common of the antisynthetases is the anti–Jo-1 antibody. Other well-characterized antibodies include the anti–signal recognition particle that targets a cytoplasmic ribonucleoprotein complex and is associated with cardiac involvement and poor prognosis. In addition, anti–Mi-2, which targets a helicase involved in regulating transcription, is associated with classic DM skin manifestations. Other immunologic features include the presence of ANA, increased levels of immunoglobulins in the serum, and the infiltration of muscle by lymphocytes.

Diagnosis

The diagnosis of PM and DM is based on history and physical examination, focusing on identifying proximal muscle weakness, and on the laboratory markers of muscle damage, especially creatine kinase. Electromyography reveals changes characteristic of myopathy, such as insertional irritability, positive sharp waves, fibrillations, and polyphasic small-amplitude muscle unit potentials. A common approach is to perform electromyography unilaterally and then obtain muscle biopsy specimens on the contralateral side in the muscle groups with the most abnormal findings to avoid the artifact of inflammatory cells in the muscle into which an electromyographic needle has been inserted. Alternatively, MRI can be used to direct muscle biopsy because it can demonstrate areas of active muscle inflammation, thus reducing the risk of sampling error. A muscle biopsy specimen with inflammatory cells, fibrosis, necrosis, regeneration, and atrophy of muscle cells confirms the diagnosis of PM. The biopsy specimen in patients with DM is more likely to demonstrate perivascular infiltration of T and B lymphocytes and perifascicular atrophy. With IBM, light microscopy shows basophil-rimmed vacuoles, and electron microscopy demonstrates cytoplasmic inclusions.

Treatment

Corticosteroids are the mainstay of treatment.[43] Initial daily prednisone doses of up to 100 mg are used and are tapered according to clinical response, serum creatine kinase levels, and side effects. Methotrexate and azathioprine are used as steroid-sparing agents, often being initiated simultaneously with high-dose corticosteroids or because of inadequate response. Up to 90% of patients with PM/DM have at least a partial response to treatment. IBM is also treated with prednisone, methotrexate, and azathioprine but with more modest expectations for clinical improvement.

In patients with resistant disease despite corticosteroids and methotrexate or azathioprine, successful treatment has been reported with the B cell–depleting drug rituximab. An alternative regimen includes IVIG infusions in addition to corticosteroid therapy. In patients with refractory PM or DM with interstitial lung disease, tacrolimus or cyclosporine can be used. Other alternative therapies for resistant disease include mycophenolate mofetil or cyclophosphamide.[43]

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Vasculitis

Carol A. Langford, MD, MHS *Cleveland, Ohio*

Vasculitis is defined by the presence of blood vessel inflammation. It can be observed in a wide variety of settings, which can be broadly grouped as secondary vasculitides, which occur in association with an underlying disease or trigger, or primary vasculitides, in which vasculitis is occurring for as-yet unknown causes. The primary systemic vasculitides comprise a range of disease entities that are uniquely identified by their clinical, histopathologic, and therapeutic characteristics. Individual diseases predominantly affect blood vessels of a particular size, which influences their clinical manifestations and has been used in their classification. The vasculitides can also differ in their severity, extending from self-limited illnesses to those that can be life-threatening in the absence of prompt initiation of treatment. Immunosuppressive agents are used to treat many vasculitic diseases. Although such approaches can be effective, the patient's long-term course can be influenced by organ damage from their initial presentation, disease relapses, and medication toxicity. Recent investigations have focused on understanding disease pathophysiology and the exploration of novel therapeutic approaches. (J Allergy Clin Immunol 2010;125:S216-25.)

Key words: *Vasculitis, arteritis, antineutrophil cytoplasmic antibody, granuloma, glucocorticoid, cyclophosphamide*

Abbreviations used

ANCA:	Antineutrophil cytoplasmic antibodies
AZA:	Azathioprine
CHCC:	Chapel Hill Consensus Conference
CNS:	Central nervous system
CSS:	Churg-Strauss syndrome
CYC:	Cyclophosphamide
GACNS:	Granulomatous angiitis of the central nervous system
GCA:	Giant cell arteritis
HCV:	Hepatitis C virus
HSP:	Henoch-Schönlein purpura
MPA:	Microscopic polyangiitis
MPO:	Myeloperoxidase
MTX:	Methotrexate
PACNS:	Primary angiitis of the central nervous system
PAN:	Polyarteritis nodosa
PMR:	Polymyalgia rheumatica
PR3:	Proteinase 3
TAK:	Takayasu arteritis
WG:	Wegener granulomatosis

Vasculitis is characterized by histologic evidence of blood vessel inflammation. When vasculitis occurs, it can lead to blood vessel stenosis/occlusion, causing organ ischemia or thinning of the blood vessel and resulting in aneurysm formation or hemorrhage. Vasculitis can be thought of in 2 broad categories: secondary vasculitides, in which blood vessel inflammation occurs in association with an underlying disease or exposure, or primary vasculitides, which are entities of unknown cause in which vasculitis is the pathologic basis of tissue injury. This review will focus on the clinical features, diagnosis, and treatment of the primary vasculitic diseases.

CLASSIFICATION

The first account of a patient who had a noninfectious vasculitis was made in 1866, when Kussmaul and Maier published a detailed report of a disorder characterized by nodular inflammation of the muscular arteries. They named this disease periarteritis nodosa, which later also became referred to as polyarteritis nodosa (PAN). The description of other necrotizing vasculitides followed, and in 1952, Zeek proposed the first classification system. The nomenclature and classification of the vasculitides has remained an evolving process as our knowledge about these diseases has grown. In 1990, the American College of Rheumatology introduced classification criteria for 7 forms of vasculitis to provide a standard way to describe groups of patients in therapeutic, epidemiologic, or other studies.[1] This was followed in 1994 by a proposal of uniform terms and definitions for the most common forms of vasculitis at the Chapel Hill Consensus Conference (CHCC; Table I).[2] Although these both represented advancements in standardization for the vasculitic diseases, they were not intended and should not be used for the purposes of diagnosing the individual patient.

PATHOPHYSIOLOGY AND ANTINEUTROPHIL CYTOPLASMIC ANTIBODIES

The pathophysiology of the vasculitides remains poorly understood and can vary between different diseases.[3,4] Clinical and laboratory-based evidence has supported the hypothesis that immunologic mechanisms appear to play an active role in mediating the necrotizing inflammation of blood vessels. Although the primary events that initiate this process remain largely unknown, recent investigators have brought us closer to understanding some of the critical pathways involved in disease and provided a rationale for the study of novel therapeutic agents (Table II).

Antineutrophil cytoplasmic antibodies (ANCA) have been a prominent focus of study in the vasculitides, not only for their possible influence in disease pathogenesis but also for their clinical applications. Two types of ANCA have been identified in patients with vasculitis: ANCA directed against the neutrophil serine protease proteinase 3 (PR3), which cause a

From the Center for Vasculitis Care and Research, Department of Rheumatic and Immunologic Diseases, Cleveland Clinic.

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Reprint requests: Carol A. Langford, MD, MHS, Center for Vasculitis Care and Research, Department of Rheumatic and Immunologic Diseases, Cleveland Clinic, 9500 Euclid Ave, A50, Cleveland OH 44195. E-mail: langfoc@ccf.org.

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TABLE I. Names and definitions of vasculitides adopted by the CHCC on the Nomenclature of Systemic Vasculitis

Large-vessel vasculitis	
Giant cell (temporal) arteritis	Granulomatous arteritis of the aorta and its major branches with a predilection for the extracranial branches of the carotid artery: often involves the temporal artery, usually occurs in patients older than 50 y, and often is associated with PMR.
TAK	Granulomatous inflammation of the aorta and its major branches: usually occurs in patients younger than 50 y.
Medium-sized vessel vasculitis	
PAN	Necrotizing inflammation of medium-sized or small arteries without glomerulonephritis or (classic PAN) vasculitis in arterioles, capillaries, or venules.
Kawasaki disease	Arteritis involving large, medium, and small arteries, and associated with mucocutaneous lymph node syndrome: coronary arteries are often involved, aorta and veins might be involved, and usually occurs in children.
Small-vessel vasculitis	
WG	Granulomatous inflammation involving the respiratory tract and necrotizing vasculitis affecting small- to medium-sized vessels (eg, capillaries, venules, arterioles, and arteries): necrotizing glomerulonephritis is common.
CSS	Eosinophil-rich and granulomatous inflammation involving the respiratory tract and necrotizing vasculitis affecting small- to medium-sized vessels often associated with asthma and eosinophilia.
MPA	Necrotizing vasculitis with few or no immune deposits affecting small vessels (ie, capillaries, [microscopic polyarteritis] venules, or arterioles): necrotizing arteritis involving small- and medium-sized arteries might be present, necrotizing glomerulonephritis is very common, and pulmonary capillaritis often occurs.
HSP	Vasculitis with IgA-dominant immune deposits affecting small vessels (ie, capillaries, venules, or arterioles): typically involves skin, gut, and glomeruli and is associated with arthralgias or arthritis.
Essential cryoglobulinemic vasculitis	Vasculitis with cryoglobulin immune deposits affecting small vessels (ie, capillaries, venules, or arterioles) and associated with cryoglobulins in serum: skin and glomeruli are often involved.
Cutaneous leukocytoclastic vasculitis	Isolated cutaneous leukocytoclastic angiitis without systemic vasculitis or glomerulonephritis.

Adapted from Jennette et al.[2]

cytoplasmic immunofluorescence pattern (cANCA) on ethanol-fixed neutrophils, and ANCA directed against the neutrophil enzyme myeloperoxidase (MPO), which result in a perinuclear immunofluorescence pattern (pANCA).[5] Because the methodology of testing can influence the interpretation, ANCA positivity determined by means of indirect immunofluorescence should be corroborated with antigen-specific testing for PR3 and MPO.

The strongest association of a disease with ANCA has been that between Wegener granulomatosis (WG) and PR3-cANCA. Although ANCA have also been described with variable frequency in other vasculitic diseases, in particular microscopic polyangiitis (MPA) and, to a lesser degree, Churg-Strauss syndrome (CSS) (Table III), many forms of vasculitis are not associated with circulating ANCA. ANCA can also be seen in association with other entities, including infection, inflammatory bowel disease, and other connective tissue diseases. In these settings ANCA are typically positive, as determined by means of indirect immunofluorescence, but negative for PR3, MPO, or both, as determined by means of antigen-specific testing, which emphasizes the importance of testing with both methodologies.

In patients with WG, the sensitivity of PR3-cANCA has been reported to be 28% to 92%, whereas specificity has been reproducibly high, ranging from 80% to 100%.[5] This raised the question as to whether ANCA measurement can be used in place of tissue biopsy for diagnosing WG. In patients with sinusitis, an active urine sediment, and pulmonary disease in which infection has been excluded, the predictive value of PR3-cANCA for WG can exceed 90%.[6] However, for other clinical presentations in which the prevalence of WG would be low, the predictive value of ANCAs is insufficient to justify the initiation of toxic therapy in the absence of a tissue diagnosis.

ANCA levels will vary during the course of WG, and from cohort studies, it was observed that patients with active disease had higher levels of ANCA compared with those who were in remission. However, changes in sequential ANCA measurements in an individual patient have not been found to be a reliable disease biomarker. In the largest prospective study published to date, increases in ANCA levels were not associated with relapse, and only 43% relapsed within 1 year of an increase in ANCA levels.[7] Given the toxicity of therapy, an increasing ANCA titer should not be used as the sole basis to start or increase immunosuppressive therapy.

TABLE II. Potential mechanisms of vessel damage in selected primary vasculitis syndromes

Immune complex formation
PAN-like vasculitis associated with hepatitis B
HSP
Cryoglobulinemic vasculitis
Production of ANCA
WG
MPA
CSS
Pathogenic T-lymphocyte responses and granuloma formation
GCA
TAK
WG
CSS

INDIVIDUAL VASCULITIC DISEASES

Giant cell arteritis

Giant cell arteritis (GCA), which has also been known as *temporal arteritis*, is a granulomatous, large-vessel vasculitis that preferentially affects the extracranial branches of the carotid artery.[8] It is the most common form of systemic vasculitis that

TABLE III. Clinical comparison of 4 forms of systemic vasculitis affecting small-sized vessels, medium-sized vessels, or both

Characteristic	WG	MPA	PAN	CSS
Upper airways disease	95%	No	No	50% to 60%
Pulmonary disease				
Asthma	No	No	No	90% to 100%
Radiographic nodule/infiltrates	70% to 85%	15% to 70%	No	40% to 70%
Alveolar hemorrhage	5% to 15%	10% to 50%	No	<5%
Glomerulonephritis	70% to 80%	75% to 90%	No	10% to 40%
Gastrointestinal	<5%	30%	14% to 53%	30% to 50%
Nervous system				
Peripheral	40% to 50%	60% to 70%	38% to 72%	70% to 80%
Central	5% to 10%	10% to 15%	3% to 30%	5% to 30%
Cardiac	10% to 25%	10% to 15%	5% to 30%	10% to 40%
Ocular	50% to 60%	<5%	<5%	<5%
Arthralgia/arthritis	60% to 70%	40% to 60%	50% to 75%	40% to 50%
Genitourinary	<2%	<5%	5% to 10%	<2%
Skin	40% to 50%	50% to 65%	28% to 60%	50% to 55%
ANCA				
PR3-cANCA	75% to 90%	10% to 50%	Rare	3% to 35%
MPO-pANCA	5% to 20%	50% to 80%	Rare	2% to 50%

affects human subjects, with an incidence of 18.8 cases per 100,000 persons in Olmsted County, Minnesota. GCA occurs almost exclusively in persons older than 50 years at a female/male ratio of up to 2:1 and is observed predominantly in persons of European ancestry.

GCA can be thought of as having 4 phenotypes that can occur alone, together, or sequentially and include cranial disease, polymyalgia rheumatica (PMR), systemic inflammatory disease, and large-vessel involvement. The most common presenting symptoms of GCA include headache, jaw or tongue claudication, scalp tenderness, weight loss, or fever (Table IV).[9] PMR, which is characterized by aching and morning stiffness in the proximal muscles of the shoulder and hip girdles, can occur in isolation but is also seen in 40% to 60% of patients with GCA. Cranial ischemic complications can occur as a result of vascular occlusion causing tissue infarction. Of these, the most dreaded complication is vision loss, which can occur in 14% of patients and is caused by optic nerve ischemia from arteritis involving vessels of the ocular circulation. Large-vessel involvement of the aorta or its primary branches occurs in 27% of cases and can present with limb claudication or complications related to an aortic aneurysm.[10]

TABLE IV. Clinical manifestations of GCA

Manifestation	Patients affected (%)
Headache	68
Weight loss/anorexia	50
Jaw claudication	45
Fever	42
Malaise/fatigue/weakness	40
PMR	39
Other musculoskeletal pain	30
Transient visual symptoms	16
Synovitis	15
CNS abnormalities	15
Fixed visual symptoms	14
Sore throat	9
Swallowing claudication/dysphagia	8
Tongue claudication	6

Adapted from Calamia and Hunder.[9]

The suspicion of GCA is raised by clinical features together with an increased erythrocyte sedimentation rate, which occurs in more than 80% of patients. The diagnosis is confirmed by means of temporal artery biopsy, which demonstrates a panmural mononuclear cell infiltration that can be granulomatous with histiocytes and giant cells. To increase yield, the length of the biopsy specimen should be at least 3 to 5 cm and sampled at multiple levels. Temporal artery biopsy specimens are positive in 50% to 80% of cases, and if the first biopsy specimen is negative, consideration should be given to a biopsy of the contralateral artery. In patients strongly suspected of having GCA, treatment should be instituted immediately to protect vision while a prompt temporal artery biopsy is being arranged. Although histologic changes can persist, a temporal artery biopsy should be performed as soon as possible after starting prednisone to obtain the best possible yield.

Glucocorticoids bring about a rapid improvement in cranial and systemic symptoms and prevent visual complications in patients with GCA. In one study the probability of loss of vision was only 1% after starting glucocorticoids.[11] Prednisone is usually initiated at a dose of 40 to 60 mg/d. After an initial dose of 60 mg/d, this can usually be reduced to 50 mg/d after 2 weeks and to 40 mg/d after 4 weeks. After that time, the dose is decreased by approximately 10% of the total daily dose every 1 to 2 weeks.[8] In patients with acute visual loss, 1 g/d methylprednisolone sodium succinate for 3 to 5 days is frequently given to protect remaining vision.[11] Isolated PMR can be effectively treated with 10 to 20 mg/d prednisone, with a rapid response to glucocorticoids being one of the diagnostic hallmarks of this disease.

The desire to identify effective treatment beyond prednisone has come from the recognition that 36% to 85% of patients have 1 or more side effects from this therapy.[12] Aspirin, 81 mg/d, has been found to reduce the risk of cranial ischemic complications and should be given together with prednisone in all patients who do not have a contraindication.[13,14] The ability of methotrexate (MTX) to decrease relapses and lessen glucocorticoids was examined in 2 randomized studies that yielded conflicting results.[15-17] At this time, neither the addition of MTX nor any other cytotoxic agent has been found to be uniformly effective in

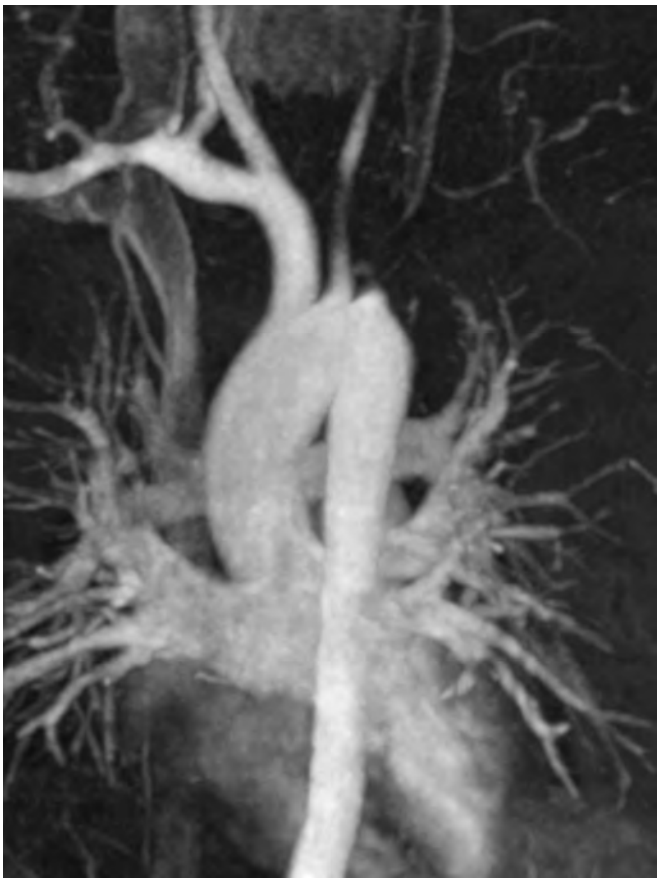

FIG 1. Magnetic resonance arteriogram in a patient with Takayasu arteritis demonstrating occlusion of the left subclavian artery coming off the aortic arch and severe stenosis of the left common carotid shortly after its origin from the arch.

reducing the use of prednisone sufficiently to decrease its risk of side effects. Randomized trials in patients with GCA and PMR did not find infliximab to provide benefit, and it is not recommended for use in these diseases.[18,19]

Acute mortality from GCA caused by stroke or myocardial infarction is uncommon, and overall, patients with GCA have a survival rate similar to that of the general population. However, thoracic aortic aneurysms might occur as a late complication of disease and can be associated with rupture and death.[10] Symptomatic relapses requiring increase or reinstitution of prednisone occur in at least 75% of patients.[16] Most patients require glucocorticoids for more than 2 years, with many receiving more than 4 years of treatment.

Takayasu arteritis

Takayasu arteritis (TAK) is a disease that affects the aorta, its main branches, and the pulmonary arteries in which granulomatous vasculitis results in stenosis, occlusion, or aneurysms of affected vessels.[20,21] Although it has been characterized as a disease affecting young women of eastern ethnicity, TAK has been observed throughout the world and can have varying clinical spectrums in different populations.

Patients with TAK can have systemic symptoms, features, or both of vascular injury. Systemic symptoms might be absent in 13% to 80% of patients and include fatigue, malaise, weight loss, night sweats, fever, arthralgias, or myalgias. Vascular symptoms are related to the location and nature of the lesion or lesions and the collateral blood flow. Hypertension occurs in 32% to 93% of patients and contributes to renal, cardiac, and cerebral injury.

A complete aortic arteriogram with visualization of all major branches is important in all patients in whom TAK is being considered as a means of diagnosis and determination of disease extent (Fig 1). The noninvasive nature of magnetic resonance and computed tomographic arteriography has made these modalities useful for serial vascular monitoring in patients with TAK, although catheter-directed dye arteriography remains valuable in providing central blood pressure measurements and precise assessment of luminal dimensions.

Disease activity is typically assessed based on clinical symptoms and signs, the erythrocyte sedimentation rate, and the presence of new arteriographic changes. However, these are not always reliable, and in one surgical series active arteritis was observed in 44% of patients who had been judged quiescent.[20]

Initial treatment of TAK usually consists of 1 mg/kg per day prednisone given for the first 1 to 3 months and then tapered to discontinuation over a 6- to 12-month period. Glucocorticoids relieve systemic symptoms in 25% to 100% of patients and might bring about an improvement in blood flow. Cytotoxic therapy is primarily used in patients who have persistent disease activity despite glucocorticoid treatment or in whom glucocorticoids cannot be tapered. MTX at 15 to 25 mg/wk in combination with glucocorticoids has been found to induce remission and minimize

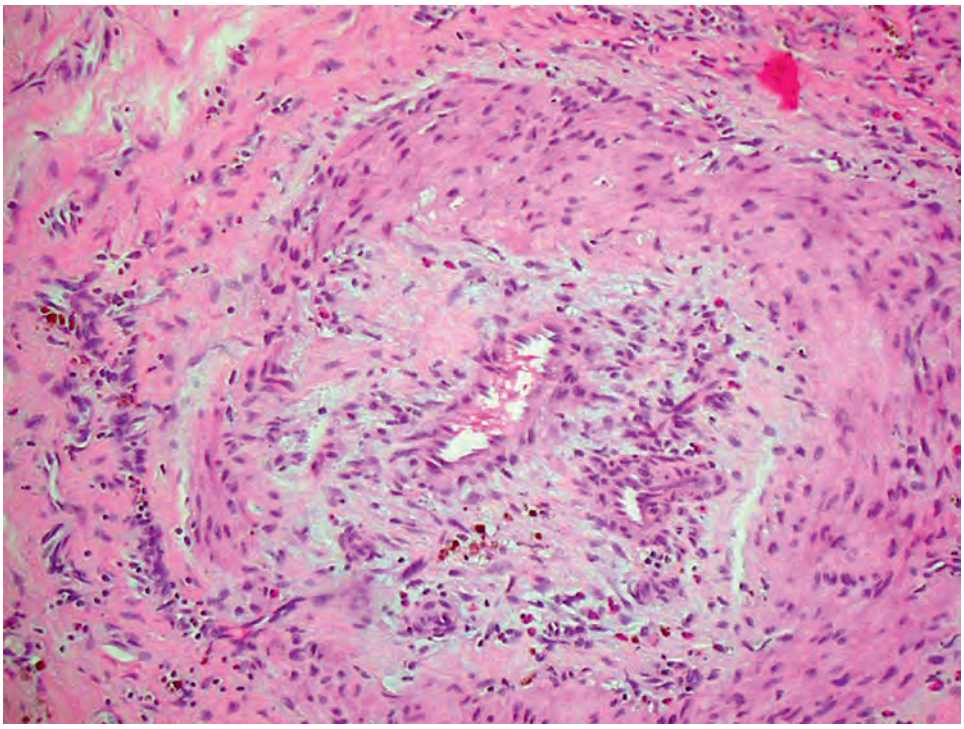

FIG 2. Medium-vessel vasculitis in a patient with PAN.

glucocorticoid therapy and toxicity.[22] Cyclophosphamide (CYC) should be reserved for patients with severe disease who cannot taper glucocorticoids and are unresponsive, intolerant, or unable to take MTX. Pilot studies have demonstrated favorable results with infliximab, but the efficacy of this therapy has not been proved from randomized trials.[23]

Nonmedical interventions have an important role in TAK in treating fixed vascular lesions that produce significant ischemia or aneurysms. The most frequent indications for surgical intervention include cerebral hypoperfusion, renovascular hypertension, limb claudication, repair of aneurysms, or valvular insufficiency. Surgical bypass has had the highest long-term patency rate, with vascular stents and angioplasty often occluding over time.[21] Nonmedical interventions should be performed in the setting of quiescent disease when possible to optimize outcome.

Patients with TAK have a low frequency of acute mortality, with the estimated 15-year survival rate being 83%. Relapses have been observed in 70% to 96% of patients, with sustained remission seen in only 28% of patients.[21]

PAN

Although PAN was the first described form of systemic vasculitis, changes in nomenclature have affected our interpretation of past literature, which included many patients who would now be considered to have MPA under the definitions of the CHCC. PAN, as it is currently defined, is estimated to be an extremely uncommon disease.

The most common clinical manifestations of PAN include hypertension, fever, musculoskeletal symptoms, and vasculitis involving the nerves, gastrointestinal tract, skin, heart, and nonglomerular renal vessels (Table III).[24] PAN is diagnosed by means of biopsy or arteriography. Biopsy specimens reveal necrotizing inflammation involving the medium-sized or small arteries, with abundant neutrophils, fibrinoid changes, and disruption of the internal elastic lamina (Fig 2). Dye arteriography is most often performed to examine the visceral and renal circulation, in which PAN would be suggested by the presence of microaneurysms, stenoses, or a beaded pattern brought about by sequential areas of arterial narrowing and dilation.

Patients with immediately life-threatening PAN affecting the gastrointestinal system, heart, or central nervous system (CNS) should be treated with 2 mg/kg per day CYC and glucocorticoids.[25] In patients in whom the disease manifestations do not pose an immediate threat to life or major organ function, glucocorticoids alone can be considered as initial therapy, with CYC being added in patients who continue to have evidence of active disease or who are unable to taper prednisone. The estimated 5-year survival rate of treated patients with PAN is 80%, with death being influenced by disease severity.[25] Relapses occur in 10% to 20% of patients.[25]

A PAN-like vasculitis can also be seen in patients infected with hepatitis B, hepatitis C, or HIV.[26] In the setting of hepatitis B or C, an antiviral agent should be part of the treatment regimen, with the goal being to contain viral replication and favor seroconversion. Patients might require glucocorticoids, alone or combined with CYC, together with plasmapheresis to initially gain control of the active vasculitis.[26]

WG

WG is a multisystem disease characterized by clinical disease involving the upper and lower respiratory tracts and kidneys with histologic evidence of granulomatous inflammation, vasculitis of the small- to medium-sized vessels, and a

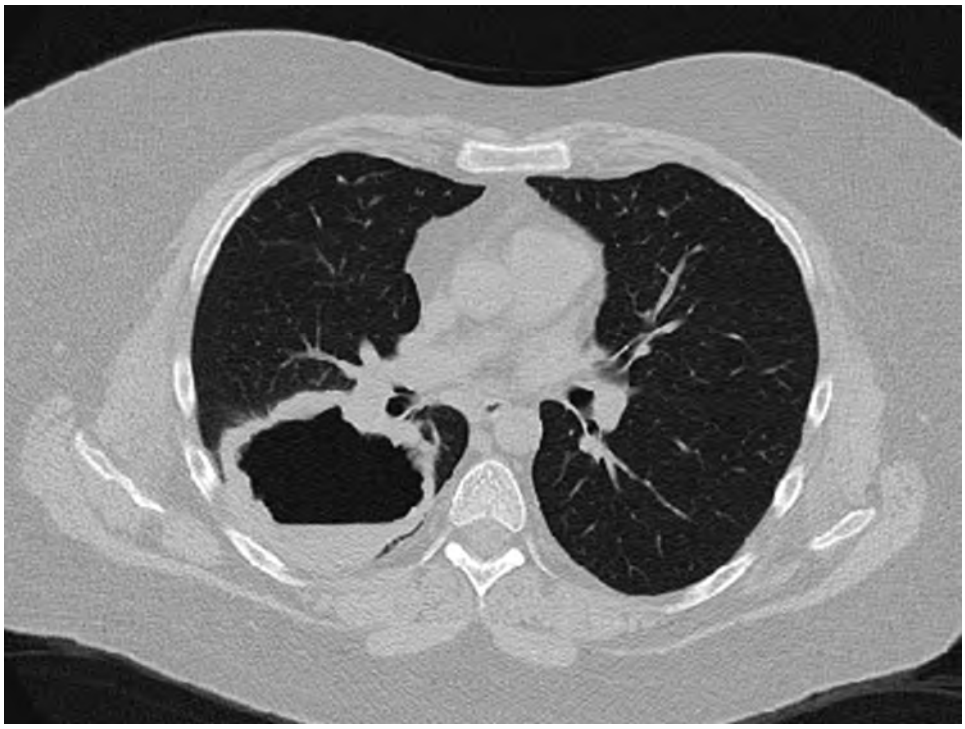

FIG 3. A large cavitary lung nodule seen on a computed tomographic scan in a patient with WG.

pauci-immune glomerulonephritis (Table III).[27] The disease can occur at any age and appears to affect men and women in equal proportions.

More than 90% of patients with WG first seek medical attention for upper airways symptoms, lower airways symptoms, or both. Nasal and sinus mucosal inflammation might result in cartilaginous ischemia with perforation of the nasal septum, saddlenose deformity, or both. Pulmonary radiographic abnormalities can include single or multiple nodules or infiltrates, cavities (Fig 3), and ground-glass infiltrates (Fig 4). Glomerulonephritis is present in 20% of patients at the time of diagnosis but develops in 80% at some point during the disease course. Renal involvement has the potential to be rapidly progressive but is asymptomatic, being detected by the presence of an active urine sediment with dysmorphic red blood cells and red blood cell casts.

The diagnosis of WG is usually based on biopsy results, with nonrenal tissues demonstrating the presence of granulomatous inflammation and necrosis, with necrotizing or granulomatous vasculitis.[27] Surgically obtained biopsy specimens of abnormal pulmonary parenchyma demonstrate diagnostic changes in 91% of cases. Biopsy of the upper airways is less invasive but demonstrates diagnostic features only 21% of the time. The characteristic renal histology is that of a focal, segmental, necrotizing, crescentic glomerulonephritis with few to no immune complexes. The clinical utility of ANCA in patients with WG is discussed in a separate section of this review.

Active WG is potentially life-threatening, and initial treatment requires glucocorticoids combined with a cytotoxic agent. Patients who have active severe WG should initially be treated with 2 mg/kg per day CYC in combination with prednisone at 1 mg/kg per day. After 4 weeks of treatment, if there is improvement, the prednisone is tapered and discontinued by 6 to 12 months. CYC is given for 3 to 6 months, after which time it is stopped and switched to a less toxic medication for remission maintenance. The 2 maintenance agents with which there has been the greatest body of data have been 20 to 25 mg/wk MTX[28] or 2 mg/kg per day azathioprine (AZA),[29,30] with a smaller experience existing with mycophenolate mofetil. In patients who have active but nonsevere disease, prednisone given together with 20 to 25 mg/wk MTX has been found to be effective at inducing and then maintaining remission.[31] In the absence of side effects, maintenance therapy is continued for at least 2 years, after which, if patients remain in remission, consideration can be made on an individual basis for tapering and discontinuation of therapy. In the setting of fulminant disease immediately threatening to life, 1 g/d methylprednisolone sodium succinate can be given in divided doses over a period of 3 days in combination with 3 to 4 mg/kg per day CYC for 3 days, after which time it is reduced to 2 mg/kg per day. Plasmapheresis has also been found to offer benefit in patients with rapidly progressive glomerulonephritis.[32]

Recognition of medication toxicity with strategies for monitoring and prevention play an important role in patient care (Table V).[33] CYC is associated with substantial toxicity, including bone marrow suppression, bladder injury, infertility, myeloproliferative disease, and transitional cell carcinoma of the bladder. Daily CYC should be taken all at once in the morning with a large amount of fluid, with monitoring of complete blood counts every 1 to 2 weeks. MTX should not be given to patients with impaired renal function (creatinine clearance, <35 mL/min) or chronic liver disease. Screening for the thiopurine methyltransferase genotype to detect patients at risk of severe neutropenia has become widely used before AZA initiation.

Recent investigations have explored the role of biologic agents. A randomized trial did not find etanercept to have any beneficial role in the induction or maintenance of WG.[34] A promising preliminary experience has been seen with rituximab (anti-CD20)

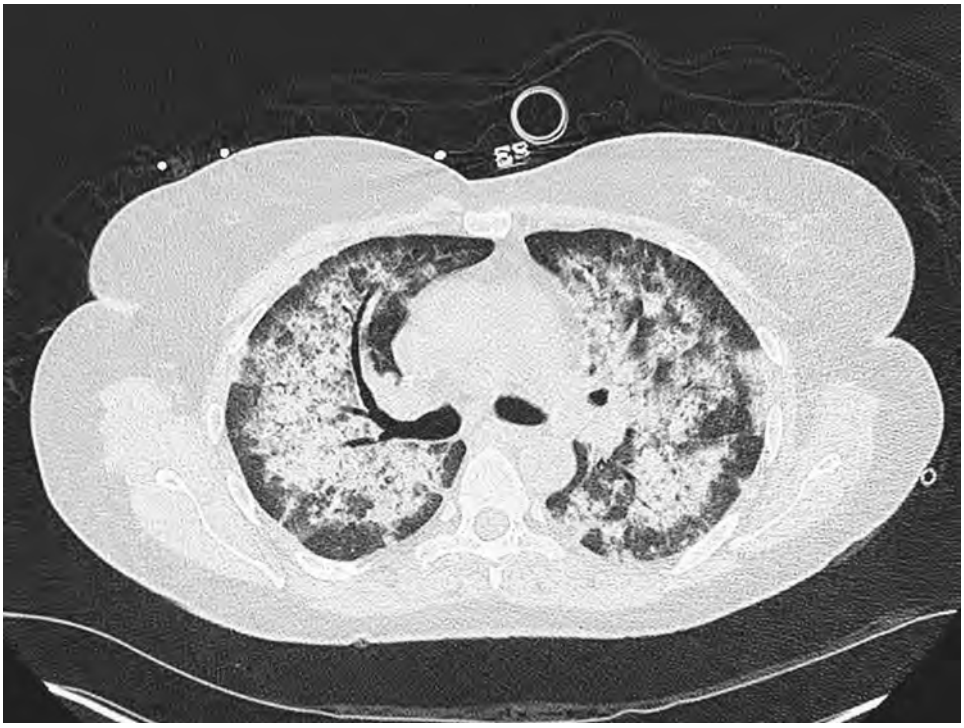

FIG 4. Computed tomographic scan demonstrating bilateral ground-glass infiltrates from alveolar hemorrhage as can occur in WG or MPA.

in patients with active severe WG or MPA.[35] This agent is currently being compared against CYC in a randomized, double-blind, placebo-controlled trial.

Before the development of treatment, patients with WG had a mean survival time of 5 months, with death occurring from pulmonary or renal failure. Current treatment regimens induce remission in 75% to 100% of patients with WG and result in the potential for long-term survival. However, relapse occurs in 50% to 70% of patients,[27] and disease-related organ damage is common.

MPA

As defined by the CHCC, MPA is characterized by necrotizing vasculitis with few or no immune deposits affecting small vessels. MPA has many similarities to WG, which has provided useful insights regarding diagnosis and management.

The cardinal features of MPA include glomerulonephritis, pulmonary hemorrhage (Fig 4), mononeuritis multiplex, and fever (Table III).[36] Approximately 75% to 85% of patients with MPA have circulating MPO-pANCA. The diagnosis of MPA is made by means of biopsy demonstration of necrotizing vasculitis of the small vessels or small- to medium-sized arteries in which granulomatous inflammation is absent. Biopsy specimens of lung tissue in the setting of pulmonary hemorrhage reveal capillaritis, hemorrhage into the alveolar space, and the absence of linear immunofluorescence, as would be seen in antiglomerular basement membrane antibody disease (Goodpasture syndrome). The renal histology is similar to that observed in WG in being a focal segmental necrotizing glomerulonephritis with few to no immune complexes.

Patients with life-threatening disease involving the lung, kidney, or nerve should initially be treated with 2 mg/kg per day CYC and 1 mg/kg per day prednisone, according to the schedule outlined for WG, followed by AZA or MTX for remission maintenance.[29,30] Patients with active nonsevere disease can be treated with MTX for remission and maintenance.[31]

In one series the estimated 5-year survival rate of MPA was 74%.[36] Like WG, MPA is a relapsing disease, with recurrences developing in at least 38% of patients.

CSS

CSS is a rare disease characterized by asthma, fever, hypereosinophilia, and systemic vasculitis.[37,38] It has been estimated to affect about 3 persons per million and has been observed in all ages equally between sexes.

CSS has been thought of as having 3 phases: a prodromal phase with allergic rhinitis and asthma, a phase characterized by peripheral eosinophilia and eosinophilic tissue infiltrates, and, ultimately, vasculitic disease that can involve the nerve, lung, heart, gastrointestinal tract, and kidney (Table III).[37] Although these phases are conceptually helpful, they might not be clinically identifiable in all patients, and they often do not occur in sequence. The histologic features of CSS include eosinophilic tissue infiltrates, extravascular "allergic" granuloma, and small-vessel necrotizing vasculitis. Vasculitis can be difficult to definitively establish, making clinical manifestations of particular importance in the diagnosis of CSS.

Prednisone, 1 mg/kg per day, is effective for many manifestations of CSS.[39] Asthma often persists after remission of the vasculitis and might limit the ability for pred to be tapered to complete discontinuation. Patients with life-threatening disease should be treated with glucocorticoids and 2 mg/kg per day CYC, as would be given for WG.[25]

Prognosis is influenced by the presence of severe disease involving sites such as the heart, gastrointestinal tract, CNS, and kidney.[37] CSS is characterized by frequent exacerbations of asthma, and relapses of vasculitic disease occur in at least 26%.[37]

Cutaneous vasculitis

Cutaneous vasculitis is the most commonly encountered vasculitic manifestation in clinical practice. Lesions most commonly consist of palpable purpura, although nodules and ulcerative lesions are also seen. Cutaneous vasculitis is histologically characterized by the presence of small-vessel inflammation within the dermis, often with leukocytoclasis.[40,41] Involvement of medium-sized vessels might be seen in cutaneous PAN.

In more than 70% of cases, cutaneous vasculitis occurs in the setting of an underlying process, such as a medication exposure, infection, malignancy, or connective tissue disease, or as a manifestation of a primary systemic vasculitis. A diagnosis of idiopathic cutaneous vasculitis should only be made after other causes have been ruled out. The course of idiopathic cutaneous vasculitis ranges from a single episode to multiple protracted recurrences. Progression to systemic vasculitis occurs infrequently.

If an underlying disease or exposure is identified, management of this process forms the primary basis for treating the cutaneous vasculitis. The therapeutic principle for idiopathic cutaneous vasculitis should be to use the least toxic yet effective regimen because there have been no standardized trials in this disease setting. Glucocorticoids are frequently used, but there remains no optimal dosage schedule. Other agents with which there has been anecdotal experience include nonsteroidal anti-inflammatory agents, antihistamines, dapsone, hydroxychloroquine, and colchicine. Cytotoxic agents should be reserved for select cases in which patients have severe disease that is unresponsive to other measures or when glucocorticoids cannot be tapered. CYC should rarely, if ever, be used to treat isolated cutaneous vasculitis.

Cryoglobulinemic vasculitis

Cryoglobulins are cold-precipitable monoclonal or polyclonal immunoglobulins that can occur in conjunction with a variety of diseases, including plasma cell or lymphoid neoplasms, chronic infection, and inflammatory diseases.[42] With the discovery of the hepatitis C virus (HCV), it became established that the majority of cases of cryoglobulinemia are related to HCV infection.[43] Cryoglobulinemia can be associated with a vasculitic illness characterized by palpable purpura, arthritis, weakness, neuropathy, and glomerulonephritis.[42] Although the presence of glomerulonephritis is associated with an overall poor prognosis, progression to end-stage renal failure is uncommon.

Combined therapy with IFN-α and ribavirin provide the best opportunity for improvement of HCV-associated cryoglobulinemic vasculitis, but long-term resolution is confined to patients who have a sustained virologic response.[44] Plasmapheresis has been used with brief responses but is not practical for long-term management. Glucocorticoids, CYC, AZA, and MTX have been applied, particularly in the case of severe disease.[44,45] Treatment with immunosuppressive drugs might transiently improve the inflammatory manifestations of cryoglobulinemic vasculitis but might also lead to an increase in HCV viremia. Although favorable results have been seen in case series with the use of rituximab, randomized controlled trials are needed to determine its efficacy.

TABLE V. Suggested toxicity laboratory monitoring schedule for prominent medications that are used in the treatment of certain vasculitides

Agent	Frequency	Investigation
Cyclophosphamide	Every 1-2 wk	CBC with differential Serum creatinine ESR Urinalysis
	Every 6-12 mo (even after treatment has been discontinued)	Urine cytology
Methotrexate	Every week during dose escalation and every 4 wk thereafter	CBC with differential Serum creatinine LFTs ESR Urinalysis
Azathioprine or mycophenolate mofetil	Every week for the first month, every 2 wk for the second month, and every 4 wk thereafter	CBC with differential Serum creatinine LFTs ESR Urinalysis

CBC, Complete blood count; *ESR*, erythrocyte sedimentation rate; *LFTs*, liver function tests.

Henoch-Schönlein purpura

Henoch-Schönlein purpura (HSP) is a small-vessel vasculitis that predominantly affects children.[46] Although adults can have HSP, 75% of cases occur before the age of 8 years. Two thirds of patients report an antecedent upper respiratory tract infection, although no predominant organism has been identified.

The 4 cardinal features of HSP are palpable purpura, arthritis, gastrointestinal involvement, and glomerulonephritis. Gastrointestinal manifestations include colicky abdominal pain, vomiting, and potentially intussusception. Renal disease, most often characterized by hematuria and proteinuria, is seen in 20% to 50% of affected children, with 2% to 5% progressing to end-stage renal failure. Less is known about HSP in adults, although several studies suggest that glomerulonephritis might be more severe and lead to renal insufficiency in up to 13% of cases.

The diagnosis of HSP is established by the pattern of clinical manifestations but can be less certain when other features precede the skin lesions. Skin biopsy reveals leukocytoclastic vasculitis with IgA deposition in blood vessel walls but is not required in most instances. Renal biopsy is rarely necessary for diagnosis but might have prognostic utility.

HSP is typically a self-limited condition that often does not require treatment. Glucocorticoids can lessen tissue edema, arthritis, and abdominal discomfort and decrease the rate of intussusception. However, they are of no proved benefit in skin or renal disease and do not appear to shorten the duration or lessen the likelihood of relapse.[47] Uncontrolled studies suggest that glucocorticoids in combination with a cytotoxic agent might be beneficial in patients with active glomerulonephritis and progressive renal insufficiency.

Outcome in patients with HSP is excellent, with disease-related death occurring in 1% to 3% of cases. Relapse occurs in up to 40% of cases, often within the first 3 months after the initial episode.

Kawasaki disease

Kawasaki disease is an acute vasculitis of childhood and represents the primary cause of acquired heart disease in children from the United States and Japan.[48] Eighty percent of children with Kawasaki disease are less than 5 years old, and boys are affected 1.5 times more often than girls.

Kawasaki disease begins as an acute febrile illness that is followed within 1 to 3 days by rash, conjunctival injection, and oral mucosal changes. Extremity changes characterized by brawny induration occur early in the disease, and 50% to 75% have cervical adenopathy. Together with fever, these 5 features constitute the criteria on which the diagnosis is based. Coronary artery lesions are responsible for most of the disease-related morbidity and mortality that occurs in patients with Kawasaki disease. Aneurysms appear 1 to 4 weeks after the onset of fever and develop in up to 25% of affected children who do not receive intravenous immunoglobulin.

Intravenous immunoglobulin, 2 g/kg, has been shown to prevent coronary aneurysm formation, lessen fever, and reduce myocardial inflammation.[49] Aspirin, 80 to 100 mg/kg per day, is given concurrently. An echocardiogram should be obtained at diagnosis and then at 2, 6, and 8 weeks to monitor for the development of coronary aneurysms. Children with multiple aneurysms, giant aneurysms, or coronary artery obstruction require close follow-up with serial ultrasonographic monitoring into adulthood and possible long-term anticoagulation.

Kawasaki disease has been reported to have a 3% mortality rate. Recurrences can develop in 1% to 3% of patients.

Behçet disease

Behçet disease is a multisystem inflammatory disease with manifestations that can affect arteries and veins of all sizes.[50,51] It occurs most commonly in 20- to 35-year-old persons of Asian and Eastern Mediterranean descent, with a male predominance in some ethnic populations.

Behçet disease is characterized by recurrent aphthous oral ulcers and at least 2 or more of the following: recurrent genital ulceration, eye lesions, cutaneous lesions, or a positive pathergy test result.[52] Among the most severe manifestations are gastrointestinal inflammation and ulceration, ocular inflammation that can lead to blindness, CNS disease with meningoencephalitis, and vascular involvement. Large venous or arterial lesions occur in 7% to 38% of patients and might include vessel thrombosis and occlusion, as well as pulmonary or peripheral artery aneurysms.

Treatment of Behçet disease is based on the disease manifestations. Aphthous lesions and mucocutaneous disease can be treated with topical or intralesional glucocorticoids, dapsone, or colchicine. Ocular and CNS disease require aggressive immunosuppression, with cyclosporine, AZA, and chlorambucil being the most commonly used agents. Preliminary studies have suggested favorable results with anti-TNF agents in patients with severe ocular inflammation.

Death occurs in 4% of patients with Behçet disease, generally as the result of gastrointestinal perforation, vascular rupture, and CNS disease. Behçet disease has the ability to remit and relapse frequently.

Primary angiitis of the CNS

Primary angiitis of the CNS (PACNS) is an uncommon disease in which patients have vasculitis isolated to the CNS without evidence of systemic vasculitis.[53,54] Granulomatous angiitis of the CNS (GACNS) represents about 50% of cases of PACNS and is a progressive disease that clinically presents with focal neurological deficits, chronic headache, or alterations in higher cortical function. More than 90% of patients with GACNS will have abnormal cerebrospinal fluid with mononuclear pleocytosis and increased protein with normal glucose levels. Results of magnetic resonance imaging are almost always abnormal, reflecting multifocal vascular insults of different ages. A cerebral arteriogram can reveal stenoses and ectasia in up to 40% of patients. Biopsy of tissue from the CNS is the diagnostic modality of choice, but results can be falsely negative in up to one fifth of patients. The diagnostic yield might be increased by taking biopsy specimens of both the leptomeninges and the underlying cortex.

In all instances a careful search must be made for processes of similar appearance, including atherosclerosis, infection, neoplasms, and drug-induced changes. An important diagnosis to distinguish from PACNS is reversible cerebral vasoconstrictive syndrome, which is characterized by a sudden onset of severe headache (thunderclap headache) with arteriographic cerebrovascular changes that have a similar appearance to vasculitis but that normalize within 12 months.[55]

GANCS is characteristically a fatal and progressive disorder but can respond to 1 mg/kg per day prednisone and 2 mg/kg per day CYC. For the 50% of patients with PACNS who do not have GACNS, treatment is based on the severity of disease manifestations and the rate of progression.

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Immunologic endocrine disorders

Aaron W. Michels, MD,[a] and George S. Eisenbarth, MD, PhD[b,c] *Aurora, Colo*

Autoimmunity affects multiple glands in the endocrine system. Animal models and human studies highlight the importance of alleles in HLA-like molecules determining tissue-specific targeting that, with the loss of tolerance, leads to organ-specific autoimmunity. Disorders such as type 1A diabetes, Graves disease, Hashimoto thyroiditis, Addison disease, and many others result from autoimmune-mediated tissue destruction. Each of these disorders can be divided into stages beginning with genetic susceptibility, environmental triggers, active autoimmunity, and finally metabolic derangements with overt symptoms of disease. With an increased understanding of the immunogenetics and immunopathogenesis of endocrine autoimmune disorders, immunotherapies are becoming prevalent, especially in patients with type 1A diabetes. Immunotherapies are being used more in multiple subspecialty fields to halt disease progression. Although therapies for autoimmune disorders stop the progress of an immune response, immunomodulatory therapies for cancer and chronic infections can also provoke an unwanted immune response. As a result, there are now iatrogenic autoimmune disorders arising from the treatment of chronic viral infections and malignancies. (J Allergy Clin Immunol 2010;125:S226-37.)

Key words: *Type 1 diabetes, HLA, autoantibodies, immunotherapy, Addison disease, autoimmune polyendocrine syndrome type 1, autoimmune polyendocrine syndrome type 2, Graves disease, polyendocrine autoimmunity, iatrogenic autoimmunity*

Multiple endocrine diseases are immune mediated and now predictable. Autoimmune disorders can cluster in individuals and their relatives. A family history of autoimmunity and screening for autoantibodies can identify at-risk subjects. Knowledge of these disorders and their disease associations can lead to earlier diagnosis and management, resulting in less morbidity and, in some cases, mortality. We will review endocrine organ-specific autoimmune diseases, autoimmune polyendocrine syndromes, and iatrogenic endocrine autoimmune disorders with an emphasis on immunopathogenesis, hopefully leading to immunotherapy for standard and experimental clinical care.

From the Departments of [a]Medicine, [b]Pediatrics, and [c]Immunology, Barbara Davis Center for Childhood Diabetes, University of Colorado Denver.

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Reprint requests: Aaron W. Michels, MD, Barbara Davis Center for Childhood Diabetes, University of Colorado Denver, 1775 Aurora Ct, MS B140, PO BOX 6511, Aurora, CO 80045. E-mail: Aaron.Michels@ucdenver.edu.

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Abbreviations used

ACTH:	Adrenocorticotropic hormone
AIRE:	Autoimmune regulator gene
APS-1:	Autoimmune polyendocrine syndrome type 1
APS-2:	Autoimmune polyendocrine syndrome type 2
CGMS:	Continuous glucose monitoring system
CTLA:	Cytotoxic T lymphocyte–associated antigen
FOXP3:	Forkhead box protein 3 gene
GAD:	Glutamic acid decarboxylase
GO:	Graves ophthalmopathy
HT:	Hashimoto thyroiditis
IA-2:	Islet-associated antigen (ICA512)
IPEX:	Immune dysfunction, polyendocrinopathy, enteropathy, X-linked
NOD:	Nonobese diabetic
POEMS:	Polyneuropathy, organomegaly, endocrinopathy, serum monoclonal protein, and skin changes
POF:	Premature ovarian failure
PTPN22:	Protein tyrosine phosphatase nonreceptor 22
TGA:	Tissue transglutaminase
TPO:	Thyroid peroxidase
TSH:	Thyroid-stimulating hormone
TSHR:	Thyroid-stimulating hormone receptor
TSI:	Thyroid-stimulating immunoglobulin
ZnT8:	Zinc T8 transporter

DIABETES MELLITUS

Background

Based on the American Diabetes Association classification, type 1A diabetes is the immune-mediated form of diabetes, whereas type 1B represents non–immune-mediated forms of diabetes with β-cell destruction, leading to absolute insulin deficiency.[1] There are additional forms of insulin-dependent diabetes with defined causes. Type 2 diabetes is overall the most common form of diabetes and is characterized by insulin resistance and less β-cell loss. In the United States, with a population of approximately 300 million, there are about 1.5 million persons with type 1A diabetes, and of these, approximately 170,000 are less than 20 years of age. The incidence of type 1A diabetes, similar to that of other immune-mediated diseases, such as asthma, is doubling approximately every 20 years.[2] Diabetes almost always develops in the setting of genetic susceptibility best defined by polymorphisms of HLA alleles.[3] Currently, there is no known cure for type 1A diabetes, and treatment for the disease consists of lifelong insulin administration. Immunotherapies aimed at preventing β-cell destruction at the time of clinical onset are actively being studied.

Genetic susceptibility

There are monogenic and polygenic forms of both immune-mediated and non–immune-mediated diabetes. Monogenic non-immune diabetes includes permanent neonatal diabetes mellitus, transient neonatal diabetes, and maturity-onset diabetes of the

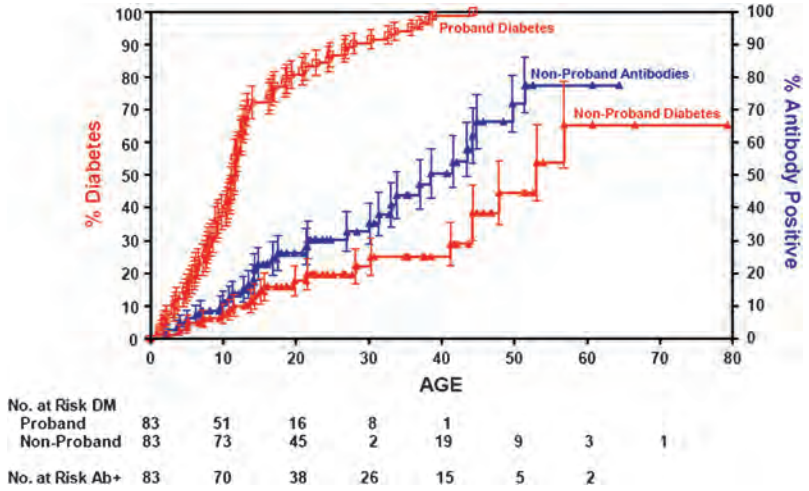

FIG 1. Progression to diabetes of initially discordant monozygotic twin siblings of patients with type 1 diabetes showing progressive conversion to diabetes. Approximately 80% become concordant for expression of anti-islet autoantibodies. *Ab*, antibody; *DM*, diabetes mellitus. Used with permission from Redondo et al.[5]

young. In general, children with these disorders lack all anti-islet autoantibodies, and therefore autoantibody assays can aid in identifying children to consider for genetic analysis. It is important to identify those who do not have type 1A diabetes, with estimates showing that approximately 1.5% of children presenting with diabetes have monogenic forms of diabetes. Several monogenic forms of diabetes are reported to be better treated with sulfonylurea therapy than with insulin (eg, mutations of the ATP-sensitive β cell–selective potassium channels and hepatocyte nuclear factor 1 alpha mutations),[4] and diabetes caused by glucokinase mutations requires no therapy at all. Approximately one half of permanent neonatal diabetes is due to mutations of the proinsulin gene that leads to β-cell loss. Two monogenic syndromes with immune-mediated diabetes are autoimmune polyendocrine syndrome type 1 (APS-1) and the immune dysfunction, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome, which will be discussed subsequently. The rest of this section will focus on the more common polygenic form of diabetes, type 1A diabetes.

Approximately 1 in 300 persons from the general population will have type 1A diabetes compared with 1 of 20 siblings of patients with type 1A diabetes. The concordance rate for monozygotic twins with type 1A diabetes is greater than 60% (Fig 1),[5] and a recent analysis of long-term twin data indicates that there is no age that an initially discordant monozygotic twin is no longer at risk.[5] Compared with monozygotic twins, initially discordant dizygotic twins are less often positive for anti-islet autoantibodies than nontwin siblings.[6] Offspring of a father with type 1A diabetes have a greater risk compared with offspring of a mother.[7]

The major determinant of genetic susceptibility to type 1A diabetes is conferred by genes in the HLA complex, which is divided into 3 regions: classes I, II, and III. Alleles of the class II genes, DQ and DR (and to a lesser extent DP), are the most important determinants of type 1A diabetes. These class II molecules are expressed on antigen-presenting cells (macrophages, dendritic cells, and B cells) and present antigens to $CD4^+$ T lymphocytes. DR3 and DR4 haplotypes are strongly associated with type 1A diabetes, with more than 90% of patients with type 1A diabetes possessing 1 or both of these haplotypes versus 40% of the US population.[8] Each unique amino acid sequence of DR and DQ is given a number. Because DRA does not vary, haplotypes can be defined by specific DRB, DQA, and DQB alleles. The highest-risk DR4 haplotypes vary at both DR (DRB1*0401, DRB1*0402, or DRB1*0405) and DQ (DQA1*0301 or DQA1*0302). DR3 haplotypes are almost always conserved with DRB1*03 combined with DQA1*0501 or DQB1*0201. The highest-risk genotype has both DR3 DQB1*0201/DR4 DQB1*0302. This genotype occurs in 30% to 50% of children with type 1A diabetes; approximately 50% of children with type 1A diabetes before the age of 5 years are DR3/4 heterozygotes versus 30% of young adults presenting with type 1A diabetes and 2.4% of the general population in Denver, Colorado. The excess risk for heterozygous haplotypes might be related to the transencoded DQ molecule (DQA and DQB encoded by different chromosomes) that can form in DR3/4 heterozygous individuals, namely DQA1*0501/DQB1*0302.[3]

In addition to HLA genes, many genetic loci contributing to diabetes risk have been implicated through genome-wide association studies (Fig 2),[9] which involves analyzing thousands of single nucleotide polymorphisms from large populations to find alleles associated with a particular disease. These alleles can increase risk (ie, high-risk alleles) or protect against a certain disease. Although HLA alleles confer the highest risk, multiple non-HLA genetic polymorphisms modify disease risk. The group of longer variable number of tandem nucleotide repeats 5′ of the insulin gene protects against diabetes. The decreased diabetes risk is associated with greater insulin message and resultant deletion of autoreactive T cells in the thymus.[10] Alleles of other identified genes primarily influence immune function, such as the protein tyrosine phosphatase nonreceptor 22, which regulates T-cell receptor signaling. The R620 W single amino acid change of protein tyrosine phosphatase nonreceptor 22 decreases T-cell receptor signaling (gain of function) and increases the risk of many autoimmune disorders, including type 1A diabetes, Addison disease, Graves disease, rheumatoid arthritis, and others.[11] Recently, a further genome-wide association study analysis identified 2 additional loci, *UBASH3A* and *BACH2*, associated with type 1A diabetes, loci having odds ratios of 1.16 and 1.13, respectively. Both of these loci were

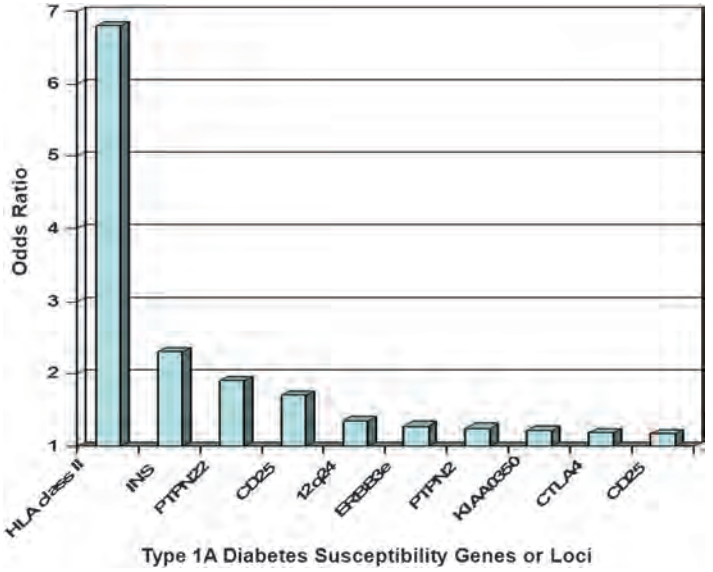

FIG 2. Summary of subsets of confirmed loci from whole-genome screens associated with type 1A diabetes and their odds ratios (from Teaching Slides at www.barbaradaviscenter.org). *CD25,* Also known as IL-2 receptor α chain; *ERBB3e,* an unidentified gene at 12q; *INS,* insulin; *KIAA 0350,* a lectin-like gene; *PTPN2,* protein tyrosine phosphatase nonreceptor 2; *PTPN22,* protein tyrosine phosphatase nonreceptor 22. Modified from Todd et al.[9]

validated from 2 separate populations, the Wellcome Trust Case-Control Consortium and the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications cohort.[12]

Environmental factors

The incidence of type 1A diabetes has been increasing dramatically at a rate of 3% to 5% per year for the last 50 years, and this rapid increase cannot be explained by genetics. There is no evidence that the epidemic of type 1A diabetes has leveled off in Finland, one of the countries with the highest incidence. The increase in the incidence of diabetes is most marked in children less than 5 years of age.[13] These observations suggest that environmental factors increasing diabetes risk have been introduced or factors decreasing risk have been removed. The Diabetes Autoimmunity Study of the Young found no evidence that bovine milk products, vaccinations, or enteroviral infections contribute to diabetes risk but has implicated decreased omega-3 fatty acid intake[14] and early cereal introduction. There appears to be a window from the ages of 4 to 6 months during which initial cereal introduction is not associated with an increased risk of islet autoimmunity because children who received initial cereal exposure at less than 3 months or after 7 months of age had a higher risk for having islet autoantibodies.[15] Omega-3 fatty acid supplementation in children with an increased genetic risk of diabetes was associated with a reduced risk of islet autoimmunity.[16] There have been several studies showing an association between vitamin D supplementation during the first year of life and a reduced risk of diabetes. A large prospective study of islet autoimmunity failed to confirm an association between type 1A diabetes risk and serum α- and γ-tocopherol concentrations, the principal forms of vitamin E in the diet and in human tissues.[17] Epigenetic influences are likely to be evaluated in future studies for diabetes risk. Hypermethylation has been associated with dietary supplements,[18] and there is discordance of methylation between monozygotic twins that increases with age.[19]

Pathogenesis

Type 1A diabetes is a T cell–mediated disease in which T cells infiltrate the islets, causing insulitis and ultimately β-cell death, decreased insulin production, and insulin-dependent diabetes. In a genetically susceptible subject, the development of diabetes occurs in stages (Fig 3). The presence of autoantibodies against islet cell antigens is the first indication for the development of diabetes, and patients retain sufficient β-cell mass initially for euglycemia. There are currently 4 autoantibodies used to predict the development of type 1A diabetes: antibodies against glutamic acid decarboxylase (GAD65), a tyrosine phosphatase–like protein (ICA512 also termed islet-associated antigen [IA-2]), insulin, and the recently discovered zinc T8 transporter (ZnT8).[20] After autoantibody development, there is progressive loss of insulin release as the autoimmune response progresses. During later stages, patients progressively experience subclinical hyperglycemia. In the final stages of development, decreased C-peptide levels cause patients to present with overt signs of diabetes.

Much of what we know about the autoimmune process in patients with diabetes comes from the study of animal models. The nonobese diabetic (NOD) mouse is a model in which type 1A diabetes and sialitis develop spontaneously, and the biobreeding rat has both diabetes and thyroiditis. As in human subjects, both models have alleles of genes within the MHC complex that influence antigen presentation to T lymphocytes and development of autoimmunity. One self-epitope in NOD mice has been shown to be a peptide of the insulin B chain, amino acids 9 to 23, that is recognized by autoreactive T lymphocytes.[21] During disease progression, activated T cells invade the pancreas and destroy β cells, resulting in insulin deficiency. Once β-cell destruction is initiated,

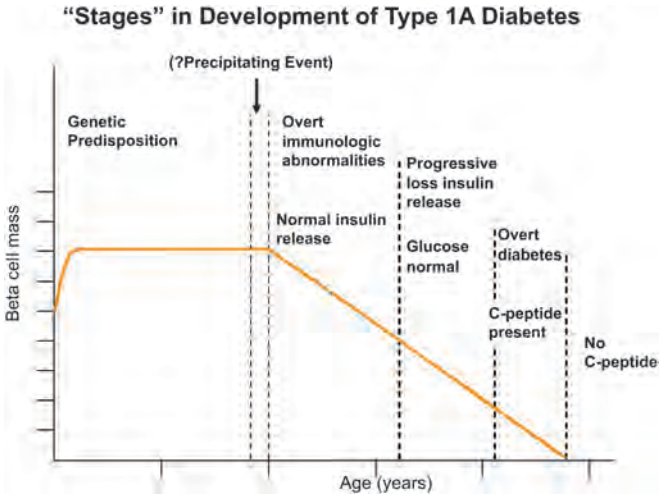

FIG 3. Hypothetic stages and loss of β cells in a patient progressing to type 1A diabetes (from Teaching Slides at www.barbaradaviscenter.org). Reproduced with permission from Eisenbarth GS.

other antigens become targets for the immune response, including islet glucose-related phosphatase, which is β cell specific.[22] Adoptive transfer of T cells from a diabetic mouse to an unaffected mouse results in diabetes. These animal models highlight the importance of having a genetic predisposition resulting in impaired immune regulation for autoimmunity to develop.

In human subjects a recent study examining postmortem pancreas specimens from patients with recent-onset type 1A diabetes showed a temporal pattern of immune cell infiltration. Initially, the inflammatory infiltrate consisted of $CD8^+$ cytotoxic T cells and macrophages.[23] $CD20^+$ B cells were not present in early insulitis but appeared in larger numbers as β-cell death progressed. $CD4^+$ T_H cells were present throughout insulitis but were not as prevalent as cytotoxic T cells and macrophages. The exact mechanism of β-cell death remains to be elucidated but likely involves cytokines, Fas/Fas ligand–induced cell death, and $CD8^+$ T cell–mediated cytotoxicity.

Diagnosis and prediction

The hallmark of type 1A diabetes is the autoimmune destruction of the pancreatic β cells by T cells. However, diagnosis is not made with T-cell assays because they are not as well developed or standardized compared with autoantibody assays. The lack of dependable assays for autoreactive T cells leads to the reliance on autoantibodies as the initial laboratory evaluation to detect an immune response against pancreatic β cells and distinguishing type 1 from type 2 diabetes.

There are several clinical scenarios in which the determination of autoantibodies is relevant. Children with transient hyperglycemia and adults presenting with hyperglycemia can present diagnostic dilemmas. Most children with transient hyperglycemia will remain healthy, but a subset will have type 1A diabetes; those with autoantibodies almost always progress to diabetes. Adults are much more likely to have type 2 diabetes, but 5% to 10% express islet autoantibodies, and these subjects progress more rapidly to insulin dependence. Independent of autoantibodies, routine monitoring of blood glucose levels is important to prevent the metabolic decompensation that can occur with many forms of diabetes.

Type 1A diabetes is a predictable disease. Autoantibodies against GAD65, insulin, IA-2, and the recently identified ZnT8 are current markers for type 1A diabetes. Relatives of patients with type 1A diabetes have been studied in detail. Expression of 2 or more autoantibodies (insulin, GAD65, or IA-2) has a positive predictive value of greater than 90% among relatives of a patient with type 1 diabetes (Fig 4)[25]; this holds true for the general population as well.[24] A single autoantibody carries a risk of approximately 20%.[25,26] With the addition of a fourth autoantibody, ZnT8, prediction will only improve because 26% of patients with autoantibody-negative type 1A diabetes in the Diabetes Autoimmunity Study in the Young study were found to have the ZnT8 autoantibodies.[27]

Treatment

The mainstay of type 1A diabetes treatment is insulin therapy. Over the last several years, multiple advances in insulin preparation, insulin delivery, and glucose monitoring have considerably improved treatment. Multiple analog insulins provide either a faster onset of action or longer duration and decrease the variability of insulin absorption. Insulin pumps allow for a more physiologic administration of insulin throughout the day. Continuous glucose monitoring systems (CGMSs) have been developed and measure interstitial fluid glucose levels. CGMSs assess blood glucose trends and provide alarms for high and low blood glucose levels. There is still a need to both calibrate the monitors and confirm low blood glucose values with fingerstick glucose determination. There is research underway with CGMS monitors controlling insulin delivery from insulin pumps.

Despite treatment with insulin therapy, long-term complications, including nephropathy, retinopathy, neuropathy, and cardiovascular disease, can result. Although the progress to complete insulin dependence occurs quickly after clinical onset, initially after diagnosis, the pancreas is able to produce a significant amount of insulin[28]; at this time, immunologic intervention can save β-cell function and reduce reliance on insulin. Two international networks conducting immunotherapy trials, the Immune Tolerance Network and TrialNet, have been established. Immunotherapies in patients with type 1A diabetes are aimed at altering the underlying immune process that results in β-cell loss. These therapies consist of agents that are non–antigen specific and those that are antigen specific. Non–antigen-specific therapies target various components of the immune system and include those directed against T cells (anti-CD3 mAbs, anti-thymocyte globulin, and cyclosporine), B cells (anti-CD20 mAbs), and other components of the immune system (Table I).[29-33] Antigen-based therapies are believed to mediate immune tolerance to antigens that result in autoimmunity to β cells. These therapies include vaccines with GAD, the B chain of insulin, and other insulin peptides (Table II).[34] Many of these therapies have reversed hyperglycemia in the NOD mouse, and several therapies show promise in altering the underlying immune process in human subjects.[35]

INSULIN AUTOIMMUNE SYNDROME

The insulin autoimmune syndrome, also known as Hirata disease, results from autoantibodies reacting with insulin. The diagnostic criteria include fasting hypoglycemia without evidence of exogenous insulin administration, high levels of serum immunoreactive insulin, and the presence of high-titer insulin

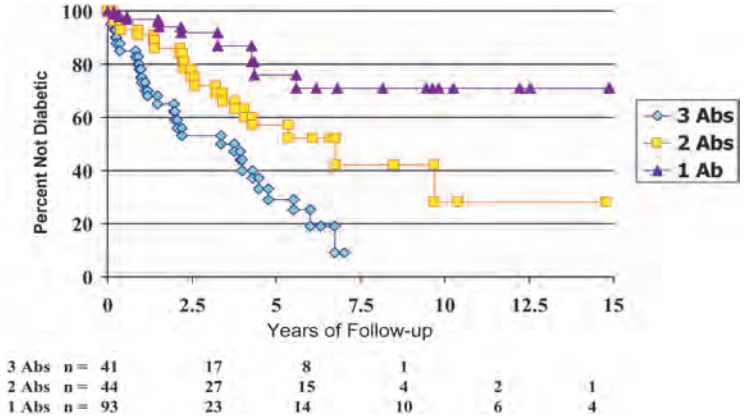

FIG 4. Progression to diabetes versus number of autoantibodies (GAD, ICA5112, and insulin). *Abs*, Antibodies. Used with permission from Verge et al.[25]

TABLE I. Non–antigen-specific immunotherapy trials for new-onset type 1A diabetes

Agent	Stage of development	Comments	References and links
Anti-CD3 mAbs	Phase II/III	Reduced insulin requirements out to 18 mo	Herold et al[85] and Keymeulen et al[86]
Anti-CD20 mAb (rituximab)	Phase II	Ongoing	www.clinicaltrials.gov/ct2/show/NCT00279305
Anti-thymocyte globulin	Phase I/II	Ongoing	www.clinicaltrials.gov/ct2/show/NCT00515099
Cyclosporine	Multiple trials	Successful remission but unacceptable side effects	Jenner et al[87]
Nicotinamide	Pilot	No effect	Elliott and Chase[88]
BCG	Pilot	No effect	Allen et al[89]
Anti-CD52 (Campath-1H)	Phase I	Withdrawn secondary to adverse events	www.clinicaltrials.gov/ct2/show/NCT00214214
CTLA-4 immunoglobulin (abatacept)	Phase I	Ongoing	www.clinicaltrials.gov/ct2/show/NCT00505375
Mycophenolate and daclizumab	Phase I	No effect	www.clinicaltrials.gov/ct2/show/NCT00100178

autoantibodies. Patients have recurrent and spontaneous hypoglycemia. The insulin autoantibodies can be monoclonal, from a B-cell lymphoma, or polyclonal. The polyclonal disorder is strongly associated with the DRB1*0406 haplotype and usually follows therapy with a sulfhydryl-containing medication, such as methimazole (an antithyroid drug used to treat Graves disease).[36]

AUTOIMMUNE THYROID DISEASE

Background

Autoimmune thyroid disease consists of Graves disease and Hashimoto thyroiditis (HT). It is very common, with a prevalence of 5% to 10% in the general population. Autoantibodies to various enzymes and proteins in the thyroid gland, thyroid peroxidase (TPO) and thyroglobulin, are the hallmark of autoimmune thyroid disease.

Graves disease

Background. Graves disease was first described by Robert Graves in 1835 as being associated with a goiter, palpitations, and exophthalmos. It is now know that the thyroid-stimulating hormone receptor (TSHR) is stimulated by autoantibodies, thyroid-stimulating immunoglobulins (TSIs), and that thyroid cells are activated, resulting in signs and symptoms of hyperthyroidism. The clinical manifestations of hyperthyroidism include a constellation of symptoms comprised of palpitations, tremor, heat intolerance, sweating, anxiety, emotional lability, and weight loss despite a normal to increased appetite. Extrathyroidal manifestations of Graves disease include Graves ophthalmopathy (GO) and dermatopathy (pretibial myxedema) with little understanding of the cause of these disease components.

Pathogenesis. Graves disease occurs in genetically susceptible individuals, with the HLA alleles contributing the greatest increase in risk, which is similar to type 1A diabetes. In white subjects HLA DR3 (HLA DRB1*03) and DQA1*0501 confer the highest risk,[37] whereas HLA DRB1*0701 is protective.[38] For monozygotic twins, the concordance rate is 20%; the rate is much lower for dizygotic twins, indicating other susceptibility factors for disease development. Female sex is the main risk factor, with smoking, lithium treatment, and low iodine consumption also associated with the disease.

Patients with Graves disease have diffuse lymphocytic infiltration of the thyroid gland and lose tolerance to multiple thyroid antigens, TSHR, thyroglobulin, TPO, and the sodium-iodine cotransporter. Autoantibodies develop when T cells recognize multiple epitopes of the TSHR.[39] The autoantibodies can either stimulate or inhibit thyroid hormone secretion. It is a balance of these autoantibodies toward thyroid cell activation that results in hyperthyroidism. Because of these various autoantibodies with differing functions, autoantibody concentrations cannot be correlated to thyroid hormone levels in patients with Graves disease. Fluctuating antibody titers can result in a thyroid yo-yo syndrome with alternating hyperthyroidism and hypothyroidism.[40] Although TSIs cause Graves disease, the serum antibody

TABLE II. Selected antigen-specific immunotherapy trials for type 1A diabetes

Agent	Stage of development	Comments	References and links
GAD65	Phase II/III	C peptide preserved at 18 mo	Ludvigsson et al[90]
Insulin B chain in incomplete Freund adjuvant	Phase I	Ongoing	www.clinicaltrials.gov/ct2/show/NCT00057499
Proinsulin-based DNA vaccine (BHT-3021)	Phase I	C peptide preserved at 12 mo	www.bayhilltx.com
Oral insulin	Prevention trial	Subset with insulin autoantibodies having a potential response	www.clinicaltrials.gov/ct2/show/NCT00419562

concentration can be low or undetectable in some patients. This could be due to assay insensitivity, misdiagnosis of the cause of hyperthyroidism, or intrathyroidal production of autoantibodies.[41]

GO is associated with Graves hyperthyroidism, but the 2 diseases can exist independently of one another. GO is clinically evident in 25% to 50% of patients with hyperthyroidism, and of these patients, 3% to 5% experience severe symptoms. GO results from increased orbital fat and muscle volume within the orbit. Histologic analysis of orbital tissue reveals lymphocytic infiltration and the inflammatory cytokines IL-4 and IL-10. Smoking is a strong risk factor for GO and worsens the symptoms of eye disease.

The association between Graves hyperthyroidism and GO suggests that the 2 disorders result from an autoimmune process to 1 or more antigens from the thyroid and orbit. Orbital fibroblasts are thought to be the antigenic target in GO. TSHR mRNA and protein expression in orbital fibroblasts has been documented in both healthy subjects and patients with GO.[42] It is possible that a form of TSHR or similar protein is expressed in the orbit and might serve as a cross-reactive target for TSIs.

Diagnosis. Graves disease is the most common cause of hyperthyroidism. Diagnosis is made with clinical and biochemical manifestations of hyperthyroidism. Thyroid function tests show low to suppressed thyroid-stimulating hormone (TSH) levels and increased thyroxine and triiodothyronine levels. Diagnosis is confirmed with a radioactive iodine uptake and scan (only tested in nonpregnant, non–breast-feeding patients) showing increased homogenous uptake. TSI levels aid in the diagnosis but are not confirmatory because patients can have Graves disease without autoantibodies present. TSI autoantibodies measured in the third trimester of pregnancy are a good predictor of neonatal Graves disease. During pregnancy, thyroid autoantibodies generally decrease, presumably because of secretion of trophoblast factors that are immunosuppressive.

Treatment. Treatment of Graves disease has changed little over the last 50 years. Treatment options include antithyroid drugs, radioactive iodine, and surgery. Antithyroid drugs block thyroid hormone synthesis, but the majority of patients experience relapses with discontinuation of therapy. Radioactive iodine ablation is the preferred treatment method in the United States. Ablation generally results in iatrogenic hypothyroidism, requiring lifelong thyroid hormone replacement. Anti-CD20 mAb has been tried in a small number of patients with Graves disease. Twenty patients received methimazole for Graves disease and were rendered euthyroid. Ten patients received anti-CD20 mAb infusions during the final 3 weeks of methimazole treatment. Fewer patients receiving anti-CD20 antibody treatment relapsed at 1 month (6/10) than those who did not (8/10).[43]

Immunotherapy trials for GO show more promise than for Graves disease alone. Agents such as anti-CD20 mAbs and anti–TNF-α mAbs have been used. In a pilot study anti-CD20 mAbs improved proptosis, soft tissue changes, and eye motility in 7 patients with moderate-to-severe GO. None of the treated patients followed to 1 year had a relapse. This was compared with 15 of 20 patients responding to methylprednisolone therapy; 10% had experienced relapses at the conclusion of the study.[44] Larger randomized controlled trials are needed to confirm these results.

HT

Background. HT is the most common endocrine autoimmune condition, affecting up to 10% of the general population. It is characterized by a gradual loss of thyroid function, goiter, and T-cell infiltration on histology. HT affects women more frequently than men, with a sex ratio of 7:1.

Pathogenesis. HT occurs in genetically susceptible populations but lacks a strong association with HLA. Mutations in the thyroglobulin gene[45] and cytotoxic T lymphocyte–associated antigen (CTLA) 4 are associated with disease.[46] T cells play a crucial role in disease pathogenesis by reacting with thyroid antigens and secreting inflammatory cytokines. Autoantibodies develop in patients with HT to TPO, thyroglobulin, and the TSHR. It is believed that these autoantibodies are secondary to thyroid follicular cell damage induced by T cells. TPO is the major autoantigen, and autoantibodies to TPO are closely associated with disease activity.

Diagnosis and treatment. The diagnosis and treatment of HT has changed very little over the last several decades. Diagnosis is made based on clinical (fatigue, weakness, cold intolerance, weight gain, constipation, dry skin, depression, and growth failure or delayed puberty in children) and biochemical manifestations of hypothyroidism. Thyroid function tests show an increased TSH level and a low thyroxine and triiodothyronine levels. Other causes of thyroiditis (postpartum, acute, subacute, and silent) need to be excluded. Treatment is with lifelong thyroxine replacement with a goal of normalizing the TSH level. Continuous monitoring of thyroid function is needed to avoid overreplacement, which can lead to premature osteoporosis and cardiac arrhythmias. Fine-needle aspiration of thyroid nodules is recommended to rule out thyroid cancer because differentiated thyroid cancer is associated with a favorable prognosis and low recurrence once detected.

ADDISON DISEASE

Background

Thomas Addison described a group of patients affected with anemia and diseased adrenal glands in 1849. Addison disease is a chronic disorder of the adrenal cortex resulting in decreased production of glucocorticoids, mineralocorticoids, and

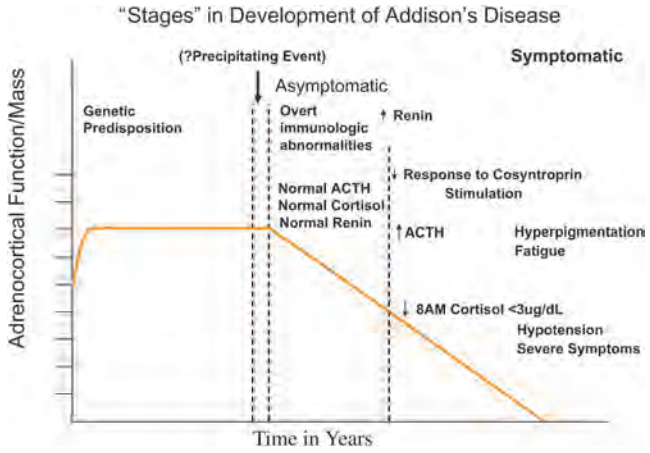

FIG 5. Stages in the development of Addison disease.[42] Adrenocortical function is lost over a period of years. In the first stage genetic predisposition is conferred by a patient's HLA genotype. In the second stage events that precipitate anti-adrenal autoimmunity occur but are currently unknown. In the third stage, which involves presymptomatic disease, 21-hydroxylase autoantibodies predict future disease. Finally, in the fourth stage overt Addison disease develops. An increased plasma renin level is one of the first metabolic abnormalities to occur and is followed by the sequential development of other metabolic abnormalities (a decreased cortisol level after cosyntropin stimulation, an increased corticotropin level, and a decreased basal cortisol level). Finally, there are severe symptoms of adrenal insufficiency, such as hypotension.

androgens. There is increased secretion of adrenocorticotropic hormone (ACTH) from the pituitary gland. Histologic examination of adrenal glands from patients with autoimmune adrenal insufficiency reveals fibrosis with a mononuclear cell infiltrate, plasma cells, and rare germinal centers.[47] The most common cause of primary adrenal insufficiency in developed countries is autoimmunity (70% to 90%), with tuberculosis the second most common cause (10% to 20%). Addison disease can be present in 3 clinical forms: part of syndromes termed APS-1 and autoimmune polyendocrine syndrome type 2 (APS-2) and as an isolated disease.

Pathogenesis and genetics

Similar to type 1A diabetes, Addison disease also can be divided into stages of disease progression. Genetically predisposed individuals have autoantibodies to the 21-hydroxylase enzyme and eventually lose the ability to produce cortisol (Fig 5).[48] Autoantibodies against 21-hydroxylase are present in more than 90% of patients with recent-onset disease. Susceptibility is conferred through the genes encoding the class II MHC, and as is the case with type 1A diabetes, there is a strong association with the DR3 haplotype. The highest-risk genotype, occurring in 30% of patients with Addison disease, consists of DR3/4, DQ2/DQ8,[49] and in this case the DRB1*0404 DR4 subtype confers highest risk on DR4 haplotypes. The MHC class I–related molecule A 5.1 allele, an atypical HLA molecule (MHC class I–related gene A), is also associated with genetic risk.[50] Polymorphisms of the MHC class I–related molecule A gene are based on the number of triplicate GCT repeats in exon 5. The translated protein interacts with the NKG2D receptor, which is important for thymic maturation of T cells.[51] NKG2D can also regulate the priming of human naive $CD8^+$ T cells.[52] The allele, designated 5.1, is associated with the insertion of a base pair, which results in a premature stop codon and loss of the membrane-binding region of the protein.

Diagnosis and treatment

The diagnosis of Addison disease is made in symptomatic patients with high levels of ACTH and a deficiency of cortisol or when serum cortisol levels do not increase after an ACTH stimulation test in the presence of increased basal ACTH levels; 21-hydroxylase autoantibodies are usually (>90%) present. The clinical manifestations are subtle (weakness, fatigue, anorexia, orthostasis, nausea, myalgias, and salt craving), and a high index of suspicion is necessary to diagnose adrenal insufficiency before an adrenal crisis. We recommend screening patients with type 1A diabetes, hypoparathyroidism, and polyendocrine autoimmunity for 21-hydroxylase autoantibodies. If present, yearly monitoring with an ACTH stimulation test is performed to allow early diagnosis and prevent an adrenal crisis. Treatment is with lifelong glucocorticoids and mineralocorticoids, with counseling about the need for stress-dose steroids for illnesses and before surgical procedures. Forty percent to 50% of patients with Addison disease will have another autoimmune disease, necessitating lifelong monitoring for associated autoimmune conditions.

IDIOPATHIC HYPOPARATHYROIDISM

Background

Idiopathic hypoparathyroidism results from a deficiency of parathyroid hormone, which regulates the serum calcium concentration and does not have an identifiable cause. This disease is a common component of APS-1 in infants and young children. It also occurs sporadically in adults, most often affecting female subjects with HT. An autoimmune basis for idiopathic hypoparathyroidism has been suggested because of its association with other autoimmune conditions.

Pathogenesis

Hypocalcemia results from parathyroid hormone deficiency. Recent work by Alimohammadi et al[53] identified a parathyroid autoantigen, NACHT leucine-rich repeat protein 5, in patients with APS-1. NACHT leucine-rich repeat protein 5 autoantibodies were identified in patients with APS-1 with hypoparathyroidism and not in healthy subjects or subjects with other autoimmune disorders. Autoantibodies to the calcium-sensing receptor on parathyroid glands have been described as well and can activate the receptor, thereby causing decreased production of parathyroid hormone.[54]

Diagnosis and treatment

Idiopathic hypoparathyroidism is diagnosed when no other causes of hypocalcemia and hypoparathyroidism can be identified. Treatment is with calcium and magnesium supplementation. To absorb calcium, active 1,25 dihydroxyvitamin D needs to be administered with calcium, and frequent monitoring of serum calcium levels is required.

PREMATURE OVARIAN FAILURE

Background

Premature ovarian failure (POF) is defined as amenorrhea, increased gonadotropin levels, and hypoestrogenism before age

40 years. POF can occur before or after puberty. Girls should begin puberty by age 13 years and menstruate within 5 years after the onset of puberty. Two distinct clinical scenarios have been identified.

Idiopathic POF with adrenal autoimmunity. Approximately 10% of female subjects with Addison disease will have POF. Steroid cell autoantibodies, directed against the enzymes 21-hydroxylase or 17-hydroxylase, cross-react with theca interna/granulosa layers of ovarian follicles. The presence of these autoantibodies correlates with the histologic diagnosis of autoimmune oophoritis.[55] MHC class II is expressed on granulosa cells of patients with POF and might potentiate a local T-cell autoimmune response.[56]

Idiopathic POF with exclusive manifestations of ovarian autoimmunity. The vast majority (>90%) of women with POF do not have Addison disease or steroid cell autoantibodies, calling into question the autoimmune component of the disease. Thyroid autoimmunity is present in about 14% of these patients. Approximately 10% of patients with isolated POF and without Addison disease will have numerous ovarian follicles intact. These patients are categorized as having resistant ovary syndrome that is insensitive to ovulation induction with exogenous gonadotropins.

Treatment

Currently, there is no treatment available to induce ovarian function or stop progression of autoimmune ovarian destruction. Treatment is focused on treating symptoms of estrogen deficiency and maintaining bone health to prevent osteoporosis. Infertility can be treated with *in vitro* fertilization with donor eggs. However, there is a relapsing and remitting component to the underlying autoimmunity, and occasionally, conceptions can be achieved. Screening for associated autoimmune conditions (type 1A diabetes, Addison disease, and thyroid autoimmunity) should be considered in patients with idiopathic POF.

LYMPHOCYTIC HYPOPHYSITIS

Background

Lymphocytic hypophysitis is a rare inflammatory lesion of the pituitary gland. Approximately 500 cases have been reported in the literature since the initial report in 1962.[57] This condition is more common in female subjects and affects women during later pregnancy and the postpartum period (eg, postpartum hypophysitis). It is strongly associated with other autoimmune disorders. Of note, ipilimumab, an mAb that blocks CTLA-4, is an immunologic therapy used in oncologic clinical trials and has induced hypophysitis.[58]

Pathogenesis

The morphologic features of hypophysitis resemble those of other autoimmune endocrinopathies. The absence of granulomas on histology distinguishes this condition from the granulomatous hypophysitis seen in association with sarcoidosis, tuberculosis, and syphilis. Anti-pituitary antibodies have been isolated in a minority of patients with disease.

Diagnosis and treatment

Presenting symptoms include fatigue, headache, and visual field deficits. Diagnosis is confirmed by means of histologic examination of a pituitary biopsy specimen. Anterior pituitary hormone deficits are common, and hormone replacement is indicated. High-dose glucocorticoid pulse therapy has been used for treatment.[59]

AUTOIMMUNE POLYENDOCRINE SYNDROMES

Background

The autoimmune polyendocrine syndromes are a constellation of disorders characterized by multiple autoimmune disorders, including endocrine gland failure or hyperactivity (Graves disease). Some of the components of the syndromes have been described previously in the review. The syndromes include APS-1; APS-2; IPEX syndrome; polyneuropathy, organomegaly, endocrinopathy, serum monoclonal protein, and skin changes (POEMS) syndrome; non–organ-specific autoimmunity (eg, lupus erythematosus) associated with anti-insulin receptor antibodies; thymic tumors with associated endocrinopathy; and Graves disease associated with insulin autoimmune syndrome. APS-1–, APS-2–, IPEX-, POEMS syndrome–, and diabetes-associated autoimmune disorders will be discussed in further detail.

APS-1

Background. APS-1/APECED (autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy) is a rare disorder generally seen in infants, and the diagnosis is made when a child has 2 or 3 of the following: mucocutaneous candidiasis, hypoparathyroidism, or Addison disease. Mucocutaneous candidiasis involving the mouth and nails is usually the first manifestation followed by the development of hypotension or fatigue from Addison disease or hypocalcemia from hypoparathyroidism. APS-1 is associated with other autoimmune disorders (type 1A diabetes, vitiligo, alopecia, hepatitis, pernicious anemia, and primary hypothyroidism) and asplenism.

Pathogenesis/genetics. APS-1 is due to a mutation in the autoimmune regulator gene *(AIRE)*, which is transmitted in an autosomal recessive manner. The *AIRE* gene encodes a transcription factor needed for the expression and presentation of self-antigens to developing lymphocytes in the thymus.[60] More than 40 mutations in *AIRE* have been described,[61] and when mutations are present, tolerance is lost to multiple self-antigens. The resulting autoreactive T cells that escape deletion in the thymus have the ability to destroy multiple specific tissues, producing a phenotype of multiple autoimmune disorders. Animal models with a knockout of the *AIRE* gene result in widespread autoimmunity, although the phenotype is mild with lymphocytic infiltration of the liver and atrophy of the adrenal and thyroid glands. The majority of mice also exhibit autoantibodies to the pancreas, adrenal glands, testes, and liver.[62] Human studies of isolated autoimmune disorders, such as Addison disease occurring without evidence of APS-1, have not found mutations in the *AIRE* gene.[63]

Diagnosis. Diagnosis is based on the presence of specific autoimmune disorders and mucocutaneous candidiasis. The known *AIRE* gene mutations can now be screened. Meager et al[64] recently reported that patients with APS-1 have multiple anti-interferon antibodies, with IFN-ω–reactive autoantibodies present in 100% of patients; assays for such autoantibodies might aid in rapid diagnosis.

Treatment. Hormone replacement is the mainstay of treatment for the endocrinopathies present in patients with APS-1.

Mucocutaneous candidiasis needs to be treated aggressively and monitored for recurrence because it can occur anywhere along the gastrointestinal tract. Untreated disease can lead to the development of epithelial cancers. Asplenism needs to be identified, and vaccinations against pneumococcus, meningococcus, and *Haemophilus influenzae* need to be administered.

A high clinical suspicion for other autoimmune disease needs to be maintained in patients with APS-1 and their relatives. Patients with APS-1 need to be followed at a center with experience monitoring and caring for these patients. Siblings need to be followed closely, and consideration should be given to screening for anti–IFN-ω autoantibodies. Recommendations are to see these patients at 6-month intervals and screen for autoantibodies.[65] If autoantibodies are present without the associated disease, functional testing is indicated. Patients with 21-hydroxylase antibodies are often followed with annual ACTH levels, 8 AM cortisol levels, and cosyntropin stimulation testing unless symptoms or signs warrant more frequent monitoring. The presence of islet cell autoantibodies warrants glucose tolerance testing to detect disease before overt clinical symptoms and education related to the symptoms of diabetes along with home glucose monitoring.

APS-2

Background. APS-2, also known as Schmidt syndrome, is the most common autoimmune polyendocrine syndrome. APS-2 has Addison disease as its defining component with either autoimmune thyroid disease or type 1A diabetes in conjunction. Women are typically affected at a higher rate than male subjects. Other diseases less commonly associated with APS-2 include celiac disease, vitiligo, pernicious anemia, myasthenia gravis, stiff man syndrome, and alopecia. Familial aggregation was demonstrated by a study looking at 10 families with APS-2, and one in 7 relatives had an undiagnosed autoimmune disease, the most common being thyroid disease.[66] Diseases can develop years to decades apart, making knowledge of the syndromes necessary to detect disease and provide treatment before morbidity and mortality. Table III compares APS-1 and APS-2.[67]

Pathogenesis/genetics. The genetics of APS-2 are governed by the HLA haplotypes, which confer disease risk to multiple autoimmune disorders. The DR3, DQA1*0501, DQB1*0201 haplotype increases the risk for type 1A diabetes, Addison disease, and celiac disease. The DR4 haplotype of patients with all 3 of these diseases is associated with DQA1*0301, DQB1*0302. If a patient with type 1A diabetes has the DRB1*0404 allele and express 21-hydroxylase antibodies, there is a 100-fold increase in the risk of Addison disease.[68] Autoimmune diseases result from a failure to develop or maintain tolerance along with a genetic predisposition, MHC alleles, controlling specific disease development. Multiple autoimmune disorders develop when tolerance is lost to a number of self-antigens.

Diagnosis and treatment. Similar to APS-1, treatment of APS-2 focuses on identifying and treating the underlying autoimmune conditions. Autoimmune thyroid disease is very common. It is prudent to screen patients with type 1A diabetes and those with Addison disease with a yearly TSH. We recommend screening patients with type 1A diabetes for 21-hyroxylase and tissue transglutaminase (TGA) autoantibodies. The optimal screening interval is not defined; however, autoantibodies can develop at any age, and repeat testing is necessary in the case of a negative test result. Relatives of patients with APS-2 need to be monitored closely.

TABLE III. Comparison of APS-1 and APS-2

APS-1	APS-2
Onset infancy	Older onset
Siblings	Multiple generations
AIRE gene mutated	
Not HLA associated	DR3/4 associated
Immunodeficiency	No defined immunodeficiency
Asplenism	
Mucocutaneous candidiasis	
18% Type 1A diabetes	20% Type 1A diabetes
100% Anti-interferon antibodies	

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IPEX syndrome

Background. The rare IPEX syndrome is caused by mutations in the forkhead box protein 3 gene *(FOXP3)*, resulting in absent or dysfunctional regulatory T cells.[69] Clinically, it presents during the first few months of life with dermatitis, growth retardation, multiple endocrinopathies, and recurrent infections. Affected neonates have overwhelming autoimmunity, including type 1A diabetes, developing as early as 2 days of age.

Pathogenesis/genetics. To date, 20 mutations in *FOXP3* have been identified in patients with IPEX syndrome.[70,71] Most of these mutations occur in the forkhead (winged-helix) domain and leucine zipper region, resulting in impaired DNA binding. The inability of FOXP3 protein to bind DNA in regulatory T cells impairs immune-suppressor function. Dysregulated T-cell function leads to overwhelming autoimmunity and recurrent infections. The scurfy mouse had a disease very similar to IPEX and has a homologous gene, scurfin, to the human *FOXP3*. The scurfy mouse model allows for understanding disease pathogenesis and provides a model to evaluate treatment modalities.[72,73] Neonatal thymectomy in male scurfy mice ameliorates disease and increases lifespan. Transfer of peripheral $CD4^+$ T cells, but not $CD8^+$ T cells, from affected mice to homologous wild-type mice results in disease, whereas bone marrow transplantation does not induce disease. Peripheral $CD4^+$ T cells appear to be hyperresponsive to antigens and have a decreased requirement for costimulation with CD28.[74] The inability of $CD4^+$ T cells to regulate the immune response from mutations in scurfin or *FOXP3* results in the IPEX syndrome.

Treatment. Children affected with IPEX usually die in the first 2 years of life of sepsis or failure to thrive. Supportive care and treatment of underlying disorders is necessary. Immunosuppressive medications have been tried in case reports or small case series. High-dose glucocorticoids, tacrolimus, cyclosporine, methotrexate, sirolimus, infliximab, and rituximab have been tried with varying degrees of success. The toxicity and infectious complications limit their dosing and use. In the scurfy mouse the disease can be cured with partial T-cell chimerism, and the same appears to be true in human subjects with normal T lymphocytes able to regulate the abnormal immune system in a dominant fashion.[75] Bone marrow transplantation can reduce symptoms and prolong survival.[76] Transplantation should be considered early in the disease to limit the autoimmune destruction to endocrine organs and possibly reduce the infectious complications from chronic immune suppression.

POEMS syndrome

POEMS syndrome has polyneuropathy, organomegaly, endocrinopathies, M-protein, and skin manifestations (hyperpigmentation and hypertrichosis) as clinical features. The causative factors of this constellation of diseases are not well defined. The syndrome is associated with plasmacytomas and osteosclerotic lesions, with radiation therapy to localized lesions being beneficial. Autologous hematopoietic stem cell transplantation has improved symptoms.[77]

Diabetes-associated autoimmune disorders

Multiple autoimmune disorders are associated with type 1A diabetes. Many of the disorders have been discussed previously in this review, and this section will focus on their relationship to type 1A diabetes.

Celiac disease is an autoimmune disorder that results in T-cell infiltration of the mucosa of the small intestine. Gliadin, a protein of wheat gluten, has been identified as the antigen responsible for inducing the autoimmune process. Like type 1A diabetes, a genetic predisposition is conferred through the HLA alleles DQ2 and DQ8. Symptoms of celiac disease can be mild but can also include diarrhea, abdominal pain, iron deficiency anemia, pubertal delay, growth failure, decreased bone mineralization, and vitamin D deficiency. TGA IgA autoantibodies are a sensitive and specific marker for the autoimmune process, more so than the older antiendomysial antibody assay. TGA autoantibodies are present in up to 16% of patients with type 1 diabetes.[78] A definitive diagnosis is made with a small-intestine biopsy showing flattened villi and intraepithelial lymphocytic infiltrates. Treatment is with a gluten-free diet, which results in reversal of the autoimmune process and normalization of the intestinal villi.[79] We recommend screening with TGA autoantibodies yearly in patients with type 1 diabetes and performing a small-intestine biopsy if the results of a repeat TGA autoantibody are positive. The biopsy specimen should be obtained close to the time of antibody measurement because the half-life of IgA antibodies are short and the titer of TGA autoantibody fluctuates with the amount of gluten in the diet. Those with a positive biopsy result are counseled on a gluten-free diet. These recommendations are based on the known risks of symptomatic celiac disease (osteoporosis, anemia, and gastrointestinal malignancy) and the rationale that the intestinal pathology is reversible with gluten avoidance.

Addison disease is present in 1 in 10,000 subjects in the general population compared with 1 in 200 in the type 1 diabetic population. One percent to 2% of patients with type 1 diabetes have 21-hydroxylase autoantibodies.[80] Many patients with Addison disease are adrenally insufficient for years before diagnosis. It is advisable to screen patients with type 1 diabetes for 21-hydroxylase autoantibodies and monitor those with positive cosyntropin (ACTH) stimulation test results.

Autoimmune thyroid disease is common in patients with type 1 diabetes. Twenty percent to 30% of patients with type 1 diabetes express TPO, thyroglobulin autoantibodies, or both, twice that of the general population. Long-term follow-up has shown 30% of patients with type 1 diabetes will have autoimmune thyroid disease.[81] It is recommend that patients with type 1 diabetes be screened for thyroid dysfunction annually with a serum TSH level.[82]

Pernicious anemia results in a macrocytic anemia from autoimmune destruction of parietal cells in the fundus and body of the stomach. The frequency of pernicious anemia in patients with type 1 diabetes has been reported to be up to 4%, with a rate of 0.12% in the general population.[83]

Vitiligo, the loss of melanocytes in the skin, is associated with many autoimmune conditions, including type 1A diabetes.

IATROGENIC ENDOCRINE AUTOIMMUNE DISORDERS

Background

Drug-induced autoimmune diseases have been recognized for years and span multiple disciplines. Iatrogenic autoimmunity is increasing in frequency as more therapies are designed to alter immune mechanisms in autoimmune conditions and cancer.

Pharmaceutical agents

IFN-α. The interferons are a group of proteins characterized by antiviral activity, growth-regulatory properties, and a variety of immunomodulatory activities. IFN-α is currently used to treat patients with the hepatitis C virus. IFN-α has been reported to cause HT and Graves disease. It is also associated with nonautoimmune thyroiditis. Approximately 5% to 10% of patients treated with IFN-α have thyroid autoimmunity, whereas another 15% have thyroid autoantibodies without clinical disease.[84] The drug also induces both islet autoantibodies and rapid progression to diabetes in a subset of patients with islet autoantibodies.[85] At a minimum, glucose levels should be monitored in patients. If these levels are abnormal, the patient should be evaluated for islet autoantibodies and monitored for diabetes, and the risks/benefits of therapy should be carefully considered.

IL-2. IL-2 induces T-cell proliferation, B-cell growth, and natural killer cell and monocyte activation. IL-2 has antitumor activity and has been used in the treatment of metastatic melanoma, renal cell carcinoma, and HIV. Thyroiditis and HT have been described with IL-2 treatment either alone or in conjunction with IFN-α in up to 16% of patients.[86]

Ipilimumab. Ipilimumab is an mAb that blocks CTLA-4, a receptor on T cells; blockade of CTLA-4 results in T-cell activation, proliferation, and differentiation. Ipilimumab has been used to treat patients with metastatic renal cell cancer and melanoma. Endocrinopathies, hypophysitis, and hypothyroidism, as well as nonendocrine autoimmune disorders, have been reported.[87] Up to 59% of participants in the National Institutes of Health studies treated with anti–CTLA-4 mAbs have presented with autoimmune toxicities. Many of the autoimmune events are transient, and some can be successfully treated with high-dose glucocorticoids. Five percent (8/163) of patients treated with anti–CTLA-4 mAbs at the National Institutes of Health have hypophysitis.[88] In the case of hypophysitis, anterior pituitary hormone deficiencies have been reported to be present for up to 2 years, despite discontinuation of therapy with ipilimumab.

Campath-1H. Campath-1H is a humanized anti-CD52 mAb that suppresses T_H1 lymphocytes. Graves disease has been associated with treatment in patients with multiple sclerosis and new-onset type 1A diabetes. In a study of 29 patients with multiple sclerosis treated with Campath-1H, 9 had Graves disease after 6 to 31 months of treatment.[89]

Highly active antiretroviral therapy. Highly active antiretroviral therapy, which is used to treat HIV infection, has been associated with Graves disease. The prevalence is rare, and it

occurs 16 to 19 months after initiation of therapy. After highly active antiretroviral therapy, there is immune reconstitution, and this is when autoimmunity develops, likely resulting from changes to $CD4^+$ T cells.[90]

CONCLUSIONS

Improved understanding of the immune pathogenesis of endocrine diseases has led to the initial development of therapies that target the underlying autoimmunity. Type 1A diabetes, one of the best-studied organ-specific autoimmune diseases, is now predictable in human subjects, and therapies are emerging to augment the underlying autoimmune destruction of β cells. With continued basic understanding of the immunologic mechanisms causing autoimmunity, better therapies can be designed to improve the quality of life for patients and their families afflicted with these disorders.

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Diagnostic testing and interpretation of tests for autoimmunity

Christine Castro, DO, and Mark Gourley, MD *Bethesda, Md*

Laboratory testing is of great value when evaluating a patient with a suspected autoimmune disease. The results can confirm a diagnosis, estimate disease severity, aid in assessing prognosis and are useful for following disease activity. Components of the laboratory examination include a complete blood count with differential, a comprehensive metabolic panel, measurement of inflammatory markers and autoantibodies, and flow cytometry. This chapter discusses these components and includes a discussion about organ-specific immunologic diseases for which immunologic laboratory testing is used. Comprehensive laboratory evaluation of a suspected autoimmune illness in conjunction with a thorough clinical evaluation provides a better understanding of a patient's immunologic disease.
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Autoimmunity involves the loss of normal immune homeostasis such that the organism produces an abnormal response to its own tissue. The hallmark of autoimmune diseases generally involves the presence of self-reactive T cells, autoantibodies, and inflammation. An area of intense research is determining why the immune system turns against its host. Over the past decade, research has greatly advanced our understanding of autoimmunity, and the scientific findings from these investigations are assisting in the creation of new clinical laboratory studies of patients to aid in diagnoses.

Examining patients for potential autoimmune diseases is fraught with difficulty because no one laboratory test establishes such a diagnosis. Typically, multiple laboratory tests are needed and include basic studies like a complete blood count (CBC), comprehensive metabolic panel, measurement of acute-phase reactants, immunologic studies, serology, flow cytometry, cytokine analysis, and HLA typing. Although some tests might be nonspecific, such as the erythrocyte sedimentation rate (ESR), they are useful for assessing disease activity. These tests can be useful in the diagnosis and management of patients with autoimmune diseases and help in providing a prognosis or indicating the severity of organ involvement or damage.

Abbreviations used

ACE:	Angiotensin-converting enzyme
AIHA:	Autoimmune hemolytic anemia
ALT:	Alanine transaminase
ANA:	Antinuclear antibody
ANCA:	Antineutrophil cytoplasmic antibody
Anti-GBM:	Anti–glomerular basement membrane
aPL:	Antiphospholipid
APS:	Anti–phospholipid antibody syndrome
AST:	Aspartate aminotransferase
cANCA:	Cytoplasmic antineutrophil cytoplasmic antibody
CBC:	Complete blood count
CCP:	Cyclic citrullinated peptide
CH50:	Plasma total hemolytic complement assay
CK:	Creatinine kinase
CRP:	C-reactive protein
CSS:	Churg-Strauss syndrome
DM:	Dermatomyositis
dsDNA:	Double-stranded DNA
ESR:	Erythrocyte sedimentation rate
IBM:	Inclusion body myositis
IIM:	Idiopathic inflammatory myopathy
JIA:	Juvenile idiopathic arthritis
LDH:	Lactate dehydrogenase
MPA:	Microscopic polyangiitis
MPO:	Myeloperoxidase
pANCA:	Perinuclear antineutrophil cytoplasmic antibody
PM:	Polymyositis
PR3:	Proteinase 3
RA:	Rheumatoid arthritis
RBC:	Red blood cell
RF:	Rheumatoid factor
RIA:	Radioimmunoassay
RNP:	Ribonucleoprotein
SLE:	Systemic lupus erythematosus
SRP:	Signal recognition particle
WBC:	White blood cell
WG:	Wegener granulomatosis

INITIAL LABORATORY EVALUATION

Inflammatory diseases will cause abnormalities in routine laboratory studies. Characteristic findings can include a normochromic normocytic anemia indicating the chronicity or severity of disease. Common hematologic parameters also include an increased or decreased platelet count, white blood cell (WBC) count, or both. Leukopenia and thrombocytopenia are common in patients with systemic lupus erythematosus (SLE).

Testing will find aberrations in serum levels of specific organ enzymes or abnormalities in metabolic processes that are reflected in the comprehensive metabolic panel. For example, autoimmune hepatitis can be manifested by increases in transaminase, bilirubin, and serum protein levels. One should be aware that these abnormalities can also be associated with drug toxicity.

From the National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health.

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Reprint requests: Christine Castro, DO, National Institutes of Health, Building 10, CRC, Room 5, SEN–5-3481, Bethesda, MD 20892. E-mail: castroc2@mail.nih.gov.

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Coagulation studies, such as a prolongation of the activated partial thromboplastin time, the prothrombin time, or both that do not correct with mixing studies, suggest an inhibitor of the clotting process is present, as seen in the antiphospholipid (aPL) syndrome. Hypercalcemia can be observed in approximately 30% of patients with sarcoidosis. An increase in muscle enzyme levels (creatinine kinase [CK], alanine transaminase [ALT], and aspartate aminotransferase [AST]) can be seen in autoimmune inflammatory myopathies (dermatomyositis [DM], polymyositis [PM], and inclusion body myositis [IBM]). Serum protein levels are helpful to screen for abnormal increases of immunoglobulin levels.

Urinalysis is commonly used to assess renal injury (glomerulonephritis and interstitial nephritis) and will show proteinuria, hematuria, or active sediment (WBC casts or red blood cell [RBC] casts). Many other illnesses, such as diabetic nephropathy, poorly controlled hypertension, or infections, can be tested similarly, but when autoimmune disease is suspected, the common laboratory evaluation will serve as an initial red flag to pursue further testing.[1]

INFLAMMATORY MARKERS

Serum proteins that are produced in response to inflammation can be referred to as inflammatory markers. These proteins are mainly produced by the liver in response to stress and can also be called acute-phase reactants. Proinflammatory cytokines, such as IL-1, IL-6, and TNF-α, induce synthesis of some acute-phase reactants that include C-reactive protein (CRP), fibrinogen, and haptoglobin. Other proteins, like albumin, are not sensitive to inflammatory cytokines for increased synthesis; instead, chronic stress (inflammation) results in a lower synthesis rate and resultant decreased serum concentrations. The inflammatory markers are not diagnostic of inflammation but reflect abnormalities that are seen in autoimmune diseases, infections, malignancies, and other illnesses.[11]

ESR

The ESR is the measure of the quantity of RBCs that precipitate in a tube in a defined time and is based on serum protein concentrations and RBC interactions with these proteins. Inflammation causes an increase in the ESR. Multiple factors influence the ESR and include the patient's age, sex, RBC morphology, hemoglobin concentration, and serum level of immunoglobulin. The sample must be handled appropriately and processed within a few hours to ensure test accuracy. Although the ESR is not a diagnostic test, it can be used to monitor disease activity and treatment response and signal that inflammatory or infectious stress is present. For example, in patients with rheumatoid arthritis (RA), the ESR correlates well with disease activity; however, normalization of the ESR often lags behind successful treatment that causes resolution of the inflammatory state.[2,3]

CRP

CRP/CRP high sensitivity was discovered and named for its reactivity to the C polysaccharide in the cell wall of *Streptococcus pneumoniae*. CRP, an innate immune protein, helps opsonize pathogens for phagocytosis and activates the complement system. CRP production is under the control of IL-1, IL-6, and TNF-α. Serum CRP concentrations change more quickly than those in the ESR, and therefore CRP might be a better reflection of current inflammation. Unlike the ESR, CRP is a fairly stable serum protein with measurement that is not time sensitive and not affected by other serum components. The magnitude of inflammation directly relates to the concentration of CRP. Levels of less than 0.2 mg/dL are considered normal, whereas those greater than 1.0 mg/dL are suggestive of inflammation, infection, or both. More recently, high-sensitivity CRP has been used. This test might better quantify lower levels of inflammation and has been important in evaluating cardiac disease and other inflammatory states.[2,3]

Ferritin

Serum ferritin is a storage protein for iron, and its synthesis is regulated by intracellular iron, cytokines (TNF-α, IL-1, and IL-6), products of oxidative stress, and growth factors. Increased levels can indicate acute or chronic sepsis, inflammation, or malignancy. Diseases such as adult Still disease, systemic-onset juvenile idiopathic arthritis (JIA), hemophagocytic lymphohistiocytosis, and iron-overload diseases, including hemochromatosis or hemosiderosis, should be considered with increased ferritin levels.[4,5]

Less common indicators of inflammatory states

Ceruloplasmin is the major copper-containing protein in the blood that plays a role in iron metabolism, and its concentration is increased in patients with acute and chronic inflammatory states, pregnancy, lymphoma, RA, and Alzheimer disease.[6,7]

Fibrinogen is a hemostatic coagulation factor produced in response to tissue injury. Fibrinogen synthesis is controlled at the transcriptional level and is increased in the presence of inflammation and stress that is mediated by IL-6.

Haptoglobin is produced in response to tissue injury. Increased levels of haptoglobin can be seen during inflammation, malignancy, surgery, trauma, peptic ulcer disease, and ulcerative colitis. Decreased levels might indicate chronic liver disease or anemia.

Albumin is a serum protein synthesized by the liver that aids body tissues in maintaining the oncotic pressure necessary for proper body fluid distribution. The average amount of albumin in the plasma is approximately 300 to 400 g, and about 15 g is produced by the liver per day. Although the rate of synthesis can double in situations of rapid albumin loss, as seen in glomerulonephritis or inflammatory bowel disease, serum levels will decrease.

AUTOANTIBODIES AND IMMUNOLOGIC STUDIES

The presence of an autoantibody in a patient does not ensure a diagnosis of an autoimmune disease. Rather, a positive serologic test result in the company of appropriate signs and symptoms helps to support a diagnosis. Serologic testing is flawed by the presence of autoantibodies in healthy subjects and other patients with nonautoimmune diseases and imperfect testing systems. Historically, many different methods were used to test for the presence of an autoantibody. Today, testing is principally done with enzyme immunosorbent assays because of cost-saving measures with mechanization.

ELISA

The ELISA is an immunometric method for detecting and measuring specific antibodies. The basic components of this laboratory method include a substrate in which an antigen is fixed (typically a 96-well microwell plate), the patient's sera, washing solutions, and a detection method in which an enzyme is linked to

an antibody that detects the antigen. In a typical double-antibody sandwich ELISA, an antibody that is attached to the bottom of a well provides both antigen capture and immune specificity while another antibody linked to an enzyme provides detection and acts as an amplification factor. This allows for accurate and sensitive detection of the antigen of interest. However, performance is largely dependent on antibody quantity, the kit's manufacturer, and the operator's skill and experience. ELISAs permit measurement of only 1 antigen at a time for a given aliquot of sample. Furthermore, ELISAs have a limited dynamic range (ie, the range over which there is a linear relationship between antigen concentration and absorbance reading); the range is narrow relative to the range for other technologies, such as multiplex assays.[8]

Rheumatoid factor and anti–cyclic citrullinated peptide antibody

Rheumatoid factor (RF) is an autoantibody that reacts to the Fc portion of polyclonal IgG, although it can be any class of immunoglobulin. Most assays detect the IgM RF. RF is helpful when evaluating patients who might have RA because the sensitivity is approximately 70%, with a specificity of approximately 70%. RF is absent in approximately 15% of patients with RA. However, approximately 15% of the healthy population might have a low titer of RF. RF-positive patients are more likely to have progressive erosive arthritis with loss of joint mobility and also have extra-articular manifestations, including rheumatoid nodules, vasculitis, Felty syndrome, and secondary Sjögren syndrome. In addition, the presence of RF is seen in other autoimmune disorders, including Sjögren syndrome, SLE, and cryoglobulinemia; in pulmonary diseases, such as interstitial fibrosis and silicosis; and in various infectious diseases.

Recently, a new biomarker for RA has been described: autoantibodies to cyclic citrullinated peptide (CCP). Inflammation activates the enzyme peptidylarginine deiminase, which incorporates citrulline into certain proteins. In patients with RA, autoantibodies are formed against the citrullinated protein (anti-CCP). The presence of serum anti-CCP antibodies is approximately 95% specific for the diagnosis of RA, with a sensitivity similar to that of RF. Testing for both anti-CCP and RF is beneficial when excluding the diagnosis of RA rather than testing for either antibody alone. In patients with early undifferentiated disease, anti-CCP–positive patients tend to go on to have more severe, erosive, and aggressive disease. Anti-CCP can also be present in other disease states, such as some cases of JIA, psoriatic arthritis, lupus, Sjögren syndrome, inflammatory myopathies, and active tuberculosis.[2,3]

Antinuclear antibody

Autoantibodies to nuclear antigens are a diverse group of antibodies that react against nuclear, nucleolar, or perinuclear antigens. These antigens represent cellular components, such as nucleic acid, histone, chromatin, and nuclear and ribonuclear proteins. Classically, the antinuclear antibody (ANA) hallmarks the serologic diagnosis of SLE, but finding an ANA is common to most other autoimmune diseases. Methods used for detection use immunofluorescence testing of the patient's serum at various dilutions with a cell substrate. Typically, screening the patient's serum for the detection of an ANA with an ELISA provides high sensitivity but lacks specificity. Results are reported as either the dilution of serum that evokes a positive test response or the degree of positivity measured by using the testing procedure. Historically, HEp2 cells (a human laryngeal epithelioma cancer cell line) have been used as the cell substrate because the result offers the advantage of detecting a nuclear fluorescent pattern. The fluorescent patterns (homogenous, diffuse, speckled, peripheral, and rim) suggest clinical associations with certain autoimmune diseases. However, because of the time and expense for testing with HEp2 cells, the assay procedures are largely done with ELISA methods.[2,3]

TABLE I. Sensitivity and specificity of specific anti-nuclear antibodies

	ANA	dsDNA	Histone	Nucleoprotein	Smith (ribonuclear)
SLE					
Sensitivity	>95%	70%	~50%	60%	25%
Specificity	60	95	50	Medium	99
RA					
Sensitivity	45	1	Low	25	1
Specificity	60			Low	
Scleroderma					
Sensitivity	60	<1	<1	<1	<1
Specificity	50				
PM/DM					
Sensitivity	60	<1	<1	<1	<1
Specificity	60				
Sjögren syndrome					
Sensitivity	50	<5	Low	Medium	<5
Specificity	50		Low	Medium	

Immunofluorescence is particularly useful as an initial screening test for those individuals suspected of having an autoimmune disease, such as SLE, Sjögren syndrome, RA, mixed connective tissue disease, scleroderma, and PM/DM. However, one must use caution when interpreting the presence of ANA because this autoantibody is found in nonrheumatic diseases, such as Hashimoto thyroiditis, Graves disease, autoimmune hepatitis, primary autoimmune cholangitis, primary pulmonary hypertension, and various infections and malignancies. Furthermore, the presence of low-titer ANA occurs more frequently in elderly populations.

Table I details the sensitivity and specificity of the various ANAs in several autoimmune diseases. Values are reported as approximate percentages, as seen in several published reviews. Table II lists specific autoantibodies and clinical disease associations.

Anti–double-stranded DNA

Autoantibodies to double-stranded DNA (dsDNA) are an important marker used in the diagnosis and monitoring of SLE. Antibodies to dsDNA are highly specific for SLE. However, some patients with other rheumatic diseases or chronic active hepatitis might have mildly or moderately increased serum titers. Previously, anti-dsDNA was typically measured by using an RIA (particularly the Farr assay). The more common current tests use an immunofluorescence assay or ELISA. The immunofluorescence assay uses a target antigen, *Crithidia luciliae*, a flagellated protozoa containing a dsDNA-containing small organelle called a kinetoplast. Antibodies to dsDNA are detected semiquantitatively by demonstrating IgG bound to the kinetoplast. In contrast, with ELISA testing, the dsDNA is bound to the solid phase of the microwell plate. The serum is incubated, and then the bound IgG is detected.[2,3]

TABLE II. Specific autoantibodies with their antigenic determinant and clinical disease associations

Autoantibody	Antigenic determinant	Clinical associations
Anti-dsDNA	dsDNA	High specificity for SLE; often correlates with active severe disease
Anti–extractable nuclear antigens Anti-Sm Anti-RNP	Smith Proteins containing U1-RNA	High specificity for SLE MCTD, SLE, RA, scleroderma, Sjögren syndrome
Anti-SSA (Ro)	RNPs	Sjögren syndrome, SLE (subacute cutaneous lupus), neonatal lupus
Anti-SSB (La)	RNPs	Sjögren syndrome, SLE, neonatal SLE
Anticentromere	Centromere/kinetochore region of chromosome	Limited scleroderma, pulmonary hypertension, primary biliary cirrhosis
Anti–Scl 70	DNA topoisomerase I	Diffuse scleroderma
Anti–Jo-1 (anti-synthetase antibodies)	Histidyl tRNA synthetase (other tRNA synthetases)	Inflammatory myopathies with interstitial lung disease, fever and arthritis
Anti-SRP	Antibody to signal recognition protein	Inflammatory myopathies with poor prognosis
Anti-PM/Scl	Antibody to nucleolar granular component	PM/scleroderma overlap syndrome
Anti–Mi-2	Antibodies to a nucleolar antigen of unknown function	DM

MCTD, Mixed connective tissue disease.

Anti–extractable nuclear antigen

The extractable nuclear antigens consist of the Smith antigen, ribonucleoprotein (RNP) or U1RNP, anti-SSA (Ro), and anti-SSB (La). They are called extractable because they are readily soluble or extractable in neutral buffers. The Smith antigen is highly specific for SLE, but it is found in only approximately 25% of patients with SLE. The U1RNP antigen is seen in patients with SLE plus systemic sclerosis and in patients with mixed connective tissue disease. The SSA (Ro) and SSB (La) nuclear antigens are often found together in patients with Sjögren syndrome. Anti-SSA and anti-SSB are also seen in some subsets of patients with SLE. This group includes those patients with subcutaneous lupus erythematosus (prominent photosensitive rashes and sometimes vasculitis) but without severe renal disease. The presence of anti-SSA and anti-SSB is associated with neonatal lupus, in which transplacental transfer of these antibodies (maternal IgG) can cause transient photosensitive rash, congenital heart block, or both.[2,3]

Anti–signal recognition particle, anti–Jo-1, anti-Mi-2 and anti-PM/Scl

Anti–signal recognition particle (anti-SRP), anti–Jo-1, anti–Mi-2 and anti-PM/Scl are termed myositis-specific antibodies because of the high specificity to the autoimmune idiopathic inflammatory myopathies (IIMs). Anti-SRP antibodies are directed toward an RNA–protein complex consisting of 6 proteins and a 300-nucleotide RNA molecule (7SL RNA). Patients with this antibody have a distinct type of IIM that is characterized by acute onset of muscle weakness, a muscle biopsy specimen that lacks inflammation, and a poor response to therapy. Anti–Jo-1 autoantibodies are the most common autoantibodies found in the group of inflammatory myopathies called the antisynthetase syndrome.

Anti–Jo-1 autoantibodies are directed against histidyl–tRNA synthetase (an enzyme that attaches histidine to growing polypeptide chains). Other less well-understood antisynthetase autoantibodies include anti-PL12, anti-EJ, anti-OJ, anti-PL7, and anti-KS. These antibodies are reportedly more common in patients with PM than in patients with DM and are rare in children. Patients with antisynthetase syndrome have disease characteristic that are very different than those of patients with anti-SRP and often present with muscle weakness, interstitial lung disease, arthritis, and fevers. The anti–Jo-1 response appears to be self-antigen driven, with isotype switching and affinity maturation.

Anti-Mi-2 antibodies recognize a major protein of a nuclear complex formed by about 7 proteins involved in transcription. Autoantibodies to Mi-2 are specific for DM and associated with acute onset, a better prognosis, and good response to therapy.

Anti-PM/Scl is an autoantibody to the nucleolar granular component. This is often seen with myositis and scleroderma overlap. Detection of such autoantibodies is done mostly through commercial blot assays using immunoblotting or dot immunoblotting methodologies in addition to traditional ELISA.[8]

Antineutrophil cytoplasmic antibody (myeloperoxidase and proteinase 3)

Antineutrophil cytoplasmic antibodies (ANCAs) react with cytoplasmic granules of neutrophils. Initial ANCA testing screens sera for the presence of ANCA, and 2 general immunofluorescent staining patterns are observed: cytoplasmic (cANCA) and perinuclear (pANCA). The immunofluorescence pattern is helpful to distinguish various ANCA-associated vasculitis syndromes. cANCA is most often seen in patients with Wegener granulomatosis (WG), microscopic polyangiitis (MPA), and Churg-Strauss syndrome (CSS). pANCA patterns were initially described in patients with MPA, but pANCA has now been observed in a variety of diseases, including other types of vasculitis, inflammatory bowel disease, SLE, RA, and JIA. Antigenic determinants of the ANCAs that are important to detect in vasculitis are proteinase 3 (PR3) and myeloperoxidase (MPO). Vasculitic states with positive test results are named PR3-ANCA positive or MPO-ANCA positive. The presence of PR3 or MPO can help the clinician to determine the type of vasculitis and activity of disease.

Antibodies to PR3 or MPO are predictive of the various vasculitis syndromes. cANCA plus PR3 has increased positive predictive value for ANCA-associated vasculitis, particularly WG. pANCA plus MPO has an increased positive predictive value for MPA and less often for CSS. ANCA plus MPO more than PR3 often leads toward a diagnosis of CSS. With increased disease activity, there is a greater likelihood that ANCA results

will be positive. ANCA titers might normalize with treatment, although persistent ANCA positivity or increasing ANCA titers do not reliably predict disease exacerbation or flare. Therefore one should not use ANCA titers to determine treatment efficacy. Furthermore, one should also be wary of ANCA positivity because this can be seen in other disease states that include infection, drug use (eg, thyroid medication, particularly propylthiouracil), and other autoimmune disease. If the suspicion for vasculitis remains, tissue biopsy should be considered.[2-4,9]

Complement

The complement cascade is a complex, tightly regulated series of proteolytic enzymes, regulatory proteins, and cell-surface receptors that mediate and augment both the complement, humoral, and cellular immune response. The classical pathway is initiated by immune complexes binding to C1q and involves C4 and C2. The alternative pathway involves factors B and D and properdin. The mannose-binding lectin pathway and classical and alternative pathways all involve cleavage of C3. This release product induces the formation of the terminal membrane attack complex (C5-C9).

Individual components, such as C3, C4, and factor B, are measured by means of nephelometry and ELISA. The plasma total hemolytic complement assay (CH50) uses a functional assay to assess the functional integrity of the classical pathway. To measure these values, diluted serum is added to sheep antibody–coated erythrocytes, and the subsequent value is the reciprocal of the highest dilution able to lyse 50% of the RBCs. CH50 is a useful screening tool to detect deficiencies of the classical pathway.

Serum levels of complement components can serve as markers of disease activity. In patients with immune complex deposition disease, serum complement proteins are consumed. and serum levels decrease. Immune complex disease results from the deposition of antigen–antibody complexes in involved organ tissues. Immune complex glomerulonephritis in patients with SLE, decreased C3 and C4 levels indicate increased consumption and disease activity. In contrast, increases of C3 and C4 levels indicate inflammatory disorders because these proteins are also acute-phase reactants. Hypocomplementemia is generally not specific for any disease and might be secondary to nonrheumatic diseases, such as subacute bacterial endocarditis or poststreptococcal glomerulonephritis. If C4 levels are low compared with C3 levels, this can indicate the presence of cryoglobulins or the genotype C4-null allele. If CH50 levels are low or undetectable, it might indicate a deficiency of 1 or more complement components. Genetic or congenital deficiencies of early complement components (C1-C4) might increase the risk for development of immune complex diseases. For example, congenital C1q deficiency, although rare in the general population, is associated with persons who have lupus.[2-4,8]

Immunoglobulins (quantitative and qualitative)

Measuring total quantitative immunoglobulin levels is a key component to any immunologic evaluation. The immunoglobulin levels reflect B-cell function (humoral production and T-cell interaction), and serum immunoglobulin levels aid in disease detection. Quantitative measurements of serum immunoglobulin levels, mainly IgG, IgA, and IgM, are done with nephelometry.

TABLE III. Differential diagnosis for immunoglobulin levels

Immunoglobulin	Increased	Decreased
IgG	Infection, inflammation, hyperimmunization, IgG multiple myeloma, liver disease, rheumatic fever, systemic rheumatic disease	Agammaglobulinemia, amyloidosis, leukemia, myeloma, preeclampsia
IgM	Early HIV infection, infectious mononucleosis, lymphoma, macroglobulinemia, myeloma, RA	Rarely agammaglobulinemia, amyloidosis, leukemia, myeloma
IgA	Chronic infections (especially of gastrointestinal tract), inflammatory bowel disease, myeloma, rheumatic fever	Agammaglobulinemia, hereditary IgA deficiency, myeloma or protein-losing enteropathy

Table III lists diseases that are associated with increased or decreased serum immunoglobulin levels.

Simple qualitative measurements of serum immunoglobulin levels reflect a subject's ability to mount a humoral immune response. Titers to tetanus, *Haemophilus influenzae* type B, and pneumococcus can easily be tested to evaluate the quality of the immune response. These levels assess the function of B cells and also detect defects that might indicate immunodeficiency. Responses to protein and polysaccharide antigens should be evaluated to assess antibody production. B-cell testing is done primarily by using *in vivo* (vaccination) studies. Protein vaccinations, such as tetanus toxoid, measure T cell–dependent responses. Polysaccharide vaccines, like Pneumovax (Merck, Whitehouse Station, NJ), measure T cell–independent responses.

Testing of specific antibody titers (eg, to influenza immunization) are reported relative to protective values. These values are based on epidemiologic data regarding protection in larger populations. For randomly acquired antibody levels, an initial comparison with protective values can be used to decide whether a proper immune response was achieved. A 4-fold increase in titers to protein vaccination indicates a normal response. A 2-fold increase in titers to a polysaccharide antigen indicates a normal response. Failure to mount an appropriate response to antigen is a clue to the physician to pursue B- and T-cell function further.[2-4,8]

Cryoglobulins

Cryoglobulins are immunoglobulins that precipitate reversibly in cold temperatures. In disease states these antibodies can bind with complement proteins and other peptides to form immune complexes and cause tissue damage. Three types of cryoglobulins exist. Type I cryoglobulins are monoclonal immunoglobulins often of the IgM isotype. Type II cryoglobulins are a mixture of polyclonal IgG and monoclonal IgM. Type III cryoglobulins are a combination of polyclonal IgG and polyclonal IgM.

At phlebotomy, whole blood is obtained, placed in prewarmed tubes without anticoagulant, and maintained at body temperature until coagulation occurs (about 1 hour). The sample is then centrifuged, and the clot is removed. The remaining serum is kept

at 4°C up to several days. The specimen is then examined daily to determine whether proteins have precipitated. Once a precipitate is present, the sample is spun again, and a cryocrit value is measured in a calibrated tube. Confirmation of the cryocrit value is seen if the precipitate redissolves when placed in a 37°C water bath.

Cryoglobulins are nonspecific indicators of disease states. Type I monoclonal cryoglobulins are associated with multiple myeloma, Waldenstrom macroglobulinemia, and lymphoproliferative disorders. Type II and III cryoglobulins can bind complement, unlike type I cryoglobulins, and are associated with hepatitis C and small-vessel vasculitis. The presence of multiple immunoglobulin components within the cryoglobulin is known as mixed cryoglobulin. Symptoms generally associated with cryoglobulins include purpura, ulcerations, Raynaud phenomenon, arthralgias, proteinuria, and renal failure. Cryoglobulins are rarely found in children.[2-4,8]

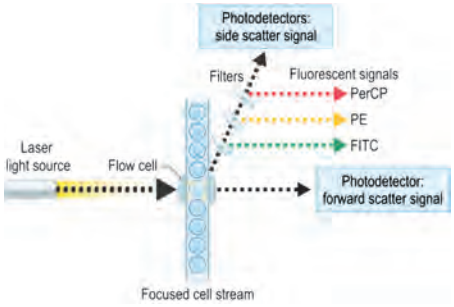

FIG 1. Flow chamber. *PerCP*, Peridinin chlorophyll protein; *PE*, phycoerythrin; *FITC*, fluorescein isothiocyanate. Reprinted with permission from Fleisher TA from Fleisher T, de Oliveira JB (2008). Flow cytometry (Ed. 3), Clinical Immunology: Principles and Practice (p. 1436). Philadelphia, PA: Elsevier Limited.

Lupus anticoagulant/anticardiolipin/aPL autoantibodies

In patients with autoimmune disease, serum antibodies that inhibit or prolong *in vitro* clinical laboratory coagulation tests are termed aPL antibodies (also called anti-cardiolipin antibodies, anti-phospholipid antibodies or lupus anticoagulant) because they are directed against phospholipids and phospholipid-binding proteins. The existence of these antibodies is associated with the anti–phospholipid antibody syndrome (APS). APS is suspected in subjects who have venous thromboses, arterial thromboses, or both; recurrent fetal loss; or thrombocytopenia. APS can occur independently of or with systemic rheumatic diseases. aPL antibodies can also be found in healthy subjects and in patients with various infections who do not have features of aPL antibody syndrome. The term anticoagulant is paradoxic in that the presence of aPL is associated with thromboses in patients. The presence of aPL can be directly measured by using enzyme-linked assays. IgG anticardiolipin has a higher predictive value than IgM or IgA of a thrombosis. β_2-Glycoprotein I has been identified as one of the major antigenic determinants of aPL antibodies.[12-15]

FLOW CYTOMETRY

Flow cytometry is a technique in which particles or tagged cells flow through laser light so that populations of particles/cells can be counted and phenotyped by using cell characteristics and surface proteins. Initial applications of flow cytometry pertained to the interest in certain cell populations, such as the numbers of lymphocytes in patients infected with HIV. The number of T cells that are $CD4^+$ is an important gauge of severity of HIV infection. However, the methodology has greatly expanded its role such that cell-cycle analysis, quantification of malignant cells, and activation status of lymphocyte subpopulations can be determined. When evaluating a patient with a suspected immunodeficiency, flow cytometry is crucial to determine the quantitative number of immune cells (typically T, B, and natural killer cells). Remember, flow cytometric testing reveals numbers of cells and does not indicate cellular function. Testing for cellular functioning involves other laboratory methods, such as measurement of quantitative immunoglobulin levels to indicate proper B-cell function.[16,17]

The markers commonly used to assess lymphocyte subsets by means of flow cytometry are listed in Table III.

The flow cytometric device consists of an illumination source, an optical bench, a fluidic system, electronic monitoring, and a computer. Cells that will flow through the cytometer are first prepared by tagging cell-surface molecules with fluorescently labeled mAbs. Illumination of the cell occurs by using air-cooled lasers that provide a monochromatic light source (argon at 488 nm or blue and helium neon at 633 nm or red). The point of illumination occurs within the flow cell. The optical bench contains lenses that shape and focus the illumination beam. Nonfluorescent and fluorescent signals generated by the labeled cell are collected and measured by using a detection system consisting of filters linked to a photodetector. Cells are injected into a moving fluid sheath to establish a focused single-file flow of cells that move through the analysis point. Differences in the magnitude of emission signal generated from each cell reflect biologic differences between the cells. Software collects data that can be used to analyze cell subpopulations based on the presence or absence of labeled antibody binding. Data are then presented as fluorescence intensity versus cell number.[8]Figures 1 and 2 illustrate the flow chamber and the presentation of data using dot plot and contour plot.

CYTOKINE STUDIES

Cytokines are molecules secreted by a variety of cells that function in cellular communication. Immunologists are keenly interested in cytokines, particularly those that influence immune function and inflammation. Commercial testing laboratories do not routinely assay most serum cytokine levels because this testing is largely done in research laboratories. Testing is laborious because of the labile nature of these small molecules. After phlebotomy, the serum needs to be quickly removed from the cellular components and frozen as quickly as possible, and testing should not be delayed. Laboratory methods commonly used to assay cytokine levels include flow cytometry and ELISA.[15,16]

Cytokines that influence inflammation include IL-1, IL-6, and TNF-α. There is extensive evidence that these cytokines promote inflammation and therefore have become targets for therapy. RA is the best example of an autoimmune illness in which anti-TNF therapy has revolutionized the natural history of the disease. Targeting TNF with proteins (fusion produced or mAbs) that antagonize TNF action results in dramatic improvement of disease activity. In fact, RA is the prototypic autoimmune disease in which the efficacy of anticytokine therapy is best demonstrated. Currently, anti-TNF, anti–IL-1, and anti–IL-6 therapies have all proved to be effective in treating RA.

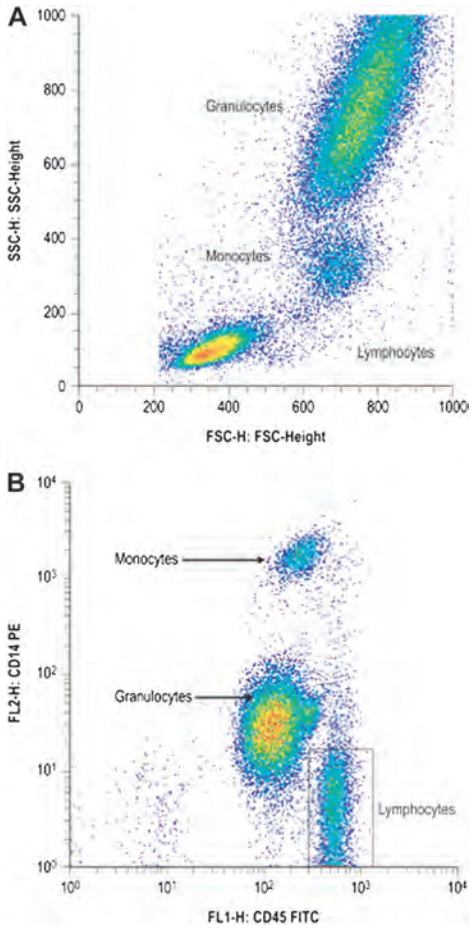

FIG 2. Presentation of data for CD8/CD4 as dot plot (A) and contour plot (B). *SSC,* Side scatter; *FSC,* forward scatter; *PE,* phycoerythrin; *FITC,* fluorescein isothiocyanate. Reprinted with permission from Fleisher TA from Fleisher T, de Oliveira JB (2008). Flow cytometry (Ed. 3), Clinical Immunology: Principles and Practice (p. 1436). Philadelphia, PA: Elsevier Limited.

MHC (HLA)

HLA is synonymous with MHC. MHC class I and II genes are the major genetic determinants of susceptibility to many autoimmune diseases. MHC class I molecules include HLA-A, HLA-B, and HLA–C. MHC class II molecules include HLA-DR, HLA-DQ, and HLA-DP. Detection of HLA type can be done routinely and can be assayed by using several methods that include gel electrophoresis, PCR, ELISA, and newer methods using high-throughput detection of nucleic acid. Many antigens of the MHC, especially of HLA class I and II, have been associated with rheumatic disorders. HLA-B27 is present in approximately 90% to 95% of white patients with ankylosing spondylitis and only 7% to 8% of the general population. HLA-DR1 and HLA-DR4 increase the risk of polyarticular JIA in many populations. HLA-DR3 and HLA-DR2 are associated with lupus in white populations, whereas much of the risk attributable to MHC is associated with variation at HLA-DRB1 in patients with RA.[3,18,19]

SPECIFIC IMMUNOLOGIC DISEASE ENTITIES

Immunologic lung disease

Sarcoidosis. Sarcoidosis is a systemic granulomatous disease characterized by noncaseating granulomas affecting multiple organ systems. The cause of sarcoidosis is not known but is believed to involve chronic inflammation with a T_H1 cellular contribution, and immunosuppressive therapy is beneficial. The organ systems most frequently involved, in decreasing order, include the lungs, skin, sinus and upper respiratory tract, eye, and musculoskeletal, abdominal, hematologic, salivary/parotid, cardiac, and neurologic organs. Biopsy of the affected tissue is vital for diagnosis, and histologic findings should show noncaseating granulomas. Imaging studies, particularly chest radiography or chest computed tomography, show bilateral hilar lymphadenopathy, interstitial infiltrates, or both. Chest computed tomography reveals nodular infiltrates that tend to be distributed along the bronchoalveolar structures.

Laboratory investigations helpful in the diagnosis of sarcoidosis include measurement of serum angiotensin-converting enzyme (ACE) and vitamin D levels. ACE levels are generally increased; however, ACE levels lack disease specificity and therefore have limited diagnostic and therapeutic utility.[4] High serum levels of 1, 25(OH)2D3 vitamin D are commonly seen in patients with granulomatous disease and are believed to induce hypercalcemia. IFN-γ produced by T_H1 cells is possibly a stimulus for 25(OH)2D3 synthesis by macrophages.

CSS. CSS is a necrotizing vasculitis affecting small and medium blood vessels characterized by eosinophilic infiltration, eosinophilic granulomas, nasal polyps, allergic rhinitis, conductive hearing loss, eye disease (scleritis, episcleritis, and uveitis), asthma, fleeting infiltrates, alveolar hemorrhage, segmental necrotizing glomerulonephritis, heart failure, and vasculitic neuropathy. Initial symptoms typically suggest a reactive airway process similar to that seen in patients with asthma. Immunologic studies that aid in the diagnosis of CSS include a CBC that reveals a peripheral eosinophilia and the serologic presence of an ANA and a pANCA directed against MPO. As is the case with any suspected vasculitis, a biopsy specimen of the involved organ showing an inflammatory destruction of the blood vessels with eosinophilic infiltrates and granuloma formation is vital for diagnosis. Characteristic of all inflammatory vasculitidies are a significantly increased ESR and CRP level.

WG and MPA. WG is characterized by systemic granulomatous vasculitic lesions of the upper and lower respiratory tract and the kidney. MPA is characterized by nongranulomatous vasculitic lesions of the lower respiratory tract, kidney, skin, and nerve. Both of these diseases can be manifested by acute renal failure, with the urinalysis showing RBCs, RBC casts, and proteinuria. Renal biopsy reveals a segmental necrotizing glomerulonephritis with a characteristic lack of immune complexes. WG can be a chronic undiagnosed illness in which a patient complains of chronic sinusitis that might cause nasal septal perforation. The auditory nerve can be inflamed, causing a conductive or sensorineural hearing loss. Cartilage inflammation will cause subglottic stenosis, saddle-nose deformity, or both; ocular inflammation leads to orbital pseudotumor, scleritis, episcleritis, or uveitis; and lung disease with nodules, infiltrates, and/or cavitary lesions, alveolar hemorrhage, or both can occur. Characteristic of vasculitis, the ESR and CRP levels will be increased. Other laboratory evaluations for WG and MPA include a serologic finding of cANCA/PR3 generally associated with WG and pANCA/MPO generally associated with MPA. Important HLA associations with WG are HLA-DR4 and HLA-DR13.

Goodpasture syndrome. Anti–glomerular basement membrane (anti-GBM) disease or Goodpasture syndrome is characterized by pulmonary hemorrhage, glomerulonephritis, or both.

Pathognomonic to Goodpasture syndrome are autoantibodies to the renal glomerular and lung alveolar basement membrane. The specific autoantigen is the 235 amino acid carboxy-terminal noncollagenous domain of type IV collagen. Urinalysis of affected subjects reveals proteinuria, dysmorphic RBCs, WBCs, and RBC cellular and granular casts. Diagnostic testing includes the detection of anti–GBM antibodies. In cases in which only the presence of diffuse alveolar hemorrhage occurs, antibodies might not be present. In those cases the diagnosis is established by demonstrating linear immunofluorescence in lung tissue.

Other autoimmune diseases with pulmonary manifestations. Many autoimmune diseases have pulmonary complications. For example, extra-articular manifestations of RA include parenchymal lung disease with nodules, diffuse interstitial lung disease, or both; obliterative bronchiolitis; or bronchiectasis. Pleural effusions and pleurisy can be bilateral in up to one quarter of cases of RA. Pleurisy is very common in patients with SLE. Other pulmonary diseases in patients with SLE include pneumonitis, pulmonary hemorrhage, and shrinking lung syndrome. These pulmonary processes can cause cough with hemoptysis and dyspnea. The aPL syndrome's pulmonary manifestations consist of pulmonary thromboembolism and pulmonary hypertension. Scleroderma pulmonary manifestations of interstitial lung disease and pulmonary hypertension are the leading cause of death in systemic sclerosis. Lastly, cases of idiopathic inflammatory myositis (PM, DB, and IBM) have varying lung disease, primarily that of interstitial lung disease.

The astute physician will be aware of the patient's symptoms so that he or she can pursue further investigations into the cause of pulmonary disease. Remembering that chronic lung disease can be associated with spurious RF autoantibodies, one must test for serum autoantibodies that are specific to a disease state. Anti-CCP antibodies help in the diagnosis of RA if accompanied by radiographic evidence demonstrating erosive disease and clinical evidence of joint involvement. High-titer ANA with anti-DNA anti–extractable nuclear antigen autoantibodies are helpful in the diagnosis of SLE, particularly if hypocomplementemia exists and the urine shows hematuria, proteinuria, and cellular elements. The presence of aPL antibodies can be confirmed with a positive lupus anticoagulant test result, anti-cardiolipin antibody, or β_2-glycoprotein antibody. The diagnosis of scleroderma is assisted with anti–SCL-70 antibodies or anti-centromere antibodies. For suspected lung disease associated with a myopathy, increased CK, AST, ALT, or aldolase levels in conjunction with the presence of a myositis-specific autoantibody are very helpful.[8]

Inflammatory or immune-mediated renal disease

Amyloidosis. Amyloidosis describes a group of disorders characterized by the extracellular tissue deposition of a variety of low-molecular-weight proteins called amyloid. Currently, there are more than 25 types of amyloid. The most frequent is type AL, which is found in primary amyloidosis, as well as in myeloma-associated amyloidosis. Type AA is found in secondary amyloidosis and is associated with chronic infections or inflammatory disease and some periodic fever syndromes, such as familial Mediterranean fever. Deposition of AL and AA occurs primarily in the kidneys (causing asymptomatic proteinuria, nephrotic syndrome, renal failure, and end-stage renal disease), heart (cardiomyopathy, systolic or diastolic dysfunction, heart block, and angina or infarct), liver (hepatomegaly), and gastrointestinal tract (gastroparesis, constipation, bacterial overgrowth, malabsorption, and intestinal pseudo-obstruction).

Amyloidosis from chronic inflammation will reveal an increased ESR and CRP level, clues to suggest inflammation is present and amyloid should be considered as a cause of organ dysfunction. Testing serum levels of type A amyloid is possible in research laboratories. Diagnosis is confirmed by means of tissue or aspiration biopsy of the affected organ, looking for birefringent material on Congo red stain.

Henoch-Schönlein purpura and IgA nephropathy. Henoch-Schönlein purpura is an immune-mediated vasculitis associated with IgA deposition and is the most common form of systemic vasculitis in children. Signs and symptoms include palpable purpura, arthritis, arthralgias, abdominal pain, and renal disease. Adults with Henoch-Schönlein purpura have presentations similar to those of children and are at increased risk for developing significant renal disease. Renal disease presents similarly to IgA nephropathy and is characterized by deposition of IgA immune complexes causing glomerulonephritis.

Diagnosis by using laboratory tests can include increased levels of serum IgA, an increased ESR and CRP level, normochromic normocytic anemia, and urinalysis showing RBCs or WBCs, cellular casts, and proteinuria. Renal biopsy discloses IgA deposition in the mesangium.

SLE. SLE is an autoimmune disease that can affect most organs. Renal involvement occurs with a high incidence, and clinical features include hematuria; proteinuria; nephrotic syndrome, nephritic syndrome, or both; acute renal failure; and chronic renal failure. Analysis of urine can show various casts (ie, RBC, WBC, granular, and waxy) in addition to oval fat bodies. In patients with active disease, both the classic and alternative complement cascades are activated, resulting in decreased serum levels of complement components (C3 and C4 are typically tested). Most patients with lupus nephritis will have high titers of anti-dsDNA autoantibodies.[20]

Anti-GBM disease. As described previously in the immunologic lung disease section of this chapter, anti-GBM disease or Goodpasture syndrome is characterized by anti-GBM autoantibodies. Patients presenting with renal involvement often have abrupt onset of oliguria or anuria, hematuria, and anemia. Renal biopsy can reveal crescents in more than 50% of the glomeruli on light microscopy (LM). Immunofluorescence demonstrates linear deposition of IgG along the glomerular capillaries and occasionally the distal tubules. Rarely, IgA or IgM can be present. Anti-GBM antibodies are also detected in the serum by means of immunofluorescence or ELISA. Many patients are also found to have positive results for ANCAs, particularly pANCA/MPO.

WG and MPA. As discussed previously, this small- to medium-sized vasculitis is able to cause inflammation in several tissues. Vasculitis often involves the kidney, causing proteinuria, hematuria, cellular casts, hypoalbuminemia, and renal failure. Serologic testing shows WG and MPA to be ANCA-associated diseases with specificity of the ANCA to PR3 in WG and the ANCA to MPO in MPA.

Other autoimmune diseases with renal manifestations. In a similar fashion discussed previously in which autoimmune diseases cause pulmonary disease, the same scenario is seen with autoimmune disease and renal involvement. RA, aPL antibody syndrome, scleroderma, and Sjögren syndrome all have known renal complications. Proteinuria might be the first clue that the kidney is involved from the result of chronic inflammation or immune complex deposition. Persistent inflammation seen in patients with

TABLE IV. Common cell markers

Marker	Cell type	Comment
CD3	T cells	Expressed on all T cells
CD4	T-cell subset	Helper/inducer T cells
CD8	T-cell subset	Cytotoxic T cells: expressed by up to one third of NK cells
CD19 or CD20	B cells	
CD16	NK cells	Some NK cells might not express CD16
CD56	NK cells	Expressed on majority of NK cells

NK, Natural killer.

RA can cause a membranous nephropathy, which is the result of reactive amyloid deposition. One must also remember that therapies to treat inflammatory disease, as seen in patients with RA, such as medications like gold or penicillamine, can cause proteinuria. Vasculitis lesions can also occur in patients with severe RA. Therefore a vigilant watch of renal function with frequent urinalysis is critically important in monitoring the autoimmune patient.[21]

Immunologic neuromuscular disease

PM, DM, and IBM constitute the IIMs. Although the cause is not well defined, muscles become inflamed as the result of both humoral and cellular immune dysfunction, causing lymphocytic infiltration and muscle damage. These myopathies differ in their cause, clinical presentation, and histology.

PM is defined by symmetric proximal muscle weakness, increases in muscle enzyme levels, characteristic electromyographic findings, and a muscle biopsy specimen that shows inflammation. The illness is progressive, with clinical symptoms that can include myalgias, dysphagia, and dyspnea. DM mimics PM with the addition of skin rashes, such as a heliotrope rash and Gottron papules. The rate of malignancy is increased in patients with IIM and more so in patients with DM and can precede, coincide with, or postdate the diagnosis. Therefore screening for malignancy is very important. IBM is the most common form of IIM in patients older than 50 years. Features that set IBM apart from other forms of myositis are asymmetry and distal involvement, particularly affecting the foot extensors and finger flexors. The disease tends to be indolent in its progression and resistant to therapy.

Diagnostic testing for myositis includes common laboratory tests (CBC and complete metabolic panel), serologies, imaging studies, and muscle biopsy. Levels of serum muscle enzyme are increased, such as CK and what are most often thought of as the subject of liver function tests: AST, ALT, and lactate dehydrogenase (LDH). The AST, ALT, and LDH actually reflect muscle disease, as well as liver disease. CK levels usually increase to 10- to 100-fold of normal values, and transaminase levels can increase to 10-fold of the normal value. Myositis-specific antibodies are found in approximately 50% of affected patients. These antibodies are listed in Table IV and are helpful in predicting the future course, prognosis, or both of disease. For example, anti-tRNA synthetase antibodies (eg, anti–Jo-1 antibodies) are strong predictors of interstitial lung disease. Genetic risk factors for the development of myositis include the alleles HLA-DRB1*0301 and HLA-DQA1*0501. Muscle biopsy is helpful to distinguish PM, DM, and IBM. PM typically demonstrates a lymphocytic infiltration seen mostly within the fascicles (endomysial inflammation), some fiber necrosis, and degenerative and regenerative fibers. MHC class I antigens on fibers identified by means of immunohistochemistry can also be seen. In patients with DM, perifascicular atrophy is common. The main features of IBM include endomysial inflammation, vacuolization ("red-rimmed vacuoles" on Gomori trichrome stain), and loss of muscle fibers. Large, atrophic, or angulated fibers are also present.[8,10,21-23]

Hematologic disease

Autoimmune hemolytic anemia. Autoimmune hemolytic anemia (AIHA) is characterized by increased erythrocyte destruction, decreased red cell survival, or both caused by autoantibodies directed against self-antigens on red cells. Two major types of AIHA exist: warm and cold. AIHA caused by the presence of warm agglutinins is almost always due to IgG antibodies that react with protein antigens on the erythrocyte surface at body temperature (37°C). The cause of warm AIHA includes infections (often in children), autoimmune disease (SLE), malignancies of the immune system (non-Hodgkin lymphoma and chronic lymphocytic leukemia), prior allogenic blood transfusion, and certain drugs (cephalosporins, hydralazine, isoniazid, sulfonamides, tetracycline, and triamterene). Signs and symptoms of mild warm AIHA include anemia, occasional jaundice, and mild-to-moderate splenomegaly, with severe cases also involving fever, tachypnea, tachycardia, angina, or heart failure. Peripheral blood examination can show leukopenia, neutropenia, or thrombocytopenia, but often patients have a normal platelet count, neutrophilia, and mild leukocytosis. Peripheral blood smear reveals polychromasia, spherocytosis, fragmented and nucleated erythrocytes, and sometimes erythrophagocytosis by monocytes in severe cases. Reticulocytosis is commonly present. Serum haptoglobin levels are decreased, and LDH levels are increased. Diagnosis depends on the demonstration of immunoglobulin, complement proteins, or both bound to the patient's erythrocytes through the direct antiglobulin test.[24-27]

The other major form of AIHA is due to cold-reactive antibodies and includes the cold agglutinin syndrome and paroxysmal cold hemoglobinuria. In patients with cold-reactive AIHA, the antibodies exhibit a greater affinity for erythrocytes at temperatures of less than 37°C and cause RBC membrane injury by activating complement. The majority of cold-reactive autoantibodies are cold agglutinins.[28]

Cold agglutinin syndrome usually occurs in middle-aged and elderly persons, with IgM the responsible immunoglobulin. Signs and symptoms are chronic anemia, dark urine, acrocyanosis, pallor, and jaundice. Laboratory testing includes mild-to-moderate anemia with prominent autoagglutination, abnormal erythrocyte morphology, reticulocytosis, jaundice, hemoglobinuria, and erythroid hyperplasia in the bone marrow.

Paroxysmal cold hemoglobinuria frequently occurs in children, often after an upper respiratory tract infection, and IgG is typically the causative immunoglobulin. Acute attacks are often severe, but the illness usually resolves spontaneously within a few days to several weeks. Signs and symptoms consist of fever, malaise, abdominal pain, dark-colored urine, jaundice, and pallor. Laboratory findings show anemia (often severe), reticulocytosis, abnormal RBC morphology, hemoglobinuria, usually erythroid hyperplasia, leukocytosis, and a platelet count that is normal or slightly increased.

Vascular thrombotic disorders. aPL syndrome is a thrombophilic disease defined by 2 major components: the presence of at least 1 type of autoantibody (aPL antibody, as mentioned earlier in this chapter), which is directed against phospholipid-binding plasma proteins, and the occurrence of at least 2 of several clinical features (recurrent fetal loss, arterial thromboses, or thrombocytopenia). Primary aPL antibody syndrome is diagnosed in the absence of other underlying diseases, and secondary aPL antibody syndrome is diagnosed if another illness (eg, SLE) is identified. Therapy consists of anticoagulation and treatment of any coexisting illness that might give rise to aPL antibodies.

Thrombocytopenia (idiopathic thrombocytopenia and posttransfusion purpura). Immune or idiopathic thrombocytopenic purpura is an acquired disorder without a clear cause. Both acute and chronic forms occur. The pathogenesis is thought to occur through a combination of increased platelet destruction and inhibition of megakaryocyte platelet production by specific IgG autoantibodies. Clinical manifestations are variable, can be abrupt or acute, and often are insidious. Symptoms include bleeding, ranging from petechiae and easy bruising to severe bleeding diathesis. Intracranial hemorrhage is quite rare. Children often exhibit symptoms after a viral or bacterial infection. Thrombocytopenia is noted on laboratory work. Bone marrow cellularity is normal, with normal erythropoiesis and myelopoiesis. Megakaryocytes are present in normal to increased numbers. However, bone marrow examination is not usually required unless another disease is suspected or if the patient is greater than 60 years of age because of concern for myelodysplasia.

Posttransfusion purpura is a rare condition that usually develops 7 to 10 days after an RBC transfusion. The syndrome is characterized by severe thrombocytopenia and bleeding caused by alloimmunization to human platelet-specific antigens after a blood component transfusion. Patients are usually middle-aged multiparous women, although posttransfusion purpura has also been reported in male subjects. Most cases occur with the formation of human platelet antigen 1a antibodies. These antibodies destroy transfused HPA-$1a^{+}$ cells and the negative recipient's cells through a poorly understood antiplatelet mechanism.[29]

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Pulmonary disorders, including vocal cord dysfunction

Paul A. Greenberger, MD, and Leslie C. Grammer, MD *Chicago, Ill*

The lung is a very complex immunologic organ and responds in a variety of ways to inhaled antigens, organic or inorganic materials, infectious or saprophytic agents, fumes, and irritants. There might be airways obstruction, restriction, neither, or both accompanied by inflammatory destruction of the pulmonary interstitium, alveoli, or bronchioles. This review focuses on diseases organized by their predominant immunologic responses, either innate or acquired. Pulmonary innate immune conditions include transfusion-related acute lung injury, World Trade Center cough, and acute respiratory distress syndrome. Adaptive immunity responses involve the systemic and mucosal immune systems, activated lymphocytes, cytokines, and antibodies that produce $CD4^+$ T_H1 phenotypes, such as for tuberculosis or acute forms of hypersensitivity pneumonitis, and $CD4^+$ T_H2 phenotypes, such as for asthma, Churg-Strauss syndrome, and allergic bronchopulmonary aspergillosis.
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Key words: *Innate, acquired, hypersensitivity, eosinophilia, lymphocyte, tuberculosis, aspergillosis, bronchopulmonary, bronchiectasis, immunologic*

Abbreviations used

ABPA:	Allergic bronchopulmonary aspergillosis
ANCA:	Antineutrophil cytoplasmic antibody
ARDS:	Acute respiratory distress syndrome
BAL:	Bronchoalveolar lavage
COPD:	Chronic obstructive pulmonary disease
CSS:	Churg-Strauss syndrome
CT:	Computed tomography
FVC:	Forced vital capacity
HDAC:	Histone deacetylase
LT:	Leukotriene
PMN:	Polymorphonuclear leukocyte
RADS:	Reactive airways dysfunction syndrome
TLR:	Toll-like receptor
TRALI:	Transfusion-related acute lung injury
VCD:	Vocal cord dysfunction

Pulmonary disorders can be organized according to whether the primary immune responses are characterized by innate or adaptive immune responses. The innate responses use complement activation or activation of polymorphonuclear leukocytes (PMNs) and occur without a period for sensitization. The adaptive responses include T_H1 or T_H2 lymphocytes, eosinophils, antibody mediated, and granuloma formation.[1] This chapter will review the various pulmonary disorders with a predominant immunologic pattern and also discuss vocal cord dysfunction (VCD), which can coexist with asthma or occur independently and results in cough, shortness of breath, and dyspnea.

INNATE IMMUNE RESPONSES

Transfusion-related acute lung injury

Transfusion-related acute lung injury (TRALI) is a nonhemolytic transfusion reaction that occurs within 10 minutes to as long as 6 hours after infusion of a blood product and causes very severe noncardiogenic pulmonary edema, cyanosis, arterial hypoxemia, and respiratory failure.[2,3] The donor plasma typically contains antibodies to human neutrophil antigens or HLA class I or II antigens.[2,3] Neutrophil alloantibodies are found in 10% to 20% of female donors and 1% to 4% of male donors, yet the incidence of TRALI is about 1:5000 transfusions.[3] Alloantibodies are generated during pregnancy, but of course that would not explain the presence of such antibodies in men. Some recipients have antineutrophil antibodies. The immediate reaction, which might resemble anaphylaxis, involves sequestration of PMNs in the pulmonary vasculature, complement activation, and generation of TGF-β, IL-8, and IL-13.[2] Immune complexes activate PMNs and cause disruption of the endothelium barrier to plasma. TRALI is extremely rare after intravenous immunoglobulin infusions but occurs with infusions of platelets (suspended in plasma), whole blood, cryoprecipitates, and fresh frozen plasma.

The immediate management includes stopping the infusion, oxygen, mechanical ventilation if indicated, and treatment of hypotension with vasopressors. Donors should be deferred from future donations. Indeed, some transfusion experts have recommended that the donor pool should not include women who have been pregnant and that donor plasma be tested for alloantibodies.[2,3] Neither of these suggestions are standard practice.

Acute respiratory distress syndrome and acute lung injury

Acute respiratory distress syndrome (ARDS) and acute lung injury represent diffuse pulmonary disease that can be fatal.[4] ARDS is a more severe form of acute lung injury. Causes include sepsis, pneumonia, trauma, or aspiration pneumonia.[4] Patients experience severe dyspnea, tachypnea, and hypoxemia. The chest roentgenogram and computed tomographic (CT) examination demonstrate bilateral infiltrates, alveolar consolidation, and "white out" of the lung. The alveoli collapse as they become filled with protein and fibrin-rich exudates (hyaline membranes), which inactivate surfactant.[4,5] Neutrophils release oxidant proteases, which damage the capillary endothelium. Bronchoalveolar lavage (BAL) reveals the presence of PMNs, procoagulant activity, IL-8 (chemotactic for PMNs), IL-2, IL-6, and TGF-β. There is reduced apoptosis of

From the Division of Allergy-Immunology, Department of Medicine, Northwestern University Feinberg School of Medicine.

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Address for reprints: Paul A. Greenberger, MD, Division of Allergy-Immunology, 676 N St Clair Street, #14018, Chicago, IL 60611. E-mail: p-greenberger@northwestern.edu.

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PMNs, which is attributable to increased concentrations of BAL fluid IL-2, IL-8, granulocyte colony-stimulating factor, GM-CSF, and growth-related oncogene α.[6] Alternatively, there is enhanced apoptosis of epithelial cells, resulting in the lack of a sufficient barrier between the alveoli and capillaries. TNF-related apoptosis-induced ligand levels are increased in BAL fluid in patients with ARDS and are recognized as proapoptotic for epithelial cells.[6]

Patients requiring mechanical ventilation benefit from smaller volumes, such as a tidal volume of 6 mL/kg, with positive end-expiratory pressures of 5 to 10 cm H_2O. Fluid replacement should be conservative. Corticosteroids and other interventions, such as nitric oxide and surfactants, are not effective.[7]

Community-acquired pneumonia

Community-acquired pneumonia presents with a productive cough, fever, pleuritic chest pain, and abnormal chest roentgenographic results.[8] On auscultation, there can be crackles and bronchial breath sounds. Most pathogens include viruses, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Mycoplasma pneumoniae*, *Staphylococcus aureus*, and *Legionella pneumophila*.[8] There might be no recovered organisms in some patients. Comorbidities influence survival.[8]

Levels of proinflammatory cytokines, such as TNF-α and IL-6, and the anti-inflammatory cytokine IL-10 are increased in those patients who succumb compared with survivors.[9] Impaired recognition of molecular patterns of bacteria is associated with decreased activation of innate immunity and worse clinical outcomes.[10] Toll-like receptors (TLRs) recognize pathogen-associated molecular patterns, and genetic polymorphisms have been identified in patients who had invasive *S pneumoniae* infections.[10] For example, polymorphisms of TLR4 impair its function in recognition of *S pneumoniae* pneumolysin, whereas polymorphisms of CD14, a coreceptor on monocytes for both TLR2 and TLR4, are associated with invasive *S pneumoniae* infections.[10] Polymorphisms in FCγRIIA increase the susceptibility to invasive disease. Current therapy includes early administration of antibiotics and supportive care. Future diagnosis might identify at-risk subjects proactively, and therapies will be able to strengthen the innate immune system.

NONINFECTIOUS PULMONARY CONDITIONS

Byssinosis occurs from the inhalation of dusts from flax, cotton, sisal, and hemp. The dusts produce bronchoconstriction, typically on the first day of the workweek, but then tachyphylaxis develops with continued exposure. Byssinosis is not asthma or hypersensitivity pneumonitis.[1] At-risk workers include those who are exposed to endotoxin during the processing of raw cotton. In contrast, workers who spin cotton are not exposed to the high concentrations of endotoxin and are considered at low risk. Long-term exposure can result in symptoms of chronic bronchitis and cough. Modest reductions in FEV_1 and forced vital capacity (FVC) have been found, but concurrent smoking appears to be the major contributor as opposed to workplace exposures. Prevention includes methods to reduce the generation of endotoxins from gram-negative bacteria by reducing exposure to waste from cotton.

In contrast to byssinosis, the organic toxic dust syndrome is a toxic alveolitis that produces influenza-type symptoms of sudden-onset headache, chills, nonproductive cough, myalgias, arthralgias, and dyspnea. Crackles can be present on lung auscultation. The onset of symptoms is within 12 hours of inhalation of organic dusts. Although the clinical presentation might mimic that of acute hypersensitivity pneumonitis, there is no requirement for prior exposure or immunologic sensitization (see the later section on hypersensitivity pneumonitis). Various circumstances of exposure have been described, such as from organic mulch, endotoxin-rich vegetables and grass seeds, and contaminated seaweed. Massive inhalation of microbial products can cause an ARDS-like presentation, and this is designated as organic dust toxic syndrome or pulmonary mycotoxicosis.[11]

In patients with silo-unloader's disease, there is inhalation of nonorganic gases, such as NO, NO_2, or N_2O_4. These nitrogen oxides then generate nitric and nitrous acids that cause noncardiac pulmonary edema and, in some patients, methemoglobinemia. Deaths can occur, whereas survivors might have bronchiolitis obliterans.

Grain-handler's disease occurs in agricultural workers with a chronic cough, symptoms of chronic bronchitis, or wheeze after exposure to grain dusts. Concurrent cigarette smoking appears to be more injurious to the lung and associated with reductions in spirometric values. Measures to reduce exposure to dust are beneficial. Because of less implementation of safety standards, there is a major concern that workers will experience grain-handler's disease and other respiratory disorders in the world's emerging economies.

Reactive airways dysfunction syndrome

The reactive airways dysfunction syndrome (RADS) describes a single unexpected inhalation of high concentrations of irritant fumes, vapors, fog, or smoke that results in acute cough, dyspnea, and wheezing within 24 hours.[12] An asthma-like syndrome begins that can last for months or years. Bronchial hyperreactivity can be demonstrated by means of methacholine challenge testing, and spirometry reveals normal or decreased FEV_1, FVC, and FEV_1/FVC ratio. There might be little to no bronchodilator response to albuterol. Bronchial biopsy specimens demonstrate loss of epithelium, subepithelial fibrosis, and infiltrates with lymphocytes but not eosinophils (as would be characteristic of asthma).

RADS might be confused with occupational asthma, where there is a sensitization period of months or years before symptoms begin, and with aggravation of underlying asthma. But RADS refers to the acute irritant-induced asthma.

World Trade Center cough

The first responders to the 2001 collapse of the World Trade Center in New York City experienced a very troublesome cough within 24 hours of beginning rescue operations.[13,14] The exposures included acrid smoke, fires that burned for 3 months, asbestos, glass fibers, lead, and aromatic compounds. Many responders did not use protective masks. Subsequent evaluations identified methacholine hyperreactivity in 24% and a reduced FEV_1/FVC ratio of less than 0.75 in 16% of affected subjects.[13] The high exposures would be consistent with a diagnosis of RADS in some subjects.[14]

VCD

VCD is a form of "functional" or nonanatomic upper airway obstruction.[15] The inspiratory tracing on a flow-volume loop is truncated (Fig 1) or incompletely performed. Other causes of nonanatomic inspiratory obstruction include vocal cord paralysis,

neuromuscular disorders, and sleep disorders.[15] In contrast, some anatomic abnormalities that cause a truncated inspiratory loop include a large goiter, tracheal stenosis, and an obstructing tumor. Symptoms of VCD include dyspnea, wheeze, tightness in the neck, shortness of breath, inability to breathe deeply or satisfactorily, and coughing. Some patients with VCD have concurrent asthma and chronic rhinosinusitis with postnasal drainage or gastroesophageal reflux or atypical (laryngopharyngeal) reflux. VCD can be intermittent and might not be present when the patient is distracted, sedated, or asleep. VCD can masquerade as or coexist with severe asthma.[16]

Recognition of VCD might begin with the truncated inspiratory loop of the flow-volume tracing, especially when the patient is symptomatic. Alternatively, it can be suspected when the patient's difficulty breathing surpasses the physical findings, such as clear chest on auscultation, wheezes over the neck but not lower airways, whispering instead of talking loudly, and refusal to inspire to total lung capacity or produce an appropriate forced expiratory maneuver. Bronchoscopy might be of value in excluding other causes. Fiberoptic laryngoscopy can help demonstrate the adduction of vocal cords during inspiration. When methacholine challenge tests are performed in patients with VCD, there might or might not be apparent flattening of the inspiratory flow-volume loop or, in fact, quite severe airways obstruction, even stridor or respiratory arrest. The latter can occur in patients with major psychiatric diagnoses and even should be anticipated in considering a methacholine challenge test in such patients with VCD.

Some patients benefit from speech therapy, which can emphasize breathing through the abdomen as opposed to thoracic breathing. Nevertheless, other patients with psychologic or psychiatric conditions might not overcome their VCD. When this is the case, it is important to avoid continued treatment with systemic corticosteroids unless it is demonstrated that there is both persistent asthma and VCD.

GRANULOMATOUS T_H1 INFLAMMATORY CONDITIONS

The granulomatous T_H1 conditions comprise sarcoidosis, tuberculosis, berylliosis, and hypersensitivity pneumonitis. CD4 T_H1 lymphocytes participate in granuloma formation. Some cytokines include IL-2, IL-12, and IFN-γ. IFN-γ, which is generated by CD4 T_H1 and $CD8^+$ lymphocytes, can be measured in patients with tuberculosis, and the US Food and Drug Administration has approved an assay that helps in the diagnosis of tuberculosis.[17] Class I MHC–restricted $CD8^+$ lymphocytes can function as memory cells to *Mycobacterium tuberculosis*.[18] In patients with advanced pulmonary tuberculosis, the BAL fluid reveals increased numbers of $CD4^+$ lymphocytes and increased $CD4^+$/$CD8^+$ ratios. There is evidence for pulmonary sequestration or compartmentalization of the $CD4^+$ lymphocytes because the peripheral blood $CD4^+$ lymphocytes can be decreased relatively and the $CD4^+$/$CD8^+$ ratio is reduced because of increases in the numbers of $CD8^+$ lymphocytes.[19] In patients with HIV/AIDS, the low numbers of $CD4^+$ lymphocytes are associated with greater susceptibility and more severe tuberculosis,[20] including decreased delayed hypersensitivity responses (type IVa1).

Granulomas help limit the replication of mycobacteria; however, lung architecture is destroyed in the process. $CD4^+CD25^+$ regulatory T cell numbers are increased in patients with tuberculosis and are thought to help control or attempt to control the intensity of the $CD4^+$ T_H1 granulomatous responses.[21] The expression of the transcription factor forkhead box protein 3 is increased and is indirect evidence of regulatory T-cell suppression of the granulomas.

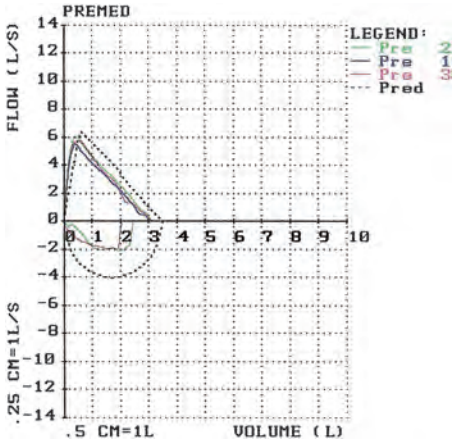

FIG 1. Flow-volume loop of a 26-year-old woman with shortness of breath, wheezing, and cough. Note blunting of the inspiratory phase versus predicted value. FVC was 3.19 L (91%), FEV_1 was 2.75 L (91%), and FEV_1/FVC ratio was 0.86. Notably, forced expiratory flow at 50%/forced inspiratory flow at 50% of FVC was increased at 1.62 (normal value is <1).

Sarcoidosis remains a disease of unknown cause that produces noncaseating, epithelioid granulomas that can affect most organ systems.[22] BAL fluid recoveries demonstrate very high numbers of activated $CD4^+$ lymphocytes, which are sustained by IL-2.[22] $CD4^+$ T_H1 lymphocytes participate in formation of the granuloma, in association with IFN-γ, and activated macrophages. IL-18, derived from monocyte/macrophages and airway epithelial cells, upregulates expression of IL-2 and supports IFN-γ production.[23] IL-18 levels are increased in BAL fluid and plasma and have been associated with progression of sarcoidosis.

Although not all patients are treated because up to two thirds have a spontaneous remission, initial pharmacotherapy is with oral corticosteroids. In an attempt to reduce the granulomatous response, TNF-α inhibitors have been administered to patients with sarcoidosis[22]; their role is not established, however. Endobronchial sarcoidosis is a rare cause of cough and wheezing.

GRANULOMATOUS T_H2 INFLAMMATORY CONDITIONS

Churg-Strauss syndrome (CSS) is a systemic, necrotizing, eosinophil-laden granulomatous vasculitis. The presentation can be that of (1) asthma with pulmonary infiltrates, (2) peripheral blood eosinophilia, (3) peripheral neuropathy (mononeuritis multiplex), or (4) palpable purpura. When a patient with asthma experiences palpable purpura on the shins or upper extremities or if foot or wrist drop occurs, CSS should be suspected. A decrease in oral corticosteroids or in high-dose inhaled corticosteroids might be associated with onset of fever and eosinophilic pneumonia, purpura, or wrist drop, any of which should raise the possibility of CSS. Histologic evidence for CSS can be obtained by means of skin biopsy or biopsy of nerves (eg, sural) or pulmonary tissue.

Laboratory findings demonstrate peripheral blood eosinophilia (20% to 60%), $CD4^+$ T_H2 lymphocytes, increased total IgE concentrations, and antineutrophil cytoplasmic antibodies (ANCAs). Approximately 60% of patients have the perinuclear pattern of

ANCAs, which on ELISA is positive for antibodies to myeloperoxidase, whereas 10% of patients have positive results for cytoplasmic staining, with antibodies directed against proteinase-3.[1] Although the presence of a perinuclear pattern of ANCAs is helpful in supporting a diagnosis, the ANCA titers do not provide prognostic information for disease management.[24,25] Similarly, eosinophil-derived major basic protein and cationic protein have not been demonstrated to have utility in guiding treatment.[24] Urinary concentrations of leukotriene (LT) E_4, the major metabolite of LTC_4 and LTD_4, and eosinophil-derived neurotoxin and 3-bromotyrosine, a marker for oxidation of eosinophils, are increased in patients with CSS.[26]

The 6-year survival has been reported to be 70%.[24] Long-term survival, up to 26 years, has also been reported.[27] The most effective therapy has been with oral corticosteroids.[24,27] Additional corticosteroid-sparing and immunosuppressive therapies include cyclophosphamide, azathioprine, IFN-α, mepolizumab (anti–IL-5), omalizumab (anti-IgE), and rituximab (anti-CD20 B lymphocytes). There are potential untoward effects from cyclophosphamide (cytopenias, hemorrhagic cystitis, and malignancy potential), azathioprine (cytopenia, nausea, and vomiting), and IFN-α (depression and progressive multifocal leukoencephalopathy). Often patients can be managed long-term with prednisone administered on an alternate-day schedule with or without immunosuppressive therapy, such as with azathioprine. Abrupt discontinuation of prednisone is not advisable because it can result in fever, eosinophilia, and pulmonary infiltrates within a few days, demonstrating that the CSS might be controlled but is not in remission.

T_H1-RELATED INFLAMMATORY CONDITIONS

Hypersensitivity pneumonitis

Hypersensitivity pneumonitis is a $CD4^+$ T_H1 and $CD8^+$ lymphocyte–predominant alveolitis that results in noncaseasting granulomas and pulmonary fibrosis. Clinical stages include acute, subacute (clinically similar to acute), and chronic. In the acute and subacute stages inhalation of organic antigens causes cough, shortness of breath, myalgias, and fever within 4 to 6 hours. The physical examination would reveal pulmonary crackles. A patient might self-treat for "flu" or be given an improper diagnosis of community-acquired pneumonia. When there is continued or repeated exposure to antigens, such as bird excreta, patients might have subacute episodes or evolve into chronic hypersensitivity pneumonitis where typical flu-like illness does not occur. The latter patients experience a nonproductive cough and progressive dyspnea and, in advanced cases, oxygen requirements. Pulmonary function tests in the acute and subacute stages typically are described as restrictive; however, especially with bird fanciers, obstructive findings can occur and mimic asthma. The restrictive findings are associated with a decreased diffusing capacity for carbon monoxide. In contrast, the diffusing capacity for carbon monoxide in patients with asthma is normal or even increased.

High-resolution CT scans demonstrate small nodules (<5 mm) that indicate alveolitis or areas of pulmonary fibrosis. Mosaic findings of fibrosis are present in patients with chronic hypersensitivity pneumonitis. An example of pulmonary fibrosis and traction bronchiectasis from avian hypersensitivity pneumonitis is shown in Fig 2.

There is striking BAL lymphocytosis of 60% to 80% from acutely ill patients.[28,29] The classic finding is a CD4/CD8 ratio of less than 1, whereas in patients with sarcoidosis, the CD4/CD8 ratio is as high as 8 because of the $CD4^+$ alveolitis.[30] In patients with hypersensitivity pneumonitis, levels of T_H1 cytokines are increased, including IL-12, IL-18, and TNF-α. $CD8^+$ lymphocytes serve as effector cells but are not sufficiently functional.[28,31,32] In contrast, in patients with chronic hypersensitivity pneumonitis, there can be an increase in the CD4/CD8 ratio as the CD4 (and T_H2) lymphocytes increase and $CD8^+$ lymphocytes decrease.[32] It has been suggested that the effector $CD8^+$ lymphocytes become "exhausted." These data suggest that chronic hypersensitivity pneumonitis is associated with "skewing" toward T_H2 lymphocytes, IL-4 production, and pulmonary fibrosis.[32] IL-17, which is proinflammatory, increases activation and numbers of neutrophils, and upregulates IL-6, IL-8, and TNF-α, might participate in hypersensitivity pneumonitis.[33,34]

Treatment includes early identification of patients with hypersensitivity pneumonitis, avoidance/remediation of the antigens involved, oral corticosteroids for short-term use, and monitoring of overall respiratory status depending on the stage that is present.

Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) is characterized by fixed dyspnea, lack of fully reversible airways obstruction, and progressive loss of FEV_1 over time. Cessation of cigarette smoking and use of oxygen have proved of value. Pharmacotherapy includes short- and long-acting bronchodilators and anticholinergic medications. For patients with moderate–to–very severe COPD, when the FEV_1 is less than 50% and the FEV_1/FVC ratio is less than 70%, combination inhaled corticosteroid/long-acting β-agonist therapy is recommended. Treatment with combination fluticasone propionate and salmeterol has resulted in fewer exacerbations but not fewer deaths.[35] In a study of patients with COPD in whom fluticasone/salmeterol or salmeterol was added to tiotropium, there was no additional benefit over tiotropium in the primary outcome of exacerbations of COPD.[36] Secondary outcomes did demonstrate increases in FEV_1, fewer hospitalizations, and improved quality-of-life measures in those patients receiving fluticasone/salmeterol.[36] An unexpected finding has been increased numbers of cases of pneumonia in patients with COPD receiving high-dose fluticasone propionate.[35,37]

The pathogenesis of COPD includes cigarette smoking (most cases), viral or bacterial infections (or a combination), genetic susceptibility, oxidative stress, and little to no response to high-dose corticosteroids. Sputum often harbors PMNs, but eosinophils can be present with either viral or combined viral and bacterial infections.[38] In patients with COPD, not only is there presence of PMNs and macrophages, there are also increases in CD4 T_H1 and CD8 lymphocyte numbers.[39]

The impaired response to corticosteroids helps differentiates COPD from asthma in most cases. After absorption, the corticosteroid binds to its receptor and traverses the cytoplasm and enters the nucleus, where it interacts with glucocorticoid response elements of DNA.[40] Then corticosteroids can reduce levels of the proinflammatory transcription factors nuclear factor κB and activator protein 1. It is thought that these transcription factors would have been upregulated by viral upper respiratory tract infections. Transcription factors can be generated as the DNA-histone complex "unwinds" during a process of acetylation by histone acetyltransferase.[40] Histone acetyltransferase levels are increased in some but not all cases of COPD, whereas in patients

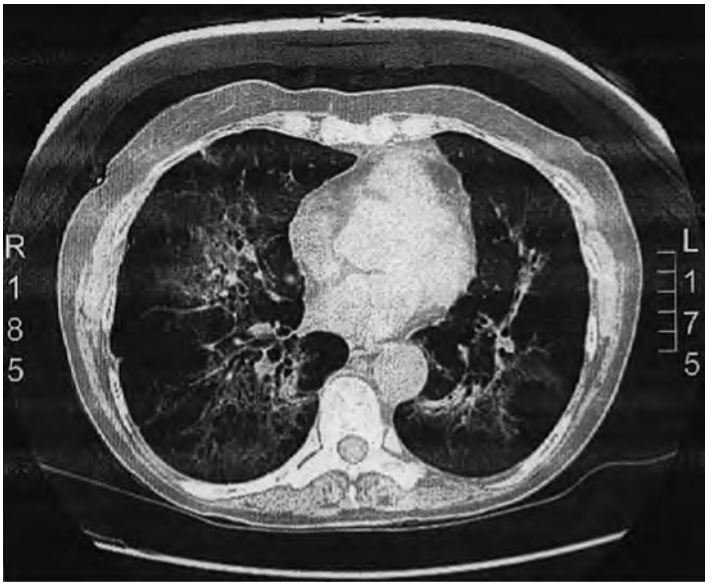

FIG 2. A 62-year-old woman who presented with "uncontrolled asthma" and had pulse oxygenation of 83% on room air reported shortness of breath for 6 years. She had 5 birds at home and worked at an exotic animal house. The CT examination revealed widened (bronchiectactic) bronchi, honeycomb fibrosis, and some opacities near the bronchi. The bronchiectasis occurred because of traction by the lung parenchyma/interstitium on the bronchi. The diffusion capacity of the lung for carbon monoxide was 39%, and the FVC was 74%. FEV_1 was 84% of predicted value, with a 6% improvement with albuterol.

with asthma, they are increased consistently.[40] Gene repression can occur when the DNA is deacetylated by histone deacetylase (HDAC) as the DNA is compacted. HDAC levels are reduced in both patients with COPD and those with asthma, but corticosteroids will increase HDAC levels in patients with asthma but not those with COPD.[40] Lack of deacetylation of the DNA in patients with COPD can favor sustained proinflammatory action and lack of response to corticosteroids, which is in contrast to what occurs in patients with asthma.

T_H2-RELATED INFLAMMATORY CONDITIONS

It has been reported that the half-life of eosinophils in peripheral blood is 8 to 18 hours and 2 to 5 days or longer in tissue.[41] In addition, perhaps there are at least 100 times as many eosinophils in tissue than in peripheral blood.[41] In the bone marrow eosinophils differentiate and proliferate from $CD34^+$ progenitors (see Chapter 6) with the major cytokines IL-3, IL-5, and GM-CSF.[42] Potent chemoattractants for eosinophils include RANTES, CCL11 (eotaxin-1), platelet-activating factor, and LTB_4.[42] The interaction of very late antigen 4 on eosinophils with vascular cell adhesion molecule 1 on endothelium results in firm adhesion to the endothelial cells. During allergic reactions, IL-4, IL-13, and TNF-α will upregulate vascular cell adhesion molecule 1, enhancing this process.

In Table I there is a list of prototype pulmonary eosinophilia syndromes or conditions. One prototype condition is allergic bronchopulmonary aspergillosis (ABPA), which complicates both asthma and cystic fibrosis.[43,44] ABPA might overlap with either hyper-IgE syndrome or chronic granulomatous disease.[45] Patients with asthma who have ABPA typically experience pneumonias or pulmonary infiltrates with eosinophilia (10% to 30%) but not peripheral blood eosinophilia as high as 40% to 60%, which occurs with CSS or parasitism. All patients have immediate skin reactivity to *Aspergillus fumigatus*. Because some commercial mixtures of *Aspergillus* species or mold mixes contain little or no *A fumigatus*, it is advisable to use a reactive extract for screening. Negative skin test results help to exclude ABPA for nearly all patients unless there is an allergic bronchopulmonary mycosis present. High-resolution CT examination demonstrates proximal bronchiectasis (inner two thirds of the lung field) in contrast to the distal bronchiectasis that occurs in some patients with COPD or recurrent infections. In patients with cystic fibrosis, there is proximal and distal bronchiectasis, and such a finding should suggest the possibility of concomitant (usually pancreatic sufficient) cystic fibrosis. In patients with ABPA, the predominant response is that of $CD4^+$ T_H2 lymphocytosis; eosinophilia; increased total serum IgE and anti–*A fumigatus* IgE, IgG, and IgA antibody levels; precipitating antibodies *to A fumigatus* and a genetically restricted susceptibility profile; and increased responsiveness to IL-4 stimulation.[43,46,47]

Treatment includes avoidance/remediation of areas in a home/workplace of obvious mold growth that can occur from unplanned water entry, oral corticosteroids to clear the pulmonary infiltrates and manage asthma, antiasthma medications as indicated, monitoring of the total serum IgE concentration because doubling over baseline values indicates a possible current new pulmonary infiltrate, and assessment of pulmonary function and respiratory status over time.[43] For initial treatment of a patient with newly diagnosed ABPA, the dose of prednisone is 0.5 mg/kg given each morning for 1 to 2 weeks, with conversion to alternate day-therapy for 2 months. The radiographic findings can be expected to clear or be reduced, as demonstrated by means of high-resolution CT examination in 2 months. Then the prednisone can be tapered and discontinued. It is not necessary to continue prednisone indefinitely in the absence of new infiltrates or development of severe (prednisone dependent) asthma. With use of the alternate-day prednisone, serious adverse effects are avoided or minimized.

TABLE I. Pulmonary eosinophilia syndromes or conditions

Asthma (allergic and nonallergic)
Asthma with atelectasis from mucus plugging
ABPA
Allergic bronchopulmonary mycosis
CSS
Collagen vascular disease (rare)
Drug allergy with pulmonary eosinophilia
Eosinophilic pneumonia
Acute (BAL fluid eosinophilia 25% to 60% with little or no peripheral blood eosinophilia)
Chronic (high peripheral blood eosinophilia)
Simple eosinophilia (Loffler syndrome)
Tropical pulmonary eosinophilia
Hypereosinophilic syndromes (interstitial infiltrates and pleural effusions, thromboembolism)
Neoplasms
Parasitism (helminthic)
Sarcoidosis (very rare)

Adapted with permission from Greenberger.[1]

Antifungal therapies have been used for the treatment of ABPA and are considered adjunctive.[48,49] A potentially good candidate for antifungal therapy is a patient with sputum plugs harboring *A fumigatus* despite prednisone therapy. There are reports of the use of omalizumab[50] for patients with ABPA, but it remains to be established whether this treatment will help to prevent new infiltrates or improve asthma symptoms.

Eosinophilic pneumonias are divided into 4 types: acute, chronic, simple, and tropical (Table I). Acute eosinophilic pneumonia can masquerade as severe community-acquired pneumonia and present with respiratory failure. When there is no or little peripheral blood eosinophilia, the diagnosis can be made with bronchoscopy and BAL showing eosinophilia of 25% to 60%. Alternatively, there might be peripheral blood eosinophilia as high as 42%.[51] Drugs, nonprescription products, parasitism, and other causes of widespread pulmonary infiltrates should be considered.

Chronic eosinophilic pneumonia is characterized by respiratory symptoms for at least 2 weeks, peripheral blood eosinophilia of at least 1000/mm^3 or BAL eosinophilia of greater than 25%, and bilateral pulmonary infiltrates.[52] In classic presentations the infiltrates are in the periphery, suggesting the photographic negative of pulmonary edema. Most patients require years of oral corticosteroid treatment. The radiographic infiltrates and surges of peripheral blood eosinophilia can be controlled with modest doses of prednisone.

Simple pneumonia (Loffler syndrome) is a mild condition lasting less than 4 weeks and has transient pulmonary infiltrates.

Tropical pulmonary eosinophilia is characterized by widespread pulmonary infiltrates and high levels of peripheral blood eosinophilia. Mediastinal lymph nodes might be enlarged and can harbor activated eosinophils. Patients typically have lived in endemic areas of parasites before tropical pulmonary eosinophilia occurs.

SUMMARY

The immunologic features of pulmonary disorders can be used to categorize various conditions and provide focus for potential innovative therapies. Although usually there is not a single treatment that antagonizes a critical component of either the innate or acquired immune system and results in clinical improvement, complex conditions might be amenable to immunologically based treatments in the future. A more ambitious goal is primary prevention of many of the pulmonary conditions discussed in this chapter. The ability to diagnose pulmonary conditions and the masquerader of asthma, VCD, continues to improve, which should result in earlier diagnoses and improved outcomes.

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Mucosal immunology, eosinophilic esophagitis, and other intestinal inflammatory diseases

Dan Atkins, MD,[a,b,d] and Glenn T. Furuta, MD[a,b,c,d] *Denver and Aurora, Colo*

The gastrointestinal mucosa constitutes the largest host-environment interface of the body. It uses both innate and adaptive immune mechanisms to provide protection from the diverse onslaught of foods, microbes, and other ingested products. The innate immune system is genetically encoded and evolutionarily ancient, possesses no memory, and lacks diversity. In contrast, the adaptive immune system is quite diverse, develops memory, and undergoes expansion after stimulation. The gastrointestinal mucosa is charged with the difficult task of mounting protective responses against invading microorganisms while simultaneously maintaining an overall state of nonresponsiveness or tolerance to innocuous substances, such as commensal bacteria and food antigens. Perturbation or malfunction of these complex protective mechanisms results in diseases, such as inflammatory bowel diseases, celiac disease, or eosinophilic gastrointestinal diseases. (J Allergy Clin Immunol 2010;125:S255-61.)

Key words: *Mucosal immunity, eosinophilic esophagitis eosinophilic gastrointestinal diseases*

Abbreviations used

DC:	Dendritic cell
EGID:	Eosinophilic gastrointestinal disease
EoE:	Eosinophilic esophagitis
FAE:	Follicle-associated epithelium
Foxp3:	Forkhead box protein 3
GALT:	Gut-associated lymphoid tissue
IBD:	Inflammatory bowel disease
IEL:	Intraepithelial lymphocyte
LP:	Lamina propria
M:	Microfold
NOD:	Nucleotide oligomerization domain
PP:	Peyer patch
TCR:	T-cell receptor
Treg:	Regulatory T

OVERVIEW OF GUT-ASSOCIATED LYMPHOID TISSUE

Mucosa-associated lymphoid tissues comprise the largest immune organ of the body and are active at multiple host-environment interfaces, such as the gastrointestinal tract and the genitourinary and bronchopulmonary systems. A discussion of the site-specific aspects of each component of the mucosa-associated lymphoid tissue is beyond the scope of this Primer, but the reader is referred to a number of outstanding reviews on these topics.[1-6] Here we will briefly review the gastrointestinal mucosal immune system and its gut-associated lymphoid tissue (GALT).

The human gastrointestinal tract is presented daily with a seemingly overwhelming load of diverse substances, including commensal bacteria and dietary antigens. Typically, the GALT is able to discriminate pathogens that require an immediate immune response from normal microbial flora or nutritive products. This process of maintaining a state of nonresponsiveness is known as oral tolerance. The mechanisms that govern tolerance are not only interesting and important aspects of this homeostatic process but are being potentially harnessed as therapeutic approaches for the treatment of certain autoimmune and inflammatory diseases.

The mucosal system is characterized as a site where antigen is selectively sampled and tolerance develops to maintain a state of controlled and protective inflammation. To accomplish these goals, the mucosa is composed of luminal protective molecules, the epithelial barrier, and the immunologically rich lamina propria (LP; Table I). The overall anatomy of the GALT is presented in Fig 1. This general overview shows important elements of the system, including the sampling of luminal antigens by microfold (M) cells, dendritic cells (DCs), and epithelia and the antigen-driven priming and maturation of naive T and B lymphocytes.

ANATOMY OF GALT

Although the primary responsibility of the intestinal epithelial cell is nutrient absorption, its role in mucosal immunity has previously been relegated to barrier function and the transport of secretory IgA. However, it is now clear that epithelia possess the ability to actively participate in mucosal immune responses.[7] Intestinal epithelial cells act as nonprofessional antigen-presenting cells, recognize and respond to bacterial and viral motifs by virtue of the expression of nucleotide oligomerization domain (NOD) and Toll-like receptors, and produce cytokines/chemokines that influence immune responses.[7] In addition, intestinal epithelial cells likely influence expansion of intestinal regulatory T (Treg) cells and cytokine expression.[8,9]

The epithelial surface overlying the Peyer patches (PPs) and lymphoid follicles is composed of a single layer of columnar cells termed the follicle-associated epithelium (FAE). Within the FAE reside specialized M cells derived from enterocytes under the influence of lymphotoxin. Human M cells differ from absorptive epithelium in that they do not harbor microvilli or membrane-

From [a]the Department of Pediatrics, National Jewish Health, [b]the Department of Pediatrics, University of Colorado Medical School, and [c]the Section of Pediatric Gastroenterology, Hepatology and Nutrition, [d]the Gastrointestinal Eosinophilic Diseases Program, National Jewish Health, the Children's Hospital, Denver, Aurora, Colo.

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Reprint requests: Glenn T. Furuta, MD, Pediatric Gastroenterology, the Children's Hospital, Denver, 13123 East 16th Ave B290, Aurora, CO 80045. E-mail: furuta.glenn@tchden.org.

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TABLE I. Elements of the mucosal immune system

Innate mechanisms of defense
Mucus
Trefoil factors
IgA
Peristalsis
Tight junctional proteins
Antimicrobial peptides
Adaptive elements of defense
B cells
$CD4^+$ T cells
$CD8^+$ T cells
Treg cells
DCs
Cellular components
Eosinophils
Mast cells
Neurons
Epithelial cells

associated hydrolytic enzymes and contain less glycocalyx but do express cathepsin E and Toll-like receptors. Regional differences in M cells (ie, differences in M cells in the colon compared with those of the small intestine) are thought to exist, suggesting accommodations to changing microflora; however, the functional significance of this is unknown. A distinctive characteristic of the M cell is the presence of an invaginated subdomain at the basolateral membrane forming an intraepithelial "pocket."[10] At this site, predominantly $CD4^+$ CD45RO memory T cells and both naive ($sIgD^+$) and memory ($sIgD^-$) B cells interact with the M cell.

The major function of M cells is the transepithelial vesicular transport of antigens from the lumen directly to the subepithelial lymphoid tissues. M cells have been shown to transport particulate proteins, bacteria, viruses, and noninfectious particles.[11] This sampling of luminal antigens and microorganisms is thought to be important in the development of immune responses and tolerance. Although various pathogenic organisms can exploit the propensity for vectorial transport of M cells as a mechanism to gain entry for infection, M cells also transport commensal flora as a potential mechanism to regulate immune responses to endogenous flora.

Microenvironmental anatomic differences within the different parts of the gastrointestinal tract are well described. Although the esophagus is lined by a stratified squamous epithelium, M cells have not yet been identified, and no resident eosinophils are present in the mucosal surface (see the eosinophilic esophagitis [EoE] section below). In contrast, the small intestine and colon are lined by a columnar epithelium, and the cellular components of the GALT are localized within microenvironments, such as PPs or interstitial lymphoid follicles. Formation of PPs is dependent on several factors, such as the IL-7 receptor and TNF, along with TNF receptor family members. These miniorgans are covered by M cells and FAE that participate in antigen trafficking, as described above. Within the barrier are also unique cell types, including intraepithelial lymphocytes (IELs) and the antimicrobial-filled Paneth cells that reside at the crypt base. The LP is populated by T and B cells, along with unique populations of DCs. Mesenteric lymph nodes are a robust site of antigen processing and form a filter that separates the mucosa from other mucosal organs.

T lymphocytes localize in the small intestine as a result of selective expression of $\alpha4\beta7$ and CCR9. $CD4^+$ and $CD8^+$ T cells occupy the LP, whereas $CD8^+$ T cells preferentially reside in the intraepithelial space. IELs are a heterogeneous population of lymphocytes that are predominantly effector/effector memory cells made up of $\gamma\delta$ T-cell receptor (TCR) $CD8^+$ T cells and 2 distinct subsets of $\alpha\beta$ TCR cells: $\alpha\beta$ TCR $CD4^+$ or $CD8^+$ cells and those that lack coreceptor expression, the so-called double-negative cells.

One subset of T cells receiving recent recognition is the Treg cell.[12] Treg cells generally have suppressive capacities that participate in the maintenance of self-tolerance. Surface marker studies have identified several subtypes, including the forkhead box protein 3 (Foxp3)–positive T cell. Mutations in the gene encoding Foxp3, a Treg-specific transcription factor, have been associated with autoimmunity in murine models and a clinical syndrome termed immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome. Patients with this disease have severe diarrhea and small and large intestinal inflammation.

B cells secreting IgA^+ originate in the PPs, ultimately taking up residence in the intestinal LP. This journey is regulated by the interaction of site-specific adhesion molecules, an $\alpha4\beta7$ on the lymphocytes, and mucosal addressin cell-adhesion molecule 1 on the high endothelial venules in the LP. A smaller percentage of IgA-producing cells in the gut (about 25%) are derived from peritoneal B1 lymphocytes driven by commensal bacteria in a T cell–dependent manner and are thought to be important in modulating the mucosal immune response to bacterial flora.

Mast cells are abundant throughout the gastrointestinal tract, and although important in the host response to parasitic infection, they might participate also in innate immune responses to bacteria.[13] LP mast cells and lymphocytes interface with the enteric nervous system, providing another pathway that can influence mucosal immune responses.[14]

Eosinophils are absent in the normal esophagus but are resident cells of the stomach and small and large bowel. Chemotactic factors contributing to this population include the constitutive expression of eotaxin-1.[15,16] The exact numbers that define normalcy are open to debate but likely depend on a number of different factors. Like mast cells, eosinophils can perform important effector functions during parasitic infection and allergic responses but can also contribute to normal gut homeostasis.[17]

INNATE MECHANISMS OF DEFENSE

Often ignored are a host of mediators that participate in the innate defense mechanisms.[18] These molecules participate in nonspecific actions that limit antigens and microbes from communicating with the epithelium and LP. Mucus is composed of a number of glycoproteins that form a viscoelastic blanket that covers the epithelial surface. The inner mucus layer ranges between 50 and 100 μm, whereas the outer layer measures up to 500 μm. This mucus blanket is primarily composed of mucin-2 but also harbors a number of different mediators, including trefoil factors, secretory IgA, and antimicrobial peptides.

Trefoil factors are shamrock-shaped proteins held together by 3 disulfide bonds.[19] Several types of trefoil factors have been described that localize to different mucosal surfaces to assist in barrier repair and wound healing. Numerous stimuli induce the production of trefoil factors, including hypoxia and epithelial disruption.

IgA antibodies are divided into 2 subclasses, IgA1 and IgA2, with IgA2 representing the predominant form on intestinal surfaces. Secretory IgA, which is secreted by B cells, binds to

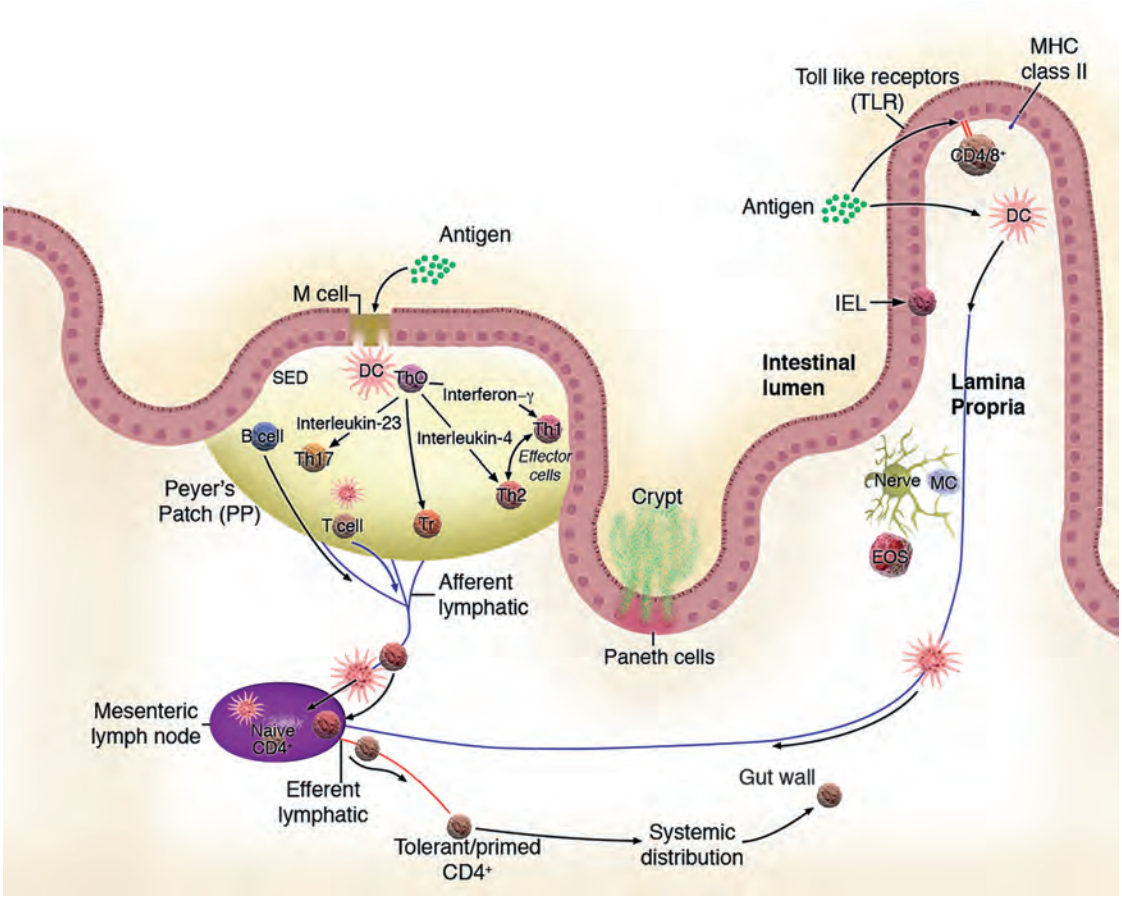

FIG 1. Anatomy of the gastrointestinal mucosa. Antigen can cross the epithelium through the M cell or DC. The subepithelial dome *(SED)* is occupied by a number of lymphocytes, including T_H0 cells that, under the direction of specific cytokines, differentiate into T_H1, T_H2, Treg, or T_H17 cells. Additional lymphocyte populations include the IELs that reside juxtaposed to the intestinal epithelial cells. Other resident cells in the LP that likely participate in the immune response include mast cells *(MC)* and eosinophils *(EOS)*.

and forms a covalent complex with the polymeric immunoglobulin receptor expressed on basolateral aspects of intestinal epithelia. Within the epithelia, IgA forms dimers that are connected by a J segment. This complex is actively transported across the epithelia to the apical surface, where it is released after proteolytic cleavage from the polymeric immunoglobulin receptor. Its exact function is unclear, but sIgA has been shown to bind microbes and toxins, preventing them from contacting the apical surface of the epithelium. Newer observations suggest that IgA might also regulate the composition of the microbial environment of the gut and limit local inflammation induced by pathogen-associated molecular patterns, such as LPS.[20,21]

Antimicrobial proteins are composed of a number of highly charged proteins called defensins.[22,23] These molecules are synthesized by Paneth cells and the epithelia. The antimicrobial properties of these highly charged molecules are attributed to their ability to increase bacterial membrane permeability. Six human α-defensins have been identified that possess selective activity against gram-positive and gram-negative bacteria and possibly viruses.[24] These cells likely participate in innate immunity, as was demonstrated in mice deficient in a Paneth cell–processing enzyme, rendering them unable to produce mature α-defensins. These mice were more susceptible to orally administered *Salmonella typhimurium* than wild-type mice.

INDUCTION OF A MUCOSAL IMMUNE RESPONSE

PPs are well-defined lymphoid aggregates composed of a large B-cell follicle surrounded by an interfollicular T-cell region. Interspersed throughout this organ are numerous macrophages and DCs. The subepithelial dome is an area rich in T and B lymphocytes and DCs. DCs migrate to basolateral surfaces of the M cell to acquire antigen and then travel to the interfollicular zone T-cell area, presumably where they participate in antigen presentation. DCs can migrate to distant sites, including mesenteric lymph nodes and the intestinal LP, where they can orchestrate an effector immune response. Experimental evidence supports an immunomodulatory role for DCs that includes both induction of oral tolerance and protective immune responses,[25] as described in a recent report in which $CD103^+$ DCs participated in the conversion of intestinal naive T cells to $Foxp3^+$ T cells.[26]

DISEASES

Inflammatory bowel diseases

Clinical description. Inflammatory bowel diseases (IBDs) are a heterogeneous group of diseases characterized by signs and symptoms related to immune-mediated inflammation of the gastrointestinal tract. The incidence of IBD ranges from 5 to 10 per 10,000 persons depending on the population examined.[27]

Typical symptoms include abdominal pain and bloody diarrhea in addition to other extraintestinal symptoms, such as fever, fatigue, arthralgias, and uveitis. In children growth failure can be an early sign. Physical examination reveals abdominal tenderness, particularly in the right lower quadrant.

Mucosal inflammation associated with Crohn disease can occur anywhere along the length of the gastrointestinal tract, with preponderance in the terminal ileum. Histologic hallmarks of tissues affected by Crohn disease include transmural inflammation and often noncaseating granulomas. Endoscopic features include skip lesions consisting of aphthous ulcerations, and radiographic studies show terminal ileal narrowing.

Suggestive laboratory abnormalities include anemia, increased sedimentation rate or C-reactive protein level, hypoalbuminemia, and increased liver enzyme levels. Ulcerative colitis has many clinical features in common with Crohn disease, but intestinal involvement is limited to the colon. In addition, the intestinal inflammation is limited to the superficial mucosa without granulomas, involves the rectum, and extends proximally. Other forms of IBD include microscopic colitis, lymphocytic colitis, and diversion colitis. Long-term complications include colorectal dysplasia and cancer.

Neutrophilic crypt abscesses are one of the most characteristic histologic features of both forms of IBD. In addition, eosinophils might be present, although to a seemingly lesser degree.

Pathophysiology. A complete review of the pathophysiology of IBD is beyond the scope of this Primer; the reader is referred to excellent reviews for more detailed descriptions.[5,18,28-32] Although the exact pathophysiology of IBD has not been determined, it is thought to develop when a genetically predisposed host is exposed to a luminal/environmental trigger. Over the course of the last few years, a number of genes have been identified in patients with Crohn disease, in particular genes linked to epithelial responses to luminal bacteria, autophagy, IL-10, and IL-23/IL-17 pathways. For instance, pathogen recognition receptors are present on the epithelial surface. One group of pathogen recognition receptors, the NOD molecules, recognize pathogen-associated molecular patterns that are present on bacterial membranes. A specific mutation of the *NOD2* gene allows for inappropriate sensing of bacteria with subsequent epithelial activation, leading to increased proinflammatory cytokine production within the mucosa. One gene associated with autophagy, *ATG16L1*, has been associated with Crohn disease. IL-10 suppresses deleterious intestinal inflammation, and recent studies have linked *IL10* mutations to IBD.[33] IL-23 and IL-17 mediate innate microbial defense and are linked to IBD.[34] In addition, loss of tolerance might also play a role because the mucosa affected by IBD contains fewer Treg cells than the healthy mucosa. IgE and food allergies are not thought to play a role in the underlying pathogenesis of these diseases.

Treatment. Goals of treatment of IBD include reducing inflammation, maintaining remission, enhancing quality of life, and avoiding the potential toxicity associated with treatments.[35-37] With this in mind, acute exacerbations are typically managed with systemic corticosteroids, whereas remission is addressed with the use of either aminosalicylates or immunomodulators, such as mercaptopurine or azathioprine. Recently, biological treatments, including anti–TNF-α antibodies (ie, infliximab and adalimumab), have significantly affected the clinicopathological features of IBD. A number of other agents, such as antidiarrheal agents, bile binders, and antispasmodics, might enhance quality of life.

Celiac disease

Clinical description. Celiac disease is an immune-mediated enteropathy that occurs as a result of gluten sensitivity in genetically predisposed individuals (DQ2/DQ8-positive HLA).[38] The incidence is 1 in 133 persons in the United States. Manifestations include those related to the gastrointestinal tract, such as chronic/recurrent diarrhea, abdominal pain, constipation, and slow growth, and nongastrointestinal symptoms, including dermatitis herpetiformis, seizures with occipital calcifications, dental hypoplasia, osteopenia, short stature, iron deficiency anemia, hepatitis, infertility, and arthritis.[39,40]

Other conditions associated with celiac disease include type 1 diabetes; Williams, Down, and Turner syndromes; IgA deficiency; autoimmune diseases; and a family history of first-degree relatives with celiac disease. Without treatment, there is an increased risk of intestinal lymphoma. Although serologic test results, such as increased IgA anti-endomysial antibody or tissue transglutaminase levels, provide strong evidence for celiac disease, the diagnosis rests on the finding of villous blunting and increased IEL numbers in mucosal biopsy specimens of the duodenal mucosa.

Pathophysiology. Recent studies have shown that a 33-amino-acid peptide in gliadin that is resistant to digestion contains the epitopes critical to the development of abnormal small intestinal mucosa in patients with celiac disease.[41,42] After uptake by the epithelium, processing of this 33-mer leads to activation of $CD4^+$ LP T cells, upregulation of the IL-2 receptor, increased production of IFN-γ and IL-15, and infiltration of the epithelia with γδ T cells. The resultant inflammatory process leads to villous blunting, crypt elongation, and loss of absorptive surfaces. Celiac disease is a cell-mediated and not IgE-mediated food allergic disease.

Treatment. Complete elimination of gliadin from the diet is the primary treatment of celiac disease.[43] Of paramount importance is attention to education and support of patients with respect to dietary elimination of gluten-containing products, review of alternative diets, adequacy of caloric and nutrient intake, and psychological support. For instance, although a number of foods should obviously be avoided, a number of products, including candies, gravies, food colorings, soy sauce, medications, play dough, and cosmetics, contain gluten in quantities sufficient to cause inflammation resulting in symptoms. In addition, many products that have been deemed wheat free, such as oats, are frequently contaminated with gliadin and should not be ingested by patients with celiac disease. Patients might also have iron, zinc, folic acid, and B complex vitamin deficiencies.

Eosinophilic gastrointestinal diseases

Clinical description. Eosinophilic gastrointestinal diseases (EGIDs) are heterogeneous diseases characterized by a diverse set of symptoms that occur in association with intestinal eosinophilia.[44] These diseases have been termed EoE, eosinophilic gastritis, eosinophilic gastroenteritis, and eosinophilic colitis depending on the anatomic location in which eosinophil numbers are increased. Over the last decade, EoE has been recognized as the most common EGID. The remainder of this section will focus on EoE. For further information, the reader is referred to recent reviews on other EGIDs.[44-46] Recent reports have expanded the association of esophageal eosinophilia with other diseases,

including celiac disease; the exact pathogenetic mechanisms and therapeutic implications of this are uncertain.[47-50]

EoE is a clinicopathological disease characterized by upper intestinal symptoms that occur in association with dense esophageal eosinophilia; other potential causes must have been ruled out as causes of symptoms and eosinophilia.[51] Children with EoE present with a wide range of symptoms, including vomiting, abdominal pain, feeding dysfunction, and dysphagia.[46,52] Feeding dysfunction is often overlooked and requires specific questioning regarding how patients eat foods (eg, dysphagia, food sticking, requiring water to wash food down, and prolonged chewing).[53] Adults present with stereotypical features of food impaction or dysphagia.[54] Patients presenting with food impaction, especially when recurrent, should be evaluated for a diagnosis of EoE. EoE occurs in all age groups and has been reported in all continents except Africa, with a reported prevalence of EoE ranging between 1 and 4 per 10,000 persons. Although the natural history is unknown, the one identified complication is esophageal stricture or narrowing.[55]

The physical examination should be directed toward excluding other causes of esophageal eosinophilia, such as IBDs, celiac disease, and connective tissue diseases. No single marker, including peripheral eosinophilia, provides diagnostic support for or against the diagnosis of EoE, although one study suggests that the combination of peripheral eosinophilia and increased serum eotaxin-3 and eosinophil-derived neurotoxin levels correlates with esophageal eosinophil density.[56] Upper gastrointestinal series can screen for other causes of vomiting and for evidence of esophageal stricture or long-segment narrowing, features associated with EoE.

Pathophysiology. Esophageal eosinophilia is a nonspecific finding that reflects a state of injury. Although a variety of diseases have been associated with this type of inflammation, including gastroesophageal reflux disease, EoE, celiac disease, infections, and IBDs among others, the exact mechanism driving this response is not certain.[44] For instance, recent evidence suggests that specific cytokines, including IL-6 and IL-1, might participate in acid-induced injury.[57] In contrast, as discussed below, IL-5 is critical to this response in murine models, and eotaxin-3 contributes to human disease.[58,59] The acute inflammatory infiltrate in patients with EoE is exclusively composed of eosinophils, with the virtual complete absence of neutrophils.

A series of recent studies have identified potential mechanisms for the pathogenesis of EoE. Mishra et al[59] provided the first murine model of aeroallergen-induced esophageal eosinophilia by sensitizing and challenging with *Aspergillus fumigatus*. By applying this system to IL-5 null mice, the investigators were able to demonstrate that esophageal eosinophilia was dependent on IL-5, as well as T cells.[59] The inflamed mucosa contains increased $CD4^+$ effector T cells and decreased Treg cells.[60,61] In addition, the same investigators have determined the effect of IL-5 on tissue remodeling associated with this eosinophilic inflammation.[62]- Together, these findings provided support for the development of therapeutics targeting IL-5 in the treatment of this disease. Addressing this need are 2 ongoing studies of anti–IL-5 in the treatment of pediatric EoE.

Translational studies have also brought increased understanding of EoE. For instance, a number of studies have begun to define the immunomicroenvironment of the esophageal mucosa. Although diagnostic criteria have solely focused on eosinophil numbers, other studies are examining associated inflammatory features, including eosinophil degranulation, that appear to be increased compared with those seen in gastroesophageal reflux disease.[63] In addition, the mucosa from patients with EoE contains increased T_H1 and T_H2 proinflammatory cytokines (IL-5 and TNF-α), CD8 lymphocytes (CD8 and CD1a), B cells, and mast cell and basophil infiltration.[64] The exact role of Treg cells in EoE is uncertain because one study demonstrated immunohistochemical evidence of $Foxp3^+$ cells in both patients with EoE and those with gastroesophageal reflux disease.[60] One genome-wide microarray analysis revealed that the most upregulated gene in the esophageal epithelia was eotaxin-3, a chemokine critical for eosinophil migration.[65] Another study identified increased eotaxin expression in the affected mucosa, providing further support for eotaxin's role in EoE's pathogenesis.[66]

Other studies have focused on remodeling in EoE, showing an increased level of esophageal fibrosis in children with EoE.[67,68] Although the exact mechanisms of this response are not certain, Aceves et al[68] showed increased TGF-β expression with activation of the SMAD pathway. Therapeutic studies suggest that fibrosis might be reversible.[69]

IgE and non-IgE immune mechanisms might participate in the pathogenesis of EoE, an important point to be kept in mind when evaluating these patients for allergen sensitization. Although skin prick testing and the measurement of food allergen–specific IgE levels are often useful in identifying potential culprit foods, they are not helpful in the detection of causative foods in non–IgE-mediated reactions. However, atopy patch testing to foods has been proposed as a useful method to potentially identify foods causing symptoms through a non–IgE-mediated immune mechanism.[70,71]

In summary, evidence to date supports a role for both IgE-mediated and non–IgE-mediated mechanisms in the pathogenesis of EoE, with eotaxin-3 and IL-5 being central mediators and fibrosis being one of the potential outcomes.

Treatment. Treatment goals have been directed toward symptom elimination and reduction/normalization of esophageal inflammation. The rationale for the later end point has been that complete histologic remission might reduce the incidence of complications. To date, the incidence of esophageal complications is unknown, and potential emotional and developmental effects of chronic treatments and repeated endoscopic analyses are beginning to be recognized.[72] Prospective studies will provide data to illuminate this area of controversy.

Despite this issue, at least 2 effective treatments, corticosteroids and dietary elimination of suspected culprit foods, have been identified. The reader is referred to a number of recent reviews on this topic for further details regarding the specifics of each treatment.[46,73] Regardless, evidence to date suggests that EoE is a chronic disease, and without continuous treatment, symptoms and inflammation will persist or return. To date, no medical maintenance treatment has been identified.[51]

SUMMARY

The mucosal immune system has a unique anatomy and physiology aimed at providing a mechanism that will allow tolerance to food antigens and commensal bacteria along with the capacity to respond to pathogenic microbes, other injurious agents, or both. The monolayered epithelium forms the initial interface between the environment and host that forms not only a barrier but also a sensor providing bidirectional communication

with other resident mucosal lymphoid cells. The lymphocytes, DCs, mast cells, and eosinophils in the LP interact to form a pluripotent network that orchestrates an innate and adaptive immune response to potential pathogens. Further delineation of the mechanisms governing the normal responses of the mucosal immune system will provide insight into disease states, such as food allergies, IBDs, and EGIDs.

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Complement disorders and hereditary angioedema

Michael M. Frank, MD *Durham, NC*

The term *complement* was introduced more than 100 years ago to refer to a group of plasma factors important in host defense and in the destruction of microorganisms. We now know that there are 3 separate activation pathways that appeared at different times in evolution: the classical, alternative, and lectin pathways. Two of these appear before the evolution of the adaptive immune system and do not require antibody for initiation. All pathways come together to activate C3, the principle opsonic protein of the complement cascade, and all continue together to the generation of biologically active factors, such as C5a, and to lysis of cells and microbes. In general, complete deficiencies of complement proteins are rare, although partial or complete deficiencies of one of the proteins that initiates the lectin pathway, mannose-binding lectin, are far more common. Although genetically controlled complement defects are rare, defects in the proteins in the circulation and on cell membranes that downregulate complement so as to limit uncontrolled inflammation are more common. A number of these are discussed, and because new methods of treatment are currently being introduced, one of these defects, CI inhibitor deficiency associated with hereditary angioedema, is discussed in some detail. (J Allergy Clin Immunol 2010;125:S262-71.)

Key words: *Complement, complement deficiencies, hereditary angioedema, atypical hemolytic uremic syndrome*

Abbreviations used
C1-INH: C1 inhibitor
C': complement
FDA: US Food and Drug Administration
HAE: Hereditary angioedema
iC3b: Inactivated C3b
MASP: Mannose-binding lectin–associated serine protease
MBL: Mannose-binding lectin
MCP: Membrane cofactor protein
PNH: Paroxysmal nocturnal hemoglobinuria

Complement is a term originally introduced a hundred years ago to define a group of factors present in fresh plasma that, when activated by a specific antibody, were able to kill microorganisms.[1] Later work showed the bacteria studied were lysed and that the killing principle was heat labile. We now define complement as a collective term for a group of about 30 known proteins and protein regulators, some of which circulate in the blood and some of which are cell membrane bound. The complement proteins play a major role in host defense and innate immunity. Although all of the early studies focused on the role of complement in host defense, in recent years, we have learned that complement is also important in the generation of a normal immune response. Phylogenetically, the complement proteins are ancient, serving a host defense function even in primitive animals in the absence of any adaptive immune system. The adaptive immune system appears in evolution at the level of the fish, and by this point in evolution, all the various complement proteins are arrayed to produce their regulatory and host defense functions.[2]

We have come to recognize 3 pathways of complement activation (Fig 1).[3,4] The first pathway was defined almost a century ago and, for this reason, is termed the *classical pathway*. This pathway is usually activated by antibody and was the first pathway identified because of its ability to kill antibody-sensitized bacteria. A second pathway, now termed the *alternative pathway*, was first observed in the 1950s but was studied in detail in the 1970s and 1980s.[5] The alternative pathway has been shown to be phylogenetically older than the classical pathway. It does not require antibody to function and is found in organisms as primitive as sea squirts.[2] Although antibody is not required for its function, the presence of antibody usually allows this pathway to function more efficiently. A third pathway described in the past 2 decades, the *lectin pathway*, is still being defined in detail. This pathway appears in development sometime after the alternative pathway and also does not require antibody to function.[6] All 3 pathways proceed through a series of proteins that are discussed below to the activation and binding of the plasma protein C3, which is central to all 3 pathways. The pathways then proceed together through the binding of an additional series of proteins to the lytic and inflammation-promoting steps in complement action.

Most reviews focus on the 3 major effector functions of complement in host defense. First is its ability to lyse cells, second is its ability to opsonize particles (ie, to render them easy for phagocytes to engulf), and third is the ability of the proteins on activation to generate cleavage fragments that have potent inflammatory activity. For example, the small fragment of C5, C5a, can cause mast cells to degranulate and release histamine, as if they were coated with IgE and antigen. It can cause migration of phagocytic cells toward the place where the peptide is generated (ie, to induce chemotaxis) and can cause cytokine and biologically active peptide release from cells.[7,8] The biological basis of these 3 complement effector activities is defined below.

It is rare to find patients with deficiencies of classical or alternative pathway proteins, although deficiencies of some of the proteins of the lectin pathway are surprisingly more common. In most cases, when complement contributes to disease it is acting appropriately (ie, the system is being activated and causing tissue damage and cell death in a normal fashion), but it is being activated inappropriately.[9,10] Thus, for example, a patient might produce an abnormal antibody to the basement membrane of the glomerulus. The antibody can bind to the glomerulus, activate complement, and cause inflammatory damage. In this case complement is acting normally; it is the antibody that is inappropriate. Table I lists some

From the Department of Pediatrics, Duke University Medical Center.
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Reprint requests: Michael Frank, MD, Box 2611 Room 131 MSRB, Duke University Medical Center, Durham NC, 27710. E-mail: frank007@mc.duke.edu.
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diseases in which complement deficiency is associated with clinical illness. Because of the many untoward effects of inappropriate complement activation, there are many control proteins that act to downregulate activated complement proteins at each step in the various reaction cascades. The importance of these proteins is that they prevent unwanted damage of one's own tissues and cells. Although the absence of complement proteins is unusual, the absence of control proteins is more common, and many of the patients who have absent control proteins have poorly controlled inflammatory disease. Moreover, as mentioned in our discussion of the lectin pathway below, there is a sizable group of patients with allotypic variations of mannose-binding lectin (MBL) that lead to very low levels of this circulating protein.

Although effector functions of complement in host defense have received the most attention over the years, more recently, it has been found that complement also functions in the induction of adaptive immunity, but here there has been far less study, and less information is available.[11] One reason that this information is coming to light slowly is that there are so few complement-deficient subjects to study. With the advent of knockout gene technology, it has been possible to develop murine strains missing 1 or more complement proteins or complement receptors, and it has been found that these animals have defects in the development of many aspects of the normal murine immune response.[12] This is discussed further toward the end of this chapter.

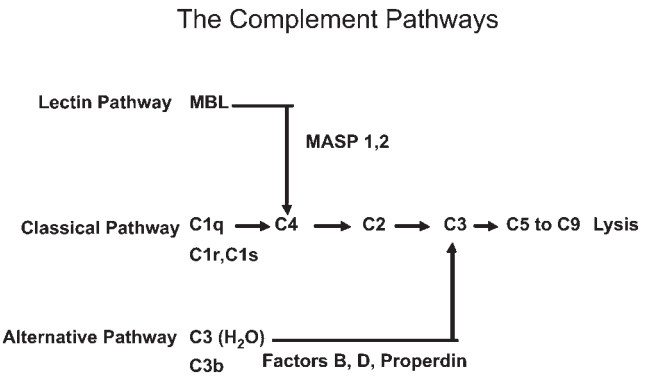

FIG 1. The 3 complement activation pathways. The classical pathway is usually activated by antibody. The lectin pathway is activated by the recognition molecule MBL binding to structures with the appropriate repetitive sugars. The ficolins are MBL-like molecules that can also activate this pathway. The alternative pathway does not have a recognition molecule as such. It is initiated by the binding of factor B to C3, which can then be cleaved by factor D. Because C3 always undergoes slow hydrolysis, the pathway is always undergoing some degree of activation. Properdin stabilizes the complex and can also initiate alternative pathway activation. C3b itself is an efficient activator of the alternative pathway, and classical pathway activation leading to C3 deposition on a target rapidly activates the alternative pathway.

THE CLASSICAL PATHWAY

The classical pathway is usually activated by antibody. IgM and the IgG subclasses IgG1, IgG2, and IgG3 bind the first component of complement, C1, to initiate activation of the classical pathway.[3] C1 exists in serum as a 3-part molecule (C1q, C1r, and C1s) held together in the presence of ionic calcium. C1q has a central protein core and 6 radiating arms, each ending in a pod-like protein domain that can bind to the Fc fragment of IgG or IgM. Each of the 6 arms is made up of 3 intertwined chains, C1q A, B, and C, and has a triple-helix structure like collagen, providing flexibility. In the case of IgG, the binding of multiple IgG molecules to an antigenic surface allows binding of multiple arms of C1q, each to an Fc fragment, with sufficient affinity of the C1q to allow C1 activation. In the case of IgM, a single molecule bound to an antigenic surface by multiple binding sites with the availability of multiple Fc fragments within this one polymeric molecule is sufficient to bind C1q and activate this pathway. On binding of C1q to antibody, a distortion of the C1q molecule takes place that in turn causes autoactivation of C1r, which then activates C1s. C1s, like C1r, acquires enzymatic activity and continues the complement cascade sequence. C1 requires calcium for self-association and therefore the classical pathway requires calcium for initiation. The function of activated C1 is to bind and cleave C4, the next protein in the classical pathway activation sequence. C4 is cleaved into a large fragment (C4b) and a small fragment (C4a). The large fragment continues the complement cascade and the small fragment, like the small fragments of C3 (C3a) and the small fragment of the next protein (C5) in the sequence (C5a), has anaphylatoxic activity. All of these fragments are able to cause mast cell degranulation with resulting histamine release.[7]

On activation of C4, a thioester-containing binding site is exposed on C4b that allows covalent attachment of C4b to the target. The nature of the binding site on C4 and C3 is similar and is discussed in further detail in the section on the alternative pathway. The site now containing C1 and C4 bound to a target allows the next protein, C2, to bind to C4b. On C2 binding to the C14b site, C1s cleaves C2 also into a large and small fragment. Again, the large fragment remains bound to the assembling protein complex. C2 binding to C4 requires the presence of ionic magnesium. The new site consisting of C4b and C2 (C4b2a) no longer requires C1 for activity. Enzymatic activity resides in the C2 fragment. This site is termed the C3 convertase of the classical pathway because it can bind the next complement protein in the sequence (C3) and, as in the earlier steps, cleave it into a large fragment (C3b) and a small fragment (C3a), which again has inflammatory activity. Just as in the case of C4b, C3b can bind covalently to the target of attack. In many cases it binds directly to C4b on the target. As mentioned earlier, C3 is the central component of all 3 complement pathways and is present at high concentration in serum, about 1.2 mg/mL.

An important function of complement is the ability to opsonize particles, which means to coat them with complement-derived protein fragments that allow them to be phagocytosed easily. Phagocytic cells have on their surface specific receptors for complement-derived peptides, often cleavage fragments of C3. When these fragments are deposited on microbes, they can link the microbe to the phagocyte receptors; the adherence facilitates the phagocytic process.

On the addition of C3b to the C4bC2 site, a new binding site is created that can bind C5, the next protein in the sequence. Again, C5 is cleaved into a large fragment and a small fragment. The large fragment, C5b, continues the complement cascade, although it does not form a covalent bond with the target and remains associated with C3b. The small fragment released, C5a, is one of the most potent inflammatory peptides released by complement activation and has strong neutrophil-aggregating activity, strong neutrophil chemotactic activity and is an excellent anaphylatoxin.[7] Injection of sufficient purified C5a into an animal

TABLE I. Functional and clinical consequences of complement deficiency

Defect	Classical pathway	Lectin pathway	Alternative pathway	C3 and factors that control C3 levels	Late-acting proteins: C5-C9
Functional Consequence	Delayed C′ activation, decreased immune response, poor antibody activation of C′	Decreased activation in the absence of antibody	Decreased C′ activation in the absence of antibody	Decreased opsonization: if control factors are abnormal, increased C′ mediated pathology	Inability to form lytic Lesions; C5 important in PMN chemotaxis
Clinical consequences	Increased incidence of autoimmune disease: infection with high-grade pathogens (eg, pneumococcus)	Infection in the newborn: question of increased rheumatic disease Question of risk of increased infection with unusual pathogens like cryptosporidium and aspergillis	Increase in infection with high-grade pathogens: some increase in *Neisseria* species infections	Marked increase in infection with high-grade pathogens: failure to downregulate C3 associated with hemolytic uremic syndrome and adult-onset macular degeneration	Marked increase in neisserial infection

may cause anaphylaxis and death from neutrophil aggregation in the circulation and massive histamine release.[7,13]

The complement cascade continues after C5b binding with the binding of C6, C7, C8, and C9. One molecule of C6 and C7 each bind to C5b on the target surface. If this binding takes place at the surface of a cell or microbe, the introduction of C7 to the binding site leads to an increase in hydrophobicity of the C5-7 complex and insertion of the complex into the lipid cytoplasmic membrane of the cell. Under these circumstances, the cell is targeted for lysis. With the binding of one molecule of C8 to the C5-7 complex, a slow leak in cells such as erythrocytes appears, and with the binding of up to 16 molecules of C9, a cylinder or donut-like structure is formed, containing all the proteins C5b through C9, that penetrates the cell membrane. The pore-like interior of the donut allows free fluid transfer and destroys the ability to maintain its osmotic equilibrium, and it lyses.

Cells protect themselves from complement attack in a variety of ways. The many complement control proteins will be discussed in greater detail below, but also the lytic C5b–9 complex can be shed from the surface of some cells or internalized and destroyed as the cell acts to protect itself from damage. Cells such as erythrocytes with little intracellular protein synthetic machinery to help repair their membranes rely on the control proteins for protection. Cells such as macrophages and endothelial cells have these extra mechanisms for clearing their membranes of deposited complement proteins.

THE LECTIN PATHWAY

The lectin pathway, unlike the classical pathway, does not require antibody to function and is developmentally more primitive than the classical pathway. It is quite similar in function to the classical pathway.[6] In the more evolved classical pathway, the recognition molecule that that sees foreign antigen with great specificity and induces complement activation is antibody. The lectin pathway does not use antibody but has its own more primitive recognition molecule. The pathway is initiated by the plasma protein MBL or by the related proteins, the ficolins. MBL has a structure remarkably similar to C1q, with a central core and a series of radiating arms composed of a flexible triple helix, each ending in a binding structure. Unlike C1q, in MBL the helix contains 3 copies of a single chain. In the case of C1q, the binding structure at the end of the arms recognizes the Fc fragment of immunoglobulin, and antibody is the recognition protein that triggers the activation sequence. In the case of MBL, there are 3 lectin-binding sites at the termination of each of the arms of the MBL. Each lectin-binding site has low affinity for sugars like mannose, but with the binding of multiple arms of the MBL, each with 3 binding sites to, for example, the repeating polysaccharides on the surface of a bacterium, the association is stabilized and the complement pathway is activated. Therefore the protein that recognizes the foreign structure is not a specific antibody but MBL itself. MBL circulates as a series of multimers and can have 2, 4, or 6 arms. In general, it is thought that the 4-arm structure predominates.

Associated with MBL in the circulation are proteins termed mannose-binding lectin–associated serine protease (MASPs). The functional structure again resembles that of C1 because C1q, the subunit with collagen-like arms that binds to antibody, also associates with serine proteases, C1r, and C1s. In the case of MBL, the associated serine proteases are MASP1, MASP2, and MASP3, as well as some other related molecules. Recent work further demonstrates the similarity of the classical and lectin pathways. C1r and C1s are reported to have some affinity for MBL, and the MASPs have an affinity for C1q. It is believed that MASP2 is the principle serine protease involved in continuation of the complement cascade, with MASP1 also active. MASP3's function is still being explored, but it might have a role in activating the alternative pathway. Currently, it is thought that the main path of activation after MBL binding is through activation of C4 by MASP2. Thus lectin pathway activation is very much like classical pathway activation. In the classical pathway antibody is the recognition molecule. It binds C1, which is then activated and cleaves C4. In the lectin pathway the recognition molecule is MBL, and MASP2 is the C1q like molecule that cleaves C4 into C4a and C4b. C4b then binds C2, the C2 is cleaved by MASP2, and the pathway continues to C9, just as in the classical pathway.

THE ALTERNATIVE PATHWAY

The alternative pathway is probably the oldest of the complement pathways in phylogenetic terms and is more difficult to understand because it operates by means of a mechanism that is fundamentally different and more primitive than that of the

classical and lectin pathways. In these latter 2 cases the pathway is specifically activated by a recognition molecule that binds to the target of attack and activates a serine protease that activates the rest of the complement sequence. In the alternative pathway C3 is itself the recognition molecule, and activation of the pathway is inefficient. C3 is a 2-chain molecule, α and β, with an internal thioester joining a cysteine at position 988 with a glutamine at position 991 in the α chain backbone. The tertiary configuration of the molecule protects the internal thioester from cleavage caused by nucleophilic attack by water; even so, it undergoes slow hydrolysis in the circulation. When water penetrates to the thioester bond, the bond is hydrolyzed, leaving a free sulfhydryl at position 988 and a hydrated carboxyl ion at position 991. This is associated with a marked change in tertiary structure, and the molecule comes to resemble C3b. Hydrolyzed C3, like C3b itself, is capable of binding factor B, a protein of the alternative pathway very much like C2 of the classical pathway. On binding to hydrated C3 or C3b, factor B can be cleaved by a serine protease, very much like C1s of the classical pathway, termed factor D. Thus a protein complex is formed consisting of hydrated C3 or C3b and the large fragment of cleaved factor B, termed Bb, with the release of the small fragment Ba. This complex is the C3 convertase of the alternative pathway. It can bind a new molecule of C3 and cleave it into C3a and C3b. The major difference between the C3 convertase (C3 cleaving enzyme) of the alternative pathway and the C3 convertase of the classical and lectin pathways is that there is no C4b in the convertase of the alternative pathway. C3b itself takes the place of C4b, with factor B acting like C2 and factor D acting like C1. A second difference is that factor D, the molecule that resembles C1s of the classical pathway and MASP2 of the lectin pathway, is not physically bound to the active site but acts as a fluid-phase enzyme.

In summary, the initial alternative pathway C3 convertase ($C3[H_2O]$,Bb), which can form slowly and spontaneously in the circulation, can bind and cleave another molecule of C3. When C3a is cleaved from the C3 to form C3b, the thioester becomes immediately available. If this cleavage occurs close to the surface of a cell or microbe, the carboxyl on the C3b generated can form an ester or amide bond with the surface of a cell or microbe. This target-bound C3b can accept another factor B molecule and, in the presence of factor D, can cleave more C3 into C3a and C3b, with more C3b becoming target bound. In the case of the classical and lectin pathways, the C42 complex is unstable and slowly decays. In the case of the alternative pathway, the C3bBb complex is also unstable. It rapidly decays and is stabilized in the circulation by yet another protein termed properdin. Properdin binds C3b, and it has recently been suggested that properdin bound to a substrate can also bind C3b and initiate alternative pathway attack. Like the classical pathway convertase, the alternative pathway convertase requires magnesium ion to function. Presumably the first pathway to develop in the complement system, in terms of phylogenetic development, was the alternative pathway. Because pathway initiation is not directed and requires the chance hydrolysis of a C3 close to the target of destruction, its binding, and then binding of additional C3 to the target, it is very inefficient. It is believed that the lectin pathway evolved to recognize the target more directly by binding to sugar groups on its surface. With the appearance of antibody, the target could be even more specifically identified. C3b deposited on a target by the lectin or classical pathway can also engage proteins of the alternative pathway to further amplify C3 deposition.

C3b undergoes a complex sequence of degradation steps, with each degradation product having different biological activity. Because these steps are regulated by control molecules, they are considered in the sections below.

COMPLEMENT RECEPTORS AND COMPLEMENT CONTROL MOLECULES

By definition, complement receptors recognize and bind various complement proteins and fragments. As with other receptors, this can cause cellular activation. However, unlike most cellular receptors, some of the complement receptors also act as control molecules and interact with the molecule they bind to allow for further degradation of the bound fragment. In performing this function, the receptors act like the complement control molecules that regulate the degradation of complement proteins to control their biological function. These many receptors and control molecules are discussed below. At virtually each step of the complement cascade, control points are established to downregulate the possibility of untoward complement activation. A few of the control molecules linked to disease are listed in Table II.

Control of activity of C1 and MASPs. In the classical pathway the activation of C1 with cleavage of C4 is downregulated by C1 inhibitor (C1-INH).[14] This single-chain molecule is a serpin (serine protease inhibitor). Enzyme inhibitors of this class present a bait sequence to the enzyme to be inhibited that looks like the enzyme's substrate. When an enzyme cleaves the inhibitor at the site of the bait sequence (amino acid 444 of the C1-INH), the inhibitor springs apart, uncovering a highly reactive site that forms a covalent bond with the active site on the enzyme. C1-INH inhibits C1r and C1s of the classical pathway and MASPs 1 and 2 of the lectin pathway. C1-INH has been termed a suicide inhibitor because it is used up during the inhibition process. During the process of C1 inhibition, the C1 molecule is taken apart, C1r and C1s are removed, and C1q is left bound to the antibody site. As discussed in a later section, C1-INH inhibits enzymes in a number of other mediator pathways in plasma, including the kinin-generating pathway, and patients with abnormalities in even one of the genes for normal C1-INH have hereditary angioedema (HAE), a swelling disorder.

Control of the activity of C4 and C2. The next steps in the classical and lectin complement pathway, the interaction of C4 and C2, are also under the control of a circulating protein, C4-binding protein.[15] This protein binds to C4b, preventing its interaction with C2 and accelerating the decay of the C4b, C2 site once formed. It also is capable of binding to C3b when these reactants are present at high concentration. As discussed, the C4b, C2 site is further controlled because it is subject to spontaneous degradation over time, losing its activity. Loss of activity is accompanied by the release of C2 from the C4b site. The C4b site can accept another C2 and, in the presence of C1, can regenerate the C4b/2 site.

Control molecules and cellular receptors that interact with C3. As an essential component in the lytic pathway, C3 functions in the classical, lectin, and alternative pathways. C3b bound to a target can not only continue the complement cascade but also acts as a potent opsonin by binding its receptor CD35, on phagocytes, aiding the phagocytic process. Because C3b, if deposited on tissue cells, can become a focus of tissue damage, its formation and degradation are under tight regulation. It is

TABLE II. Some regulators of complement activation and their role in disease

C1-INH downregulates the complement, kinin-generating, clotting, and fibrinolytic pathways. Heterozygous deficient individuals have HAE.
MCP (CD46) is a cofactor for the cleavage of C3. Homozygous or heterozygous defects can lead to aHUS.
Factor H is a cofactor for the cleavage of C3. Complete deficiency is associated with glomerulonephritis. Partial and complete deficiencies are associated with aHUS. Polymorphism is associated with age-related macular degeneration and HELLP syndrome.
Factor I is a cofactor for the cleavage of C4 and C3. Complete deficiency is associated with low levels of C3 and infection. Deficiencies are associated with aHUS.
CD59 downregulates formation of the membrane attack complex. Acquired deficiency by hematopoietic progenitors leads to paroxysmal nocturnal hemoglobinuria.

aHUS, Atypical hemolytic uremic syndrome; *HELLP*, hemolytic anemia, elevated liver enzymes, and low platelets, occurring during pregnancy.

simplest to describe the steps in degradation in plasma and then the effect of the receptors (Fig 2).

There are a number circulating proteins and cell-surface receptors that can interact with C3b, and the results of the interaction might differ depending on the set of control proteins with which it interacts.[16,17] Virtually all normal cells have these control molecules. Two plasma proteins, factors H and I, are critical regulators of C3b in plasma and to some extent on certain cells, such as erythrocytes. When C3b is generated, it will bind factor H, and the complex of C3b and H can be attacked by the circulating complement enzyme factor I, which can then cleave the C3b α chain, leading to the formation of inactivated C3b (iC3b). iC3b no longer functions as a C3 or C5 convertase, but it remains cell bound and remains a potent opsonin. The rare patients missing factor I have low C3 levels in the circulation because the alternative pathway stays active and cleaves C3, and these patients also have an increased incidence of infection.

It is interesting that the complement system attempts to discriminate self from nonself in an attempt to minimize unwanted tissue damage. C3b deposited on one's own tissues or cells is often close to a sialic acid which is present in relatively large amounts in normal tissues and cellular membrane carbohydrates. Factor H binding and activity is facilitated by sialic acid. Any C3b deposited on one's own cells therefore tends to be cleaved by factor I, preventing further complement activation. Most microorganism surfaces are not rich in sialic acid. Factor H function is not facilitated. C3b remains on the organism surface, and the C3 convertase of the alternative pathway continues to deposit additional C3b on the microbe to promote phagocytosis. Many pathogens have evolved mechanisms to incorporate sialic acid into surface structures to protect themselves in part from complement attack. For example, *Escherichia coli* K1 has developed sialic acid–containing capsules to mimic the surface of the normal cell and thus protect the bacterium from destruction.[18]

Five different cellular receptors are important in the binding and phagocytosis of C3-coated particles. CD35 (also termed CR1) recognizes C3b, as does a recently described receptor, which is present on Kupffer cells and some monocytes, termed CRIg.[19] The β_2-integrins (CD11b/CD18, which is also termed CR3, and CD11c/CD18, which is also termed CR4) recognize target-bound iC3b, the product formed by the action of factors H and I acting on C3b, and mediate phagocytosis. Receptors for iC3b are present on all phagocytes and dendritic cells, although they are not present on lymphocytes. The β_2-integrins are 2-chain molecules (α and β chain).[20] The α chain (CD11b or CD11c) provides the ligand recognition, and the β chain (CD18) is required for transport of the 2-chain complex to the cell surface. Patients with leukocyte adhesion deficiency have a defect leading to their inability to express these molecules on the cell surface and are highly susceptible to infection. CD11c/CD18 is the signature receptor used in identification of monocytic dendritic cells. Presumably this receptor, acting through complement bound to antigen, is of critical importance in processing of antigen for presentation to the immune system.

The C3b receptor CD35 (CR1) is present on erythrocytes, phagocytes, dendritic cells, and all B cells.[21] As mentioned, binding of a particle to a phagocyte surface by CD35 aids in the phagocytic process. However, if an immune complex forms in the circulation and binds C3b, most often it will bind not to the surface of a phagocyte but to the surface of an erythrocyte through erythrocyte CD35 because of the large number of erythrocytes in the circulation. The immune complex, bound to the surface of the red cell, is effectively out of the circulation and cannot easily leave the intravascular space to be deposited in tissues, such as the kidneys. As the erythrocyte circulates through the liver and spleen, the immune complex comes in contact with the fixed phagocytes in the sinusoids of these organs and is removed from the red cell surface and phagocytosed. The red cell exits the liver or spleen free of the complex and continues to have normal survival. During this process, some of the CD35 is removed from the red cell as the immune complex is removed. The infusion of normal erythrocytes into patients with active systemic lupus erythematosus with circulating immune complexes is followed by those erythrocytes gradually losing their CD35 as the CD35 on the infused erythrocytes binds the circulating immune complexes and transports them to the liver and spleen.

CD35 itself acts as a cofactor protein for degradation of C3, but its function is different from that of the proteins listed above. Like C3b that has bound factor H, C3b bound to CD35 can be cleaved by factor I, but the cleavage leads to a different fragmentation pattern. Cleavage of the α chain leads first to the formation of iC3b, but the process does not stop at this step. Further cleavage of the α chain leads to release from the target-bound C3b of the largest part of the iC3b, C3c, with retention of a 40-kd fragment of the α chain of iC3b, C3dg, which is bound to the target. This fragment can be further trimmed by proteases to C3d. C3dg and C3d do not bind to CD35 or to the β_2-integrins, but do bind to CD21 (CR2), which is present on all B cells, a T-cell subset, and follicular dendritic cells. Because β_2-integrins are not on B cells and CD21 is not present on most phagocytes, the fragmentation pattern of C3 mediated by the various cofactor proteins can direct targets of attack or antigens to phagocytes, antigen-presenting cells, or B cells.

A group of other complement control molecules on the membrane of normal cells also act to dampen the activity of C3 if it is accidently deposited.[21] Thus membrane cofactor protein (MCP; CD46) acts as a cofactor for the cleavage of C3b by factor I, just as factor H does. Another molecule present on most cells, which is bound to the cells by a phosphatidylinositol linkage, decay-accelerating factor (CD55), interacts with both the classical and alternative pathway C3 convertase to increase the rate of degradation of the convertase, destroying its activity. It is interesting that these 2 molecules, which are widely distributed on cells of the body, together have much of the activity of CD35 on immune cells

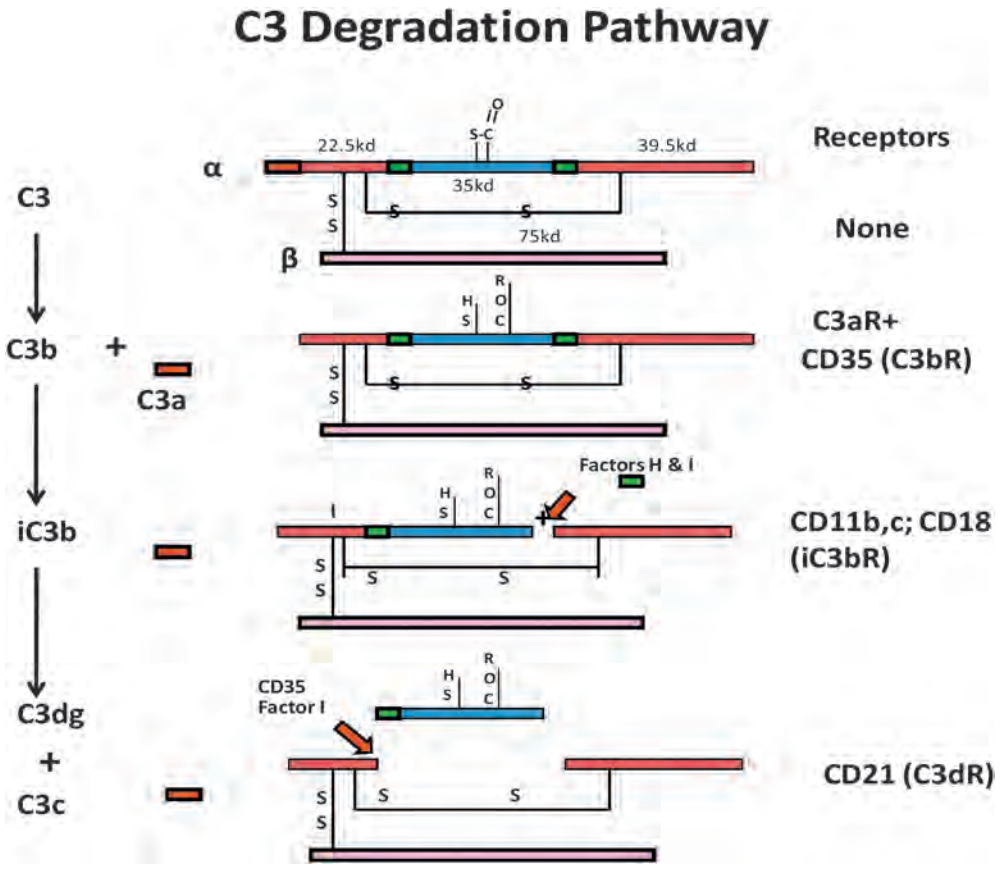

FIG 2. The 2-chain molecule C3 is shown first. There are no receptors that recognize this molecule. The C3 convertase of the classical or alternative pathway cleaves off C3a, an anaphylatoxin. The remainder of the molecule C3b undergoes a marked molecular rearrangement and now is recognized by CD35 (CR1), as well as by the recently recognized receptor on Kupffer cells, CRIg. C3b binds factor H and now can be cleaved by factor I to iC3b. iC3b is recognized by CD11b/CD18 and CD11c/CD18. These 2-chain receptors are on all phagocytes and dendritic cells. They aid in the processing of antigen. In serum the cleavage of C3 stops at this point, but when an immune complex is bound to cellular CD35 or when C3 is deposited on a cell with CD35 or other membrane-bound complement control molecules, such as CD46, it is cleaved further by factor I to C3c and C3dg. C3dg can be trimmed to C3d and C3g. C3d and C3dg are recognized by CD21 found on B cells and dendritic cells. Antigen with multiple bound C3d molecules can interact with both CD21 and the B-cell receptor, which can augment the immune response.

and phagocytes. CD35 has both decay-accelerating and cofactor activity in the same receptor molecule, and these activities are separated and slightly changed in CD46 and CD55.

As discussed, the complement system has been present over much of mammalian evolution, and microorganisms have evolved mechanisms for using these proteins as docking sites for entry into cells. Thus MCP has been shown to be a docking site for measles virus, for certain adenoviruses, and for some *Neisseria* species organisms; CD21 is a docking site for EBV. Each year, the list grows of control molecules that are found to be docking sites for various viruses or bacteria.

Several of the complement receptors are thought to aid directly in cellular activation or inhibition. CD35 has been discussed above as a facilitator of phagocytosis. The β_2-integrins CD11b and c/CD18 are the principle iC3b receptors and, like CD35, provide a signal for phagocytosis. These receptors are present on all phagocytes and natural killer cells. As mentioned, CD11c/CD18 is used as an identifying marker of dendritic cells. Follicular dendritic cells, B cells, and some T cells have CD21 (CR2) on their surface. This receptor recognizes C3d, C3dg, and polymerized iC3b. It is believed that antigens with C3d on their surface can cross-link CD21 with the B-cell receptor, augmenting the ability of antigen to activate B cells by as much as a thousand fold.[22]

As mentioned earlier in the chapter, inherited defects in the control molecules are more common than inherited defects in the complement proteins themselves. Factor H abnormalities have been reported in 2 important medical situations. Lack of normal factor H activity plays a critical role in the development of familial, atypical, hemolytic uremic syndrome, that is hemolytic uremic syndrome that occurs spontaneously and is not associated with bacterial infection and diarrhea.[23] In fact, investigation has shown that 3 different molecules, each of which plays a role in C3 degradation, can be abnormal in various subgroups of these patients. The 3 proteins are factor H, factor I, and MCP. The defects in the proteins can be present in either the heterozygous or homozygous state, probably reflecting the fact that half the normal number of C3 control molecules is not sufficient to protect against untoward immunologic activation. One way of thinking about the pathogenesis of this syndrome is that a toxin enters the circulation and is deposited on endothelial cells, particularly in the kidney, and on

erythrocytes. As the subject makes an immune response to the toxin, in the absence of sufficient control molecules, antibody binds to the toxin, and cells with toxin and antibody are destroyed by poorly regulated complement activation. In truth, no one has shown that this is the mechanism of disease, but it places the disease in a framework that allows the pathophysiology to make sense.

It has also recently been reported that the largest risk factor in the development of macular degeneration in the elderly is an alteration of the amino acid at position 402 in factor H from a tyrosine to a histadine.[24] It is believed from statistical studies of DNA sequences from pedigrees of families with inherited macular degeneration that approximately 50% of cases are associated with this alteration in one amino acid, although the factor H allele with histadine in position 402 is fairly common in the population, and other factors must be involved.

Receptors for the anaphylatoxins C3a, C4a, and C5a

Of the anaphylatoxins, C5a has been studied in the greatest detail.[7] It is a potent chemotactic factor causing the directed migration of phagocytes. It contracts smooth muscle cells and causes mast cells to degranulate in the absence of IgE antibody. It causes neutrophils to adhere to one another and to endothelium in vessels. It clearly plays a part in the damage observed during the course of immunologic lung disease. Mice with a defect in the C5a receptor do not experience all of the manifestations of immunologic or allergic lung disease. It is likely that far more information will become available about this important receptor in the development of asthma. There is less information available on C4a and C3a binding. The membrane receptor for C3a is clearly different from that of C5a and can be triggered to cause mucus secretion in the airways, but its role in immunologic airways disease is still speculative.

Control of the late steps in the complement cascade

The later steps in the complement cascade are also under tight control. The site composed of the C3 convertase with bound C5 will decay if it does not bind C6 rapidly, and there are a series of molecules that downregulate the late-acting proteins both in serum and on cells. S-protein, a plasma protein, interacts with C7 as the C5, C6, and C7 complex forms and becomes hydrophobic.[21] On binding S-protein, this complex is neutralized and can no longer bind to cell surfaces. Similarly, clusterin, another plasma protein, binds to the forming C5-9 complex and prevents its activation and completion. Most cells in the body have membrane-bound CD59, which interacts with the C5b-8 site, decreasing the binding of C9 and preventing polymerization of C9. It protects the cell by preventing effective pore formation. All of these control molecules are important in maintaining homoeostasis, and loss of the control molecules often leads to disease. CD59, like CD55, is linked to cell membranes by a phosphatidylinositol linkage. By not having a transmembrane domain, the protein is free to move rapidly in the fatty hydrophobic plane of the cell membrane to intercept forming C5b-9 and prevent cell lysis. Almost all patients with the disease paroxysmal nocturnal hemoglobinuria (PNH) have an acquired bone marrow defect in which they have a mutation in bone marrow stem cells of the gene *PIGA* (phosphatidylinositol glycan class A), the first enzyme in the development of phosphatidylinositol linkages.[25] A single patient with a genetic deficiency of *CD59* has been reported, and this patient also had PNH. This gene is present on the X-chromosome, and a single gene defect in a bone marrow stem cell leads to an inability to synthesize the first intermediate in this linkage pathway and therefore the failure to have phosphatidylinositol-linked proteins on the cell membrane. A failure to generate hematopoietic cells with CD59 causes all hematopoietic cells of bone marrow origin derived from the abnormal clone to be easily lysed by complement. As mentioned, alternative pathway proteins in the circulation undergo slow activation; CD59 is critical for neutralizing membrane attack proteins when they bind to our own cells. In patients with PNH, this mechanism is defective, and patients have a hemolytic anemia, often thrombocytopenia, and often a low neutrophil count. Recently eculizumab, a humanized monoclonal anti-C5 protein was approved for the treatment of PNH.[26] This antibody binds C5 and prevents complement-mediated lysis while allowing opsonization that occurs at the earlier C3 step to proceed. This is the first medication that improves cell survival in this patient group with a disease that has a generally grim prognosis.

The role of complement in the generation of immunologic lung disease is of particular interest. For many years, it was taken as gospel that complement plays no role in IgE-mediated lung disease or asthma. Recent work has suggested that this might not be the case. Complement can play a number of interesting functions in the generation of lung pathology.

First, it has been suggested that complement functions importantly in directing immune responses toward T_H1- or T_H2-type immunity. T_H1 immunity is generally considered most important in prevention of infection, and T_H2 immunity is associated with asthma and other allergic diseases. It is believed that the activation of C5 and the generation of C5a are important in directing the immune response toward a T_H1 phenotype, and lack of C5 therefore skews the system toward the generation of T_H2 immunity.[27,28] On the other hand, once immunity or allergy is established, it is believed that C5a might be generated during immunologic responses in the lung and, acting as an anaphylatoxin, might cause mast cell degranulation, smooth muscle contraction, and so on, thereby contributing to the asthmatic response.

COMPLEMENT IN THE AFFERENT LIMB OF THE ADAPTIVE IMMUNE RESPONSE

In recent years, attention has turned to the role of complement in the development of immunity.[29,30] This discussion has focused so far on the efferent limb of the response and how tissue damage is caused or controlled by complement. As mentioned early in the chapter, the complement system is phylogenetically older than the adaptive immune system, and many of the complement proteins existed as the adaptive immune system evolved.[31] Therefore it is not surprising that elements of the complement system were incorporated into the adaptive immune system, and these elements are only now being slowly identified. As mentioned in an earlier section, the binding of complement to an antigen allowing cross-linking of CD21 and the B-cell receptor increases antigenicity by up to 1000-fold. In this case complement augments the immune response. It is also known that subjects deficient in complement, although rare, often have major defects in adaptive immunity. Animals deficient in C1q, C4, C3, and CR1/2 make a poor immune response, particularly to T-dependent antigens; have poor germinal center formation; and have poor immunologic memory. Complement aids in the localization and retention of antigens within the germinal center, and it is believed that this localization of antigen to the germinal center facilitates an ongoing immune

response. Perhaps surprisingly, patients deficient in C1, C4, and, to a lesser extent, C2, have a high propensity toward systemic lupus erythematosus.[32,33] In fact, of the relatively few C1q-deficient subjects who have been described, 96% have had systemic lupus. Of the relatively few C4-deficient subjects who have been described, 75% have had lupus. Even heterozygosity of the genes for C4 predispose subjects to the development of lupus. This propensity to cause systemic lupus erythematosus seems to be independent of the genetic localization of C4, C3, and factor B in the major histocompatibility locus as class III genes and therefore their linkage to the MHC. In addition to the above, animals, particularly those deficient in C1q and C4, do not develop normal tolerance as well, although animals deficient in C3 and CR1/2 do not appear to have this defect. Although these are intriguing findings and have been repeated in many laboratories, it is still not completely clear how complement functions in the afferent limb of the adaptive immune response. It is quite likely that this question will be clarified over the next few years.

COMPLEMENT DEFICIENCIES AND CLINICAL ILLNESS

In the preceding paragraphs we have mentioned many diseases associated with defects in complement activation or control. Although recent research has demonstrated that HAE is not a disease whose clinical manifestations are due to defects in complement activation, it has typically been considered in this group. Because enormous progress has been made in defining its pathogenesis and treatment, it is given more detailed consideration.[34,35]

HAE is an inherited disease caused by low functional levels of the complement control plasma protein C1-INH. Patients have spontaneous episodic attacks of angioedema or deep localized swelling, most commonly of a hand or foot, that begin during childhood and become much more severe during adolescence. The edema is nonpitting and nonpruritic and is not associated with urticaria. Patients usually have a prodrome, a tightness or tingling in the area that will swell, lasting most frequently for several hours, followed by the development of angioedema. The swelling typically becomes more severe over about 1½ days and then resolves over about the same time period. In some patients attacks are preceded by the development of an erythematous rash that is not raised and not pruritic: erythema marginatum. The second major symptom complex noted by these patients is attacks of severe abdominal pain caused by edema of the mucosa of any portion of the gastrointestinal tract. The intensity of the pain can approximate that of an acute abdomen, often resulting in unnecessary surgical intervention. The gastrointestinal edema generally follows the same time course to resolution as the cutaneous attacks.

Laryngeal edema is the most feared complication of HAE and can cause complete respiratory obstruction. Although life-threatening attacks are infrequent, more than half the patients with HAE have laryngeal involvement at some time during their lives. Dental work with the injection of a topical anesthetic into the gums is a common precipitant, but laryngeal edema is often spontaneous. The clinical condition can deteriorate rapidly, progressing through mild discomfort to complete airway obstruction over a period of hours. Soft tissue edema can be difficult to see when it involves the larynx. If the swelling progresses to difficulty swallowing secretions or a change in the tone of the voice, this should be considered an emergency and might require emergency intubation or even tracheostomy to ensure an adequate airway. Other presentations are less common, but genital swelling is sometimes noted in male and female patients.

In most cases the cause of the attack is unknown, but some patients note that trauma or emotional stress precipitates attacks. In some female patients menstruation also regularly induces attacks and estrogens increase attack frequency. The frequency of attacks varies greatly among affected subjects and at different times in the same subject, with some experiencing weekly episodes, whereas others might go years between attacks, and attacks can start at any age.

As noted above, C1-INH is a serpin that inactivates its target by forming a stable one-to-one complex with the enzyme to be inhibited. Although hepatocytes are the primary source of C1-INH, the protein is also synthesized by monocytes. The regulation of the protein production is not completely understood, but because patients respond clinically to attenuated androgens with increased serum levels of C1-INH, it is believed that these androgens may stimulate C1-INH synthesis. HAE is an autosomal dominant disease, with as many as 25% of patients providing no family history. Presumably, most of these cases are caused by new gene mutations. Because all C1-INH–deficient patients are heterozygous for this gene defect, it is believed that half the normal level of C1-INH is not sufficient to prevent attacks.

Although named for its action on the first component of complement (C1 esterase), C1-INH also inhibits proteins of the fibrinolytic, clotting, and kinin pathways (Fig 3). Specifically, C1-INH inactivates plasmin-activated Hageman factor (factor XII) and its fragments, activated factor XI, tissue plasminogen activator, and kallikrein. Within the complement system, C1-INH blocks the activation and activity of C1 of the classical pathway and MASPs 1 and 2 of the lectin pathway. Without C1-INH, unchecked activation of complement causes cleavage of the C4 and C2 proteins in the complement sequence, and patients often have low levels of these proteins. Levels of the next protein in the complement cascade, C3, are normal. The major factor responsible for the edema formation is now known to be bradykinin, an important nonapeptide mediator that can induce leakage of post capillary venules. Bradykinin is derived from cleavage of the circulating protein high-molecular-weight kininogen by the plasma enzyme kallikrein, the activity of which is controlled by C1-INH.

There are 2 genetic types of C1-INH deficiency that result in essentially the same phenotypic expression. The C1-INH gene serping 1 is located on chromosome 11 in the p11-q13 region. The inheritance is autosomal dominant with incomplete penetrance. Type 1 is the most common form and accounts for approximately 85% of cases. Synthesis of or secretion C1-INH is blocked at the site of a faulty allele but occurs at the normal allele. The result is transcription of the normal protein, yielding quantitative serum concentrations of C1-INH that are approximately 10% to 40% of normal values. Type 2 HAE accounts for approximately 15% of cases. Mutations near the active site of the inhibitor lead to synthesis and secretion of nonfunctional C1-INH protein. These patients also have a normal functioning allele. Patients with type II HAE have either normal or increased concentrations of the protein.

A clinical syndrome resembling HAE and termed type 3 HAE has been described that affects mostly woman. In this condition no abnormalities of complement or of C1-INH have been described, but one third of patients have been found to have a gain-of-function abnormality of clotting factor XII, and it appears that many of the other patients with type III disease have defects in the proteins that cause normal bradykinin degradation.

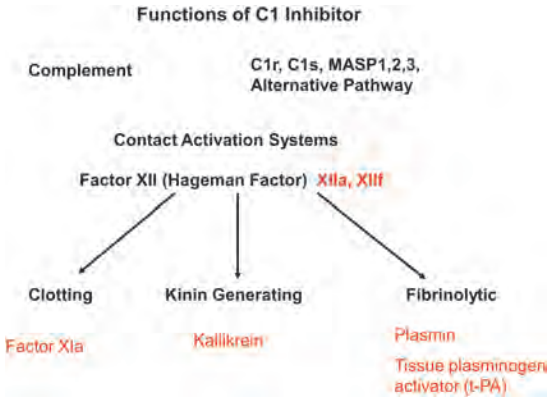

FIG 3. Functions of C1-INH.

In America 3 treatment regimens are available for prophylaxis, and within the last months US Food and Drug Administration (FDA) has approved treatments for acute angioedema attacks. Impeded androgens, such as the gonadotropin inhibitor danazol, have been found to reliably prevent attacks in the vast majority of patients. Impeded or weak androgens have many side effects that, although usually mild, preclude their use in some patients and they are not effective in everyone. In children they can cause premature closure of boney epiphyses, and they are not used in pregnant women. The fibrinolysis inhibitor ϵ aminocaproic acid is also effective in preventing attacks and is often used in children, but its use is attended by the development of severe fatigue and muscle weakness over time.

Recently, purified C1-INH, prepared from human plasma (trade name Cinryze, Viropharma US), given IV has been approved for prophylaxis of HAE, but the half-life of this protein is short, on the order of 40 hours. In clinical trials it was administered intravenously (1000 U) 2 to 3 times a week. A second plasma C1-INH preparation (trade name Berinert, CSL Behring, Australia) at 20 U/kg was recently approved for acute treatment of attacks by the FDA. Recombinant C1-INH (Rhucin) is also in development. Kalbitor, Dyax US (Ecallantide) a 60 amino acid kallikrein antagonist given SQ was recently approved for treatment of acute attacks, and a bradykinin type 2 receptor antagonist (Firazyr, Shize, US) are also reported to be effective in the treatment of acute attacks in preliminary double-blind studies and are in various stages of applying for FDA approval. Thus it is likely that treatment will be greatly modified with the availability of these new agents in the next few years.

Both patients and animals deficient in the classical pathway factors and C3 have an increased propensity to infection, particularly with high-grade pathogenic bacteria like pneumococci, as opposed to viruses (Table I).[9,36] Patients with late component defects, such as of C5-9, have a propensity toward systemic *Neisseria* species infections with *Neisseria gonorrhoeae* or *Neisseria meningitides*. Why opsonization, which only requires complement through C3, is not sufficient to protect against these 2 groups of organisms is not clear, but repeated infection with either of these 2 organisms is often an excellent tip to the clinician that a late complement protein deficiency is present. Alternative pathway defects are rarer, and in fact, no factor B deficiency has ever been described.[37] The few patients with factor D deficiency also have a propensity toward infection, but autoimmunity has not been seen in either animals or patients with defects in this pathway. Defects in the lectin pathway are being defined currently.[6] As discussed earlier in the chapter, MBL has a central core and a series of radiating arms ending in the lectin-binding sites. The radiating arms have the structure of collagen and, like collagen, are composed of 3 intertwined chains; however, unlike collagen, the chains are identical. It has been noted that single-gene defects affecting these chains can lead to improper winding of the chains about one another during the formation of the protein, leading to low levels of MBL. This protein is present normally at very low levels, 2 μg/mL, and patients, commonly with one of 3 genetic defects in the *MBL* gene, even when present in the heterozygous state, have inefficient chain matching and as little as one tenth of the normal level of MBL. Moreover, defects in the promoter region of the gene have been shown to lead to low MBL levels in some patients. It is reported from Europe that children with these defects have a high frequency of infection, although few studies have been done in America to confirm this finding. It is reported that the incidence of other rare infectious disease is increased in this patient group. It is also reported that subjects with MBL abnormalities often die early during the course of cystic fibrosis. Because patients with cystic fibrosis typically have high-titer antibody to their organisms, it is not known why the MBL deficiency should lead to early death. It is also suggested that MBL deficiencies facilitate the pathogenesis of rheumatic disease. All of these observations are intriguing, and all require considerably more study before we understand both the observations and their meaning.

It should be clear from this brief review that complement proteins are capable of having important biological effects and can influence the expression of a wide variety of autoimmune and allergic diseases. We believe that as we develop a clearer understanding of the complex interactions involved in pathogenesis, we will develop a far more insightful approach to the treatment of these important illnesses.

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Immune responses to malignancies

Theresa L. Whiteside, PhD, ABMLI *Pittsburgh, Pa*

Immune responses to tumor-associated antigens (TAs) are often detectable in tumor-bearing hosts, but they fail to eliminate malignant cells or prevent the development of metastases. Patients with cancer generate robust immune responses to infectious agents (bacteria and viruses) perceived as a "danger signal" but only ineffective weak responses to TAs, which are considered as "self." This fundamental difference in responses to self versus nonself is further magnified by the ability of tumors to subvert the host immune system. Tumors induce dysfunction and apoptosis in $CD8^+$ antitumor effector cells and promote expansion of regulatory T cells, myeloid-derived suppressor cells, or both, which downregulate antitumor immunity, allowing tumors to escape from the host immune system. The tumor escape is mediated by several distinct molecular mechanisms. Recent insights into these mechanisms encourage expectations that a more effective control of tumor-induced immune dysfunction will be developed in the near future. Novel strategies for immunotherapy of cancer are aimed at the protection and survival of antitumor effector cells and also of central memory T cells in the tumor microenvironment. (J Allergy Clin Immunol 2010;125:S272-83.)

Key words: *Cancer, immunity, tumor escape, immune suppression, effector T cells*

Abbreviations used

Anx:	Annexin V
APC:	Antigen-presenting cell
APM:	Antigen-processing machinery
β2 m:	β_2-microglobulin
CTL:	Cytolytic T lymphocyte
DC:	Dendritic cell
FasL:	Fas ligand
FOXP3:	Forkhead box protein 3
iNOS:	Inducible nitric oxide synthase
MDSC:	Myeloid-derived suppressor cell
NK:	Natural killer
PD-1:	Programmed death 1
PD-L1:	Programmed death ligand 1
PGE_2:	Prostaglandin E_2
ROS:	Reactive oxygen species
STAT3:	Signal transducer and activator of transcription 3
TA:	Tumor-associated antigen
TAM:	Tumor-associated macrophage
TCR:	T-cell receptor
TIL:	Tumor-infiltrating lymphocyte
Treg:	Regulatory T
VEGF:	Vascular endothelial growth factor

Evidence accumulated over the last few years convincingly shows that the host immune system is involved in cancer development and progression, as well as control of metastasis. The presence of antitumor cellular responses, humoral responses, or both to tumor-associated antigens (TAs) has been observed in many, but not all, patients with cancer.[1,2] The evidence for such pre-existing antitumor immunity in patients with cancer confirms that the tumor-bearing host is capable of mounting an immune response to TAs. Tumor progression from a single transformed cell to a mass of malignant cells is a multistep process involving a series of genetic changes occurring in human subjects over a period of months or years and culminating in the established tumor.[3] During this period, neither the host immune system nor the developing tumor are idle: those newly emerging tumor cells that are recognized by the immune system are eliminated only to be replaced by genetic tumor variants resistant to immune intervention and giving rise to a heterogenous population of malignant cells found in any tumor. Tumors are genetically unstable, and the emergence of new genetic variants, which is responsible for the tumor heterogeneity, ensures that the tumor survives in the face of the host immune system. Only the tumor cells that manage to avoid recognition escape and survive, whereas those that are recognized by the immune system are eliminated as soon as they arise. The tumor development involves a prolonged series of checks and balances between the host attempting to curtail tumor growth and the tumor benefiting from genetic changes, altering its microenvironment and avoiding immune elimination. Thus the tumor becomes resistant to immune effector cells.

The interactions between the host and the tumor have been referred to as "immune surveillance," a concept that originated many years ago with F. M. Burnett and that introduced his vision of a vigilant host immune system able to spot, recognize, and eliminate tumor cells. A modern version of the immune surveillance theory not only emphasizes the ability of the host immune system to recognize and destroy tumor cells but also its contribution to "immune selection" of resistant tumor variants. Thus the "immune editing" hypothesis[2,4] has been advanced to suggest that by means of elimination of tumor cells sensitive to immune intervention, the host immune system edits for survival of tumors that become resistant to immune cells. An alternative hypothesis allows for the progressing tumor to develop immunosuppressive mechanisms that will thwart any attempt of immune tumor elimination and in effect will induce a state of tumor-specific tolerance.[5] In the first instance the immune system initiates the selection of resistant tumor variants, and in the second the tumor becomes a perpetrator of immune unresponsiveness. Central to the paradigms of immune selection or immune editing and

From the University of Pittsburgh Cancer Institute and the Departments of Pathology, Immunology, and Otolaryngology, University of Pittsburgh School of Medicine.

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Reprint requests: Theresa L. Whiteside, PhD, University of Pittsburgh Cancer Institute, 5171 Centre Ave, Pittsburgh, PA 15213. E-mail: whitesidetl@msx.upmc.edu.

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immune suppression is the premise that the tumors acquiring new mutations are able to avoid immune intervention and are capable of both escaping and disabling the host immune system. Neither of the 2 hypotheses has been completely accepted today, and there are those who believe that tumors progress because of the genetic instability and others who favor tumor-specific tolerance of the immune system, which enables the tumor to take advantage of the tissue microenvironment regardless of the immune system and benefit from it. This controversy regarding the significance of the immune system in tumor development and progression underscores the complexity of interactions between the tumor and the immune cells. It surmises that these interactions might be bidirectional, are influenced by the local microenvironment, and not infrequently might result in demise not of the tumor but of tumor-reactive immune cells.

In this chapter the nature and components of the host immune response against tumors will be discussed, including the reasons for the failure of the immune system to contain tumor growth and metastasis. It is this latter aspect of the immunobiology of human malignancies that will be emphasized, largely because it directly affects cancer immunotherapy. A relatively recent realization that tumors have devised multiple and remarkably effective mechanisms for disarming the host immune system has opened a way for the introduction of novel therapeutic strategies aimed at eliminating tumor escape. If the tricks tumors use for protection from immune intervention by the host are responsible for their progression, then it could be surmised that a limited success of current immune therapies for cancer can be reversed by therapies that target the escape mechanisms, and because these escape mechanisms might be unique for each tumor rather than generalized, the future challenge will be to identify the "immunologic signature" of each tumor and then use selective therapies to eliminate the tricks and restore vigorous antitumor immunity.

TUMOR PROGRESSION AND THE HOST IMMUNE RESPONSE

There are several lines of evidence that point to an early, as well as late, involvement of the immune system in tumor development. Early tumor lesions, and even premalignant foci, such as melanocytic nevi, are frequently infiltrated with hematopoietic cells, including lymphocytes, macrophages, and occasionally granulocytes.[6,7] The presence of immune cells in the tumor at later stages of development (ie, the abundance of tumor-infiltrating lymphocytes [TILs]) has been associated with improved patient survival in several early studies (reviewed in Whiteside[8]). More recently, studies by Fridman's group performed a comprehensive multivariate analysis of cellular interactions in the tumor microenvironment based on the type, density, localization, and function of immune cells present within human colorectal cancer and demonstrated that immune reactivity at the tumor site influences clinical outcome.[9-11] Thus increased densities of T-cell infiltrates with a high proportion of $CD8^+$ T cells within primary colorectal carcinomas were associated with a significant protection against tumor recurrence.[11] Furthermore, the same group also showed that coexpression of genes mediating cytotoxicity and T_H1 adaptive immune responses accurately predicted survival in patients with colorectal carcinoma independently of the metastatic status.[12] In aggregate these multiparameter analyses of tumor-infiltrating cells *in situ* suggest that immune cells can and indeed often do play a role in tumor control but that both intrinsic and extrinsic factors in the tumor microenvironment alter the balance required for optimal control.[12]

In many patients with cancer, it is possible to expand in culture and *in vitro* test functions of tumor-specific cytolytic T lymphocytes (CTLs) from the peripheral blood or TILs.[8] This finding, which has been reproduced in many laboratories, suggests that precursors of such CTLs exist in the circulation or at the tumor site in patients with cancer and can be induced to proliferate when autologous dendritic cells (DCs) pulsed with relevant tumor epitopes and used as antigen-presenting cells (APCs). More recent experiments, using tetramers and flow cytometry, have directly demonstrated the presence of tumor peptide–specific T cells in the circulation of patients with cancer.[1,13,14] Furthermore, the frequency of such peptide-specific T cells appears to be higher in the circulation of patients with cancer than in healthy subjects.[15] Finally, the SEREX technology, based on the presence of tumor-specific antibodies in sera of patients with cancer, has been successfully used for tumor-antigen discovery in many laboratories.[16] These findings, as well as recent identification of numerous TAs that appear to be immunogenic in that they induce humoral immune responses, cellular immune responses, or both *in vitro* by using human immune cells and *in vivo* in animal models of tumor growth, strongly support the notion that the host immune system recognizes the presence of the tumor and responds to it by generating both local and systemic immune responses.

If the tumors are not ignored by the immune system, why do they progress? Several answers to this question can be considered. First, there is the old argument for the lack of a "danger signal"[17] in tumors akin to those presented by pathogens invading tissues during an infection. Recognition by DCs of pathogen-associated molecular patterns through the ubiquitous Toll-like receptors leads to efficient DC activation and maturation. It promotes generation of vigorous cellular and antibody responses to bacterial or viral antigens, presumably because the immune system perceives an infection as a danger signal[17] benefiting the host. However, functional Toll-like receptors are known to be expressed by many human solid tumors,[18] and recent data indicate that tumors use them to promote their own growth; for protection from spontaneous, immune-mediated, or drug-induced apoptosis; or both.[18,19]

Second, TAs are perceived by the immune system as "self" or "altered self" antigens, which evoke weak immune responses because tolerance prevents generation of immune responses to self. The only "unique" TAs are mutated antigens, and these are strongly immunogenic and elicit robust immune responses.[20] However, only a handful of such mutated TAs are known, and the vast majority of TAs are poorly immunogenic or simply tolerogenic. In this context cancer can be viewed as an autoimmune phenomenon in which tolerance to self prevents effective immune responses to TAs Patients with cancer who have not been treated with chemotherapy or radiotherapy generally have normal immune responses to viral or bacterial antigens, yet they are unable to respond to their own TAs. Except for late-stage disease, they generally have normal delayed-type hypersensitivity responses to recall antigens but are anergic to autologous TAs. Although tolerance to self is a detriment to the generation of antitumor responses in patients with cancer, another factor that exerts an overwhelming effect on these responses is the tumor microenvironment. Each tumor creates its own milieu characterized by the presence of immunosuppressive factors and by the excess of

TAs produced and released by the growing tumor. Evidence suggests that tumors produce a broad array of immunoinhibitory factors, which exert either local or systemic effects on the host antitumor immune responses.[5] Therefore it is not surprising that antitumor immunity might be weak, inefficient, or even absent in patients with cancer, depending on the nature of tumor-host interactions, as well as the robustness of regulatory mechanisms in control of immune tolerance.

Immune antitumor responses could be influenced by the gradual deterioration of the immune system with age.[21] The increased incidence of cancer present in the elderly might be due to immunosenescence (ie, progressive remodeling of the immune system with a reduced ability of immune cells to respond to activating stimuli and increased responsiveness to tolerogenic signals).[21] Immunosenescence can significantly interfere with the effectiveness of cancer immunotherapies, and it has been suggested that clinical trials testing immunopotentiating agents in patients with cancer should be conducted in elderly subjects.[21]

Recent multiparameter analyses of primary and metastatic human tumors (eg, colorectal carcinoma) recognize several major immune "coordination profiles," the presence of which is influenced by the balance between tumor escape and immune antitumor responses and that are subject to host-tumor cross-talk.[12] In this context it is important to consider differences between primary and metastatic tumors. Not only are metastatic tumors more immunosuppressive, but also they appear to be less readily recognized by TA-specific immune effector cells. The latter could be due to defects in the expression levels of antigen-processing machinery (APM) components, MHC molecules, or both in the tumor and its metastases.[22] Because different copy numbers of distinct trimolecular peptide–β_2-microglobulin (β2 m)–MHC complexes presented on the tumor surface might lead to differential T-cell recognition, this aspect of tumor–immune cell interactions is critical.[22,23] A recent comparison of primary renal cell carcinoma, renal cell carcinoma metastases, and normal renal tissue with respect to HLA ligand presentation and gene expression demonstrated a greater similarity between primary tumor and metastasis than between the tumor and normal tissue.[24] This observation provides a good rationale for peptide-based immunotherapy because it is likely to preferentially target the tumor and its metastases and not the normal tissue.

NATURAL VERSUS ADAPTIVE IMMUNE RESPONSES TO MALIGNANCIES

Antitumor immune responses can be innate (natural) or acquired (adaptive). Innate immunity is mediated by cells or soluble factors that naturally exist in tissues or body fluids and can interfere with tumor growth or survival. Among hematopoietic cells, macrophages, granulocytes, natural killer (NK) cells ($CD3^-CD56^+$), non–MHC-restricted T cells ($CD3^+CD56^-$), and $\gamma\delta$ T cells have the natural capability to eliminate tumor cell targets.[21] In addition, natural antibodies with specificities directed at surface components of tumor cells might be present in the sera of patients with cancer.[16] Other serum factors, including complement components, C-reactive protein, mannose-binding protein, and serum amyloid protein, also play a role in innate immunity.[25] Adaptive immune responses to tumors are mediated by $CD3^+$ T-cell receptor (TCR^+) T cells when they recognize tumor-derived peptides bound to self-MHC molecules expressed on APCs. Little is currently known about the molecular signals and cellular steps involved in directing APCs, such as DCs, to execute a tolerogenic versus immunogenic program in response to antigens. As indicated above, tumors can also serve as APCs, although low levels of MHC class I molecule expression, MHC class II molecule expression, or both on the surface of tumor cells makes this an inefficient process.[22] More likely, TAs are taken up by DCs present at the tumor site, processed, and cross-presented to T cells in the tumor-draining lymph nodes in the form of the trimolecular peptide–β2m–MHC complexes.[23] For adaptive immune response to occur, T cells expressing correct (cognate) TCRs have to be present. Recognition of the peptide and its binding to the variable domains of the TCR initiates signaling (signal 1) that leads to T-cell activation.[26] This requirement implies prior sensitization and a clonal expansion of memory T cells in response to a cognate tumor epitope (anamnestic or recall responses). Alternatively, precursor T cells expressing the TCR can be primed by the cognate peptide–MHC ligands presented on APCs, and the subsequent development of antitumor effector cells is viewed as a primary immune response. In either case costimulatory molecules (signal 2) are necessary for an immune response to proceed,[27] and once T-cell proliferation is initiated, appropriate cytokines (signal 3) become essential for sustaining the response.[28] Recent findings stress the key importance of signal 3 for the development of immune responses and for their contraction.[28] Like all immune responses, those that are TA specific do not go on forever but peak and then contract, restoring the preactivation balance. The precise mechanisms responsible for immune contraction are not yet defined, and regulatory T (Treg) cells, as well as other mechanisms, have been proposed to regulate immune reactivity, but it is clear that events in the environment play a dominant role in this respect.

Immune responses to malignant cells can be categorized as locoregional or systemic. *In situ* or local responses refer mainly to TILs, which accumulate in most human solid tumors and the role of which in tumor progression remains highly controversial. Long considered by some an effector arm of antitumor responses, TILs are viewed by others as victims of the tumor microenvironment because their effector functions are often impaired, presumably by tumor-derived factors.[29] A failure of local antitumor responses mediated by TILs is thought to contribute to tumor progression. Systemic immunity to tumors, as measured by delayed-type hypersensitivity responses or by various *ex vivo* assays of T-cell responses in the peripheral circulation of patients with cancer, are difficult to demonstrate, and TA-specific responses have been particularly elusive. Nevertheless, by using highly sensitive multicolor flow cytometry, it has been possible to detect and measure the frequency of TA-specific $CD8^+$ and $CD4^+$ T cells in the peripheral circulation of patients with cancer.[1] Although the response levels vary, TA-specific and nonspecific proliferative or cytotoxic responses of peripheral lymphocytes in patients with cancer appear to be at least partially impaired.[29-31] Data indicate that the same functional impairments seen in TILs are found in both circulating and lymph node lymphocytes of patients with cancer.[29,32] Thus it has been concluded that, in general, human tumors exert profound suppressive effects on both local and systemic antitumor immunity in these patients.

In contrast to the failure of antitumor immune responses to control tumor progression in human subjects, a large body of experimental evidence derived from preclinical animal models of cancer suggests that the immune system can prevent tumor growth or cause its rejection.[33] In the prevention setting

vaccination of animals with TAs plus adjuvant protects them from rechallenge with tumor,[34] whereas immunotherapy of established tumors with vaccines, cytokines, adoptively transferred immune cells, or immunomodulatory agents results in tumor rejection, provided the tumor is not in an advanced stage. Remarkably, this has been a consistent pattern seen with carcinogen-induced, virally induced, and spontaneously arising tumors in mice, suggesting a fundamental difference in immune responses to tumor antigens between mice and human subjects. Indeed, it appears that the difference might be due to appreciably greater immunogenicity of murine TAs, which in most cases are virus- or carcinogen-related epitopes and thus foreign rather than self-epitopes. Alternatively, the answer might be that experimental murine tumors are established, grow, progress, and are eliminated by therapy in the very short time required for the completion of the experiment, leaving no time for the development of tumor escape mechanisms. In contrast, human tumors are diagnosed and treated after many years of coexistence with the host. An introduction or establishment of the tumor in mice is a dramatic event that mobilizes host defenses in contrast to a silent coexistence of tumor cells with the immune system for many years in human subjects. To minimize this difference, transgenic murine models have been developed, allowing for ensured, genetically driven tumor development in a "spontaneous" environment.[35] Transgenic mice have been especially useful in the design of preventive cancer vaccines,[34] and information they provide is encouraging for the development of immunoprophylaxis of cancer in human subjects. Nevertheless, to date, it has been difficult to translate the positive results seen in mice to immunotherapy of established human tumors. It is plausible that numerous and varied mechanisms of escape developed by the latter during the prolonged residence and interactions with the host provide human tumors with advantages not afforded to murine tumors established in an experimental setting.

TUMOR ASSOCIATED ANTIGENS

Recent progress in the development of cancer vaccines has been greatly facilitated by the availability of well-defined TAs, many of which have been characterized in the last decade.[36] Most of these TAs are derived from self-proteins that are either mutated or otherwise differentially expressed in normal and tumor cells, as exemplified by oncogenes or oncofetal or cancer testis antigens. The major categories of TAs that have been often used as candidates for immune therapies are listed in Table I.[36,37] A recent report provides a much longer prioritized list of well-characterized cancer antigens best suited for use in cancer vaccines.[38] The list is based on criteria generated by a panel of experts convened by the National Cancer Institute[38] and is designed to assist investigators in the field of immunotherapy in the selection of the most promising TAs for further testing in clinical trials.

As already indicated, immune responses to TAs, even to those representing altered self-antigens, are detectable in tumor-bearing hosts, although in most cases no correlations between the presence of *in vitro* responses to TAs and prognosis have been documented. This is in contrast to numerous animal tumor models, which have provided strong evidence that in the presence of effective antitumor immunity, tumors fail to progress and established tumors regress.[39] Nevertheless, human cancer vaccine trials in patients with cancer have made use of many well-characterized TAs in the hope that their presentation on appropriately polarized DCs will overcome difficulties with the generation of a strong immune response in the therapeutic setting. The most recent reports of such clinical trials in patients with cancer indicate that multiple subcutaneous injections of an immunogenic tumor peptide, such as NY-ESO-1, plus a mix of 2 potent adjuvants, such as Montanide ISA-51 and CpG7909, can be effective in inducing sustained peptide-specific immune responses and significantly prolong survival, even in patients with advanced disease, including solid tumors other than melanoma.[40] These reports, demonstrating that antitumor, antivaccine, or both immune responses correspond to clinical outcome, suggest that the optimization of vaccination strategies is likely to overcome tumor-induced suppression and to restore the immune balance altered by cancer development.

IMMUNE CELLS IN THE TUMOR MICROENVIRONMENT

Immune cells that are most frequently found in the human microenvironment are lymphocytes, which are capable of mediating both innate and adaptive immunity, although monocytes, tumor-associated macrophages (TAMs), and DCs are also commonly seen.[41] Inflammatory cells present in the tumor are in intimate contact with tumor cells, stromal fibroblasts, extracellular matrix components, and blood vessels. Proinflammatory cytokines secreted by inflammatory cells can contribute to tumor progression, and soluble factors produced by the tumor in response to nonspecific or tumor-specific signals, such as prostaglandin E_2 (PGE_2), adenosine, or TGF-β, downregulate functions of immune cells. The tumor microenvironment is created by the tumor, and it is continuously shaped and dominated by the tumor, which directs all cellular and molecular events taking place in the surrounding tissue.

Immune cells recruited to the tumor include T cells ($CD3^+TCR^+$), which are by far the largest component of mononuclear tumor infiltrates[41] and have received the most attention. Although their accumulation in the tumor might be considered evidence of immune surveillance by the host, they are largely ineffective in arresting tumor growth, although they can proliferate and mediate antitumor cytotoxicity on their removal from the tumor bed and *ex vivo* IL-2 activation.[42]

Phenotypic and functional characteristics of human TILs are listed in Table II. More current data on the status of T cells found in human tumors suggest that their phenotypic and functional profile varies depending on the microenvironment created by the tumor and that this profile or "immune signature" can influence prognosis and disease outcome.[9,12] It appears that TILs obtained from advanced or metastatic lesions are more functionally impaired than those from early lesions, suggesting that tumor burden or the potential of a tumor to suppress immune cells might determine the functional status of infiltrating T cells. Among $CD4^+$ T cells present in the tumor, a subset of $CD4^+CD25^{high}$ forkhead box protein 3 (FOXP3)–positive Treg cells is expanded to constitute from 5% to 15% of CD4 T cells in the infiltrate. Their frequency is higher in the tumor than in the peripheral circulation.[43,44] These cells suppress functions of other immune cells in the microenvironment by mechanisms that might be cell contact dependent or might involve the production of inhibitory cytokines or adenosine.[43-46] Recently, a potent proinflammatory T-cell subset, IL-17–producing T_H17 cells, were observed among $CD4^+$ cells in patients with ovarian carcinoma. The presence of

TABLE I. Human TAs that are candidates for immune therapies*

TA category	Examples
Oncofetal	Oncofetal antigen/immature laminin receptor (OFA/iLRP)
	Glypican 3 (heparan sulfate protoglycan)
	α-Fetoprotein (AFP)
	Carcinoembryonic antigen (CEA)
Oncogenes	The RAS family: p53, Her2 neu
Cancer testis (CT) antigens:	MAGE-1
	BAGE
	GAGE
	NY-ESO-1/LAGE
	SAGE
	Other 35-40 CT antigens mapping to chromosome X (CT-X) or distributed throughout the genome (non-X CT)
Human melanoma antigens	MART-1/MELAN-A
	Gp100/pmel 17
	Tyrosinase
	Tyrosinase related proteins (TRP) 1 and 2
	Chondroitin sulfate proteoglycan (CSPG4)
Human glioma antigens	IL-13 receptor α2
	Eph A2
	Survivin
	EGFR variant III (EGFRvIII)
Head and neck cancer antigens	EGFR
	Human papilloma virus (HPV 16 or 18)
	Aldehyde dehydrogenase A1 (ALDHA1)
	CSPG4
Normal overexpressed or modified antigens	MUC-1
	Cyclin-B1
	Prostate-specific antigen (pSA)
	Prostate membrane-specific Ag (PMSA)

*The actual list of TAs available for immune therapies is much longer. The reader is referred to a more comprehensive recent listing of these antigens.[36,37]

these cells was significantly correlated to enhanced survival in these patients and was found to inversely correlate with the number of $FOXP3^+$ Treg cells.[47]

Macrophages ($CD14^+$) present in tumors are referred to as TAMs. Although normal macrophages uptake antigens and play an important role in control of infections, TAMs are reprogrammed to inhibit functions of immune cells through the release of inhibitory cytokines, such as IL-10, PGE_2, or reactive oxygen species (ROS).[48] It is hypothesized that reprogramming of TAMs occurs in the tumor microenvironment as a result of tumor-driven activation. Evidence has accumulated indicating that invasiveness of tumors, such as human primary colon carcinomas, is directly related to the number of TAMs detected in the tumor. In patients with invasive breast cancer, an increased TAM count is an independent predictor of reduced relapse-free survival, as well as reduced overall survival.[49] The available data support the active role of TAMs in tumor-induced immunosuppression on the one hand and in the promotion of tumor growth on the other. Furthermore, preliminary evidence suggests that the reciprocal differentiation of Treg and T_H17 cells from an uncommitted common $CD4^+$ precursor along either a suppressive or proinflammatory pathway, respectively, is biased by TAMs.[47] Thus TAMs appear to significantly contribute to shaping of the tumor microenvironment.

A subset of myeloid-derived cells equivalent to $CD11b^+/Gr1^+$ cells in mice, which are $CD34^+CD33^+CD13^+CD15^-$ and called myeloid-derived suppressor cells (MDSCs), accumulate in human tumors.[50] They are recruited from the bone marrow by means of tumor-derived soluble factors, such as GM-CSF, vascular endothelial growth factor (VEGF), and IL-10; migrate to lymph nodes, where DCs cross-prime T cells; and interfere with this process. They also migrate to tumors, become tumor-associated MDSCs, and inhibit immune cell functions through the production of arginase 1, an enzyme involved in the L-arginine metabolism. Arginase 1 synergizes with inducible nitric oxide synthase (iNOS) to increase superoxide and nitric oxide production, inhibiting lymphocyte responses by the induction of iNOS in surrounding cells.[51] Current data support the active role of MDSCs in tumor-induced immune suppression that contributes to functional dysfunction of immune cells in the tumor, as well as the peripheral circulation of patients with cancer.

DCs ($HLA\text{-}DR^+CD86^+CD80^+CD14^-$) are nature's best APCs. They are a common component of tumor immune infiltrates and are responsible for the uptake, processing, and cross-presentation of TAs to naive or memory T cells, thus playing a crucial role in the generation of tumor-specific effector T cells.[52] In addition, DCs control the induction of Treg cells. In patients with cancer, cellular interactions between antigen-presenting DCs and T cells lead to expansion and accumulation of Treg cells at the tumor site and in the periphery.[52] The DC-derived signals that determine the outcome of DC–T-cell interactions operate at the levels of (1) antigen presentation (signal 1); (2) display of costimulatory molecules (signal 2); and (3) the presence of immunomodulatory cytokines (signal 3). Stimuli that lead to upregulation of signals 1 and 2 in the absence of signal 3 might facilitate peripheral tolerance induction.[52] At the same time, newer evidence suggests that many conditions relevant to signal 1, such as antigen

TABLE II. Morphologic, phenotypic, and functional characteristics of TILs found in human solid tumors

Morphology: small to large lymphocytes
Phenotype: $CD3^+TCR\text{-}\alpha/\beta^+$ T cells; few (<5%) $CD3^-CD56^+$ NK cells
Mix of $CD4^+$ and $CD8^+$ cells; variable CD4/CD8 ratio
Largely $CD45RO^+CCR7^-$ memory T cells
Express activation markers (CD25, HLA-DR)
Nearly all are $CD95^+$
Accumulations of Treg cells ($CD4^+CD39$ + TGF-β^+) and $CD4^+IL\text{-}17^+$ T_H17 cells
Clonality: oligoclonal, as determined based on TcR Vβ gene expression
Specificity: autotumor-specific T cells detectable in some tumors at a low frequency
Functions: Low or absent ζ chain expression: inefficient TCR signaling
Suppressed nuclear factor κB activation
Decreased locomotion, proliferation, cytotoxicity
Cytokine profile: T_H2 type with IL-4, IL-5, and IL-13 production and no/little IL-2 or IFN-γ production; excess of IL-10 or TGF-β
In vitro response to IL-2 variable but more decreased in TILs recovered from metastatic rather than primary lesions
Increased levels of caspase-3 activity
Apoptosis of $CD8^+$ T cells (TUNEL+; Anx^+)

TUNEL, Terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling.

dose, determine whether Treg or T_H2 effector (Teff) cells are induced, irrespective of the maturation state of DCs.[52] In addition, insights into the APM in DCs and evidence that some of the components of APM, including MHC class II molecules, might be downregulated or altered in patients with cancer,[23] suggest that Treg cell induction might be influenced not only by the nature and dose of the antigen but also by its processing and its presentation to T cells.

Tumor-associated DCs directly exposed to tumor cells, tumor-derived factors, or both have been shown to readily undergo apoptosis and to have impaired maturation.[53] Specifically, tumor-derived factors, such as gangliosides, were shown to inhibit DC generation and their function *in vitro*.[54] This suppressive effect of gangliosides on DCs was found to be mediated by tumor-derived VEGF, a known antidendropoietic factor.[53] The data on functional impairments of tumor-associated DCs have to be balanced by numerous reports in the literature, which suggest that the presence of DCs in tumors is associated with improved prognosis and prolonged patient survival, as well as a reduced incidence of recurrent or metastatic disease.[55] In contrast, patients with lesions reported to be scarcely infiltrated with DCs have a relatively poor prognosis.[56] Fewer DCs were observed in metastatic than in primary lesions. In one study it was shown that the number of DCs present in the tumor was by far the strongest independent predictor of overall survival, as well as disease-free survival and time to recurrence, in a large cohort ($n = 132$) of patients with oral carcinoma compared with such well-established prognostic factors as disease stage or lymph node involvement.[55] It appears that not only the number of DCs but also the presence of functionally unimpaired, normally signaling T cells in the tumor microenvironment are important for overall survival of patients with cancer.[55]

NK cells ($CD3^-CD56^+CD16^+$), which mediate innate immunity and contain both perforin-rich and granzyme-rich granules, are well equipped to mediate lysis of tumor cells. Although NK cells represent "the first line of defense" against pathogens,[57] most human tumor cells are resistant to perforin-mediated NK cell lysis, and NK cells are rarely found among TILs.[41] This is despite the fact that tumor cells often downregulate MHC antigen expression and are enriched in MICA and MICB molecules.[58] There might be several reasons for the paucity of NK cells in tumors, including the possibility that NK cells are present in premalignant or early lesions and absent from advanced tumors, which is consistent with their role in immune surveillance rather than killing of cancer cells at the tumor site.[41] More recent data suggest that the primary biologic role of NK cells in tumor-bearing hosts might not be the elimination of tumor targets but rather the facilitation of DC–T-cell interactions and driving the immune responses to TAs.[59] Because the tumor site is not likely to be the optimal milieu for this type of immune interaction, the paucity of NK cells in tumors might fit with their physiologic functions. The *in vivo* role of NK cells in antitumor immune defense is not yet clear, and work continues to define it further.

Polymorphonuclear leukocytes are infrequently seen in infiltrates of human solid tumors, with the exception of nests of eosinophils that might be present in association with tumor cells in some cases. In human tumors granulocytes, which are a major cellular component of many murine tumors, are rare, being largely replaced by TAMs or MDSCs. This could be explained by the fact that most inflammatory infiltrates into human tumors are chronic rather than acute, with granulocytes long gone by the time human tumors are diagnosed, biopsied, and examined.

B cells ($CD19^+$, $CD20^+$) are also rare in most human tumors, with the exception of breast cancer and melanoma.[6,60] The primary function of B cells is differentiation into antibody-producing plasma cells. Although TA-specific antibodies are frequently detected in the circulation of patients with cancer, these antibodies are made and secreted in the tumor-draining lymph nodes, spleen, or other lymphoid tissues. From these sites, IgG molecules can readily be transported through plasma or lymph to tissue sites. Therefore the presence of B cells or plasma cells in tumors is not expected *a priori*, although it might be that the ability to make antibodies *in situ* could be an important aspect of host defense.

Inflammatory infiltrates present in human tumors change in composition and intensity during tumor progression. The initial acute inflammation involving the recruitment and influx of antitumor effector cells is replaced by chronic inflammation in later stages of tumor progression. Tissue hypoxia plays a major role in shaping the nature of immune infiltrates in tumors. It is created by activation of hypoxia-responsive genes in tumor cells[61] and favors the influx of granulocytes and phagocytic macrophages, which depend on the glycolytic pathway for survival.[62] These cells take up and process dying tumor cells, producing an abundance of ROS. The subsequent reoxygenation of the microenvironment is accompanied by activation of the nuclear factor κB pathway in both tumor cells and infiltrating immune cells, leading to the excessive secretion of proinflammatory cytokines.[5] Responding to this nuclear factor κB–driven cascade of proinflammatory cytokines, the tumor and stromal cells produce a variety of soluble factors with wide-ranging biologic effects, including the promotion of tumor cell proliferation.[5] In the tumor microenvironment cellular expansion, differentiation, or activation, as well as cell migration, matrix remodeling, and blood vessel growth, are reprogrammed to benefit the tumor. Thus the nature of chronic inflammatory infiltrates and functions of the tumor-infiltrating immune cells depend on how aggressively a given tumor remodels its microenvironment.

IMMUNE EFFECTOR CELLS IN THE CIRCULATION OF PATIENTS WITH CANCER

In human subjects peripheral blood is the major source of cells for studies of their antitumor functions. T lymphocytes, NK cells, monocytes, DCs, and B cells and their subsets have all been extensively evaluated in the peripheral circulation of patients with cancer by using conventional phenotypic and functional *in vitro* assays. Results indicate that signaling abnormalities, functional impairments, and apoptosis seen in immune cells obtained from the tumor microenvironment are likewise present in peripheral blood cells of patients with cancer.[63,64] The finding of $CD8^+$ T-cell apoptosis in the circulation of these patients is perhaps the most convincing evidence that all is not well with immune effector cells in cancer.[65] The proportion of $CD8^+CD95^+$ T cells that bind Annexin V (Anx) and yet are 7-amino-actinomycin D negative ($7AAD^-$) or propidium iodide (PI) negative is significantly greater in the peripheral circulation of patients with cancer, including those with head and neck, breast, and ovarian carcinomas and melanoma, than in age- or sex-matched healthy donors.[65] As indicated in Table III,[66-68] T cells that undergo spontaneous apoptosis in the circulation of these patients are $CD3^+CD95^+$, bind Anx, and have increased levels of caspase-3 activity and decreased expression of the TCR-associated ζ chain.[63,69,70] Circulating $CD8^+$ T cells, especially the effector subpopulations ($CD8^+CD45RO^+CCR7^-CD27^-$ and $CD8^+CD28^-$), have a significantly greater propensity to undergo spontaneous apoptosis than $CD4^+$ T cells in patients with cancer. This could explain the functional deficits found in $CD8^+$ effector cells, such as the downregulation in expression of signaling molecules, specifically the ζ chain. The available data suggest that functional defects in T cells might be linked to their increased sensitivity to apoptosis and that the tumor participates in engineering spontaneous or activation-induced cell death of T cells.[65] The highest proportions of $Fas^+Anx^+CD8^+$ T cells are generally seen in a subset of patients with advanced active disease.[70] In patients with cancer, the vast majority of circulating $CD8^+$ T cells are $CD95^+$, and the Fas/Fas ligand (FasL) pathway contributes to their apoptosis because human solid tumors express FasL and export it to the periphery in the form of $FasL^+$ exosomes.[71,72] However, tumor-induced apoptosis of immune cells engaging death ligand/receptor interactions is only one of many mechanisms used by tumors to engineer an immune escape.[65] Based on increasing insights into these mechanisms, it is possible to speculate that the presence of the constellation of immune defects might allow for the identification of a subset of patients with cancer who have poor prognosis because their tumors create a particularly immunosuppressive environment.

Apoptosis of Fas^+, activated $CD8^+$ T cells in the circulation of patients with cancer leads to a rapid turnover of T lymphocytes, contributing to a loss of antitumor effector cells and an aberrant lymphocyte homeostasis.[66,73] Recent data indicate that circulating Vβ-restricted $CD8^+$ T cells and tumor peptide–specific tetramer–positive $CD8^+$ T cells are especially sensitive to apoptosis.[74] By using T-cell receptor excision circle (TREC) analysis, a PCR-based technique that allows for quantification of recent thymic emigrants in the peripheral circulation, it has been determined that patients with cancer had significantly fewer recent thymic emigrants than healthy age-matched donors.[67] The results suggest that the lymphocyte turnover is faster in patients with cancer than in healthy control subjects, either because the thymic output in patients is lower or the peripheral expansion of T cells is greater, diluting T-cell receptor excision circles and enhancing the maturation rate of naive T cells.[66,73] Such rapid turnover of T cells could have detrimental effects on antitumor responses. A loss of effector subpopulations of $CD8^+$ T cells, which appear to be targeted for apoptosis in patients with cancer, might severely compromise antitumor functions of the host and contribute to tumor progression.[73]

TABLE III. Characteristics of T lymphocytes in the peripheral circulation of patients with cancer*

Predominant phenotype	
T lymphocytes:	
% $CD3^+CD95^+Anx^+$ (increased vs NC)	
% $CD3^+CD25^+$ (increased vs NC)	
% $CD3^+HLA\text{-}DR^+$ (increased vs NC)	
$CD8^+$ subset:	% $CD8^+CD95^+Anx^+$ (increased vs NC)
$CD8^+$ naive:	% $CD8^+CD45RO^-CCR7^+$ (decreased vs NC)
$CD8^+$ central memory:	% $CD8^+CD45RO^+CCR7^+$ (decreased vs NC)
$CD8^+$ peripheral memory:	% $CD8^+CD45RO^+CCR7^-$ (increased vs NC)
$CD8^+$ effector cells:	% $CD8^+CD45RO^-CCR7^-$ (increased vs NC)
$CD4^+$ subset:	% $CD4^+CCR7^+$ (decreased vs NC)
$CD4^+$ naive:	% $CD4^+CD45RO^-CCR7^+$ (decreased vs NC)
$CD4^+$ memory cells:	% $CD4^+RO45RO^+CCR7^+$ (decreased vs NC)
$CD4^+$ Treg cells:	% $CD4^+CD25^+$ (increased proportions vs NC)
Clonality: Polyclonal with various restricted TCR Vβ specificities	
Specificity: TA-specific/tetramer$^+$ T cells detectable in many cases	
Functions	
Low ζ chain expression in T and NK cells: inefficient TCR signaling	
Decreased proliferation in response to anti-CD3 antibody, PMA/ionomycin, mitogens	
Decreased antitumor cytotoxicity and NK/lymphokine-activated killer activity	
Cytokine profile: highly variable	
Apoptosis of $CD8^+$ T cells and NK cells (Anx^+)	
Increased caspase-3 activity in T cells	
Increased lymphocyte turnover	

LAK, Lymphokine-activated killer; *NC*, healthy control subjects; *PMA*, phorbol 12-myristate 13-acetate.
*The percentage of positive cells in patients with cancer compared with healthy age- and sex-matched control subjects are from Kuss et al,[66] Kim et al,[67] and Kim et al.[68]

The clinical significance of spontaneous apoptosis of $CD8^+$ effector cells in patients with cancer is currently unknown. A search for surrogate markers of prognosis or a response to therapy in patients with cancer has led to further studies of $CD8^+$ T-cell apoptosis. The level of spontaneous apoptosis discriminates between patients with cancer and healthy control subjects but not between patients with active disease versus those who are NED after oncologic therapies.[67] However, expression of CCR7, which is also a differentiation marker for T cells, by $CD8^+$ T cells was observed to protect the $CD8^+$ effector cells from apoptosis because CCR7 signaling correlated with higher Bcl-2 expression but lower Bax and Fas expression and phosphoinositide 3-kinase pathway activation in $CD8^+$ T cells.[68] The frequency of circulating $CD8^+CCR7^+$ T cells now emerges as an immune biomarker that might be predictive of survival benefits in patients with cancer. Pending validation, this immunologic biomarker that is simply defined by flow cytometry could acquire substantial clinical usefulness in the future.

Another subset of antitumor effector cells, NK cells, representing 8% to 10% of lymphocytes in the peripheral circulation, has

been credited with the ability to eliminate tumor cells in the circulation and thus prevent establishment of distant metastases.[75] Recent data suggest that in addition to mediating perforin-mediated lysis, NK cells constitutively express several ligands of the TNF family and can therefore induce apoptosis in a broad variety of tumor cell targets.[76] This mechanism of tumor cell elimination might be of greater biologic importance than secretory, granule-mediated killing, largely because most tumor cells express receptors for the TNF family ligands and are sensitive to death by apoptosis.[76] NK cells, which are able to discriminate between normal and abnormal cells based on the presence and expression levels of MHC class I molecules, are considered to play a major role in early stages of tumor development. They express receptors that enable them to survey the target for the respective ligands. These receptors are of 2 types: killer inhibitory receptors, killer activating receptors, or both.[57] NK cell functions and their interactions with other cells or extracellular matrix molecules are regulated through these receptors and Fcγ receptors.[57] In the peripheral circulation of patients with cancer, NK cells, like $CD8^+$ T cells, can also be dysfunctional. On a per-cell basis, these NK cells mediate lower levels of cytotoxicity.[77] Furthermore, some studies suggest that NK cells are also sensitive to apoptosis.[78] Among circulating NK cells in patients with breast cancer, a subset of $CD56^{bright}CD16^{dim}$ NK cells, which represents about 95% of all NK cells and is responsible for effector functions, preferentially binds Anx and thus is primed for apoptosis.[79] These patients also had significantly lower NK activity than the age- and sex-matched healthy control subjects tested in parallel. These and other data suggest that endogenous circulating NK cells have the potential to play a role in tumor surveillance, but in the presence of the tumor, their antitumor functions are subverted, and no longer control metastasis dissemination. Once the tumor is established, it especially subverts the subsets of NK cells found at the sites of metastasis and those responsible for cytotoxic functions.

In addition to NK cells, another category of nonspecific effector cells, $CD3^+CD56^+$ NK/T cells, can potentially eliminate tumor targets. They represent a very minor subset of circulating lymphocytes in healthy subjects but have been reported to be expanded in patients with cancer, as well as tumor-bearing rodents.[80] NK/T cells are also a minor component of TILs. In the presence of IL-2, NK/T cells, like $CD3^-CD56^+$ NK cells, readily differentiate into lymphokine-activated killer cells containing numerous granzyme- and perforin-containing granules and are able to mediate tumor cell lysis.[77] Both NK and NK/T cells express receptors for IL-18 and thus are activated in the presence of this cytokine as well.

REGULATORY IMMUNE CELLS IN PATIENTS WITH CANCER

The presence in the circulation of patients with cancer of suppressor lymphocytes capable of downregulating functions of other immune cells was described many years ago.[81] Today such cells are phenotypically identified as $CD4^+CD25^{high}FOXP3^+$ T cells and referred to as Treg cells.[82] They can be isolated from PBMCs or tumor sites by means of immunoselection on magnetic beads coated with antibodies to surface antigens expressed on Treg cells, such as CD25 or CD39. In mice depletion of $CD4^+CD25^+$ T cells results in the development of autoimmunity, and in tumor-bearing animals it promotes immune responses to autologous tumor. In patients with cancer, tumor-associated lymphocytes are enriched in $CD3^+CD4^+CD25^{high}$ T cells.[83] On sorting by flow, these T cells have been shown to secrete TGF-β or IL-10 and to enzymatically cleave ATP to adenosine.[45,46] The mechanisms through which these T cells regulate antitumor immune responses are being intensively investigated, and because Treg cells come in different flavors (eg, natural Treg cells, inducible T_R1 cells, $CD39^+$ Treg cells, or cytotoxic T lymphocyte–associated antigen–positive Treg cells), these mechanisms vary, likely depending on the microenvironmental context. Similarly, the microenvironment influences the induction of Treg cells; for example, T_R1 cells are preferentially induced at the tumor site, which is rich in IL-10, TGF-β, and PGE_2, all of which have been shown to promote T_R1 cell generation.[43,44] The prognostic significance of Treg cells in patients with cancer has been controversial, with many reports linking their accumulations to poor prognosis, presumably as a result of suppressed antitumor immunity,[84] and others reporting better survival in the presence of increased Treg cell frequencies,[85] possibly because of their ability to suppress tumor-promoting mechanisms or induce tumor cell death. The controversy arises because in human subjects no definite identity marker for Treg cells exists, and their functional repertoire is broad and varied. Nevertheless, their responsibility for the contraction of immune responses is critical for health.

Another subset of $CD4^+$ T cells with an origin shared with Treg cells has recently been identified. Like Treg cells, $CD4^+$ T_H17-producing T cells originate from uncommitted $CD4^+$ T-cell precursors, and the participation of TGF-β in their differentiation links them to Treg cells.[86] However, T_H17 cells produce IL-17, IL-21, and IL-IL-22, promoting tissue inflammation, and require the presence of IL-6, as well as the transcription factors signal transducer and activator of transcription 3 (STAT3), RORγ, and RORα, for differentiation.[87] Although the presence of T_H17 cells has been documented in several human carcinomas,[86] their function in tumors remains controversial. Recent reports show that $CD4^+FOXP3^+CCR6^+$ Treg cells can produce IL-17 on activation and can inhibit proliferation of $CD4^+$ responder T cells,[87] confirming a relationship between Treg and T_H17 cells that can be modulated by cytokines in the tumor microenvironment. It also emphasizes the plasticity of T-suppressor and T-effector subsets of $CD4^+$ lymphocytes.

The second major subset of regulatory cells in cancer are MDSCs ($CD34^+CD33^+CD13^+CD11b^+CD15^-$).[50] Tumors recruit MDSCs from the bone marrow through tumor-derived soluble factors, such as GM-CSF, TGF-β, IL-10, and VEGF.[5] Immature myeloid cells migrate to lymph nodes, where DCs cross-prime T cells and interfere with this process, thus suppressing CTL generation. They also migrate to the tumor site and become MDSCs able to produce arginase I and promote iNOS activation.[5,51] MDSCs also produce high levels of ROS and indoleamine-2,3-dioxygenase, an enzyme involved in the catabolism of tryptophan, an essential amino acid for T-cell proliferation and differentiation.[88] In tumor-bearing mice MDSCs accumulate in the spleen, reaching a very high frequency and exerting potent immune suppression, thereby favoring tumor growth. GM-CSF, often used as an immune adjuvant,[89] is also a product of tumor cells, which recruits MDSCs from the bone marrow and is responsible for their accumulation in patients with cancer.[90] In patients with cancer, normal physiologic functions of GM-CSF and MDSCs are subverted by the tumor to promote its development.

The tumor uses a variety of mechanisms and produces various factors and enzymes that enable it to suppress the host antitumor

immune responses. Some of these factors are listed in Table IV. Among these factors, 2 have recently been in the limelight. B7-H1 is an immunoglobulin-like immunosuppressive molecule broadly expressed in tumor cells, which signals to its counterreceptor, programmed death 1 (PD-1), on T cells.[91] Signaling delivered to T cells through B7-H1 (programmed death ligand 1 [PD-L1]) inhibits their proliferation, cytokine production, and effector functions.[92] Also, triggering by the PD-L1$^+$ tumors of PD-1 on T cells increases tumor cell resistance to immune and drug-induced death,[91] demonstrating that cancer cells can use receptors on immune cells as signals to induce resistance to therapy. Blockade of PD-L1/PD-1 interactions promotes generation of TA-specific T cells and attenuates their inhibition by Treg cells.[93] Therefore PD-1 antagonists, which are expected to augment TA-specific immune responses, might be useful in therapy of cancer.[94] Levels of the cytokine IL-17 have been shown to be increased in the tumor microenvironment.[95] Adoptive transfer studies and examination of the tumor microenvironment suggest that CD4$^+$ T cells accumulating in the tumor are the main source of IL-17 and that the enhancement of tumor growth by IL-17 is mediated by its binding to IL-17 receptors expressed on tumor cells, initiating IL-6 production, which in turn activates oncogenic STAT3, upregulating prosurvival and proangiogenic genes.[95] Thus T_H17 seems to promote tumor growth, in part through activation of an IL-6/STAT3 pathway in tumor cells. These data are contradictory to the recently reported improved survival of those patients with ovarian cancer whose tumors contained large numbers of T_H17^+ TILs.[47] This discrepancy illustrates the difficulty of dissecting the role of T_H17 in human cancer and of interpreting environmental interactions occurring in different tumor types.

NEW INSIGHTS INTO ANTITUMOR IMMUNITY

The field of tumor immunity has long suffered from a misconception that cancer cells are ignored by the immune system and that tumors are passive targets for antitumor responses. It is now certain that growing tumors attract components of both innate and adaptive host immunity.[96] Although most TAs are self-antigens that are overexpressed or altered posttranscriptionally, immune responses to TAs, including those listed in Table I, are clearly made. A growing tumor releases TAs and produces numerous cytokines/chemokines, which attract immune cells, including DCs, to the tumor site and tumor-draining lymph nodes. These DCs take up TAs, maturing into IL-12–secreting cells, and process the TAs by using the APM components for the presentation to T cells as peptide–MHC class I–β2 m complexes. These T cells develop into T_H1-type CD8$^+$ CTLs (Fig 1). DCs can also take up and process another set of TAs through the MHC class II pathway, generating T_H1-type CD4$^+$ T_H cells that produce IFN-γ and IL-2. These cells help to expand the population of TA peptide–specific CTLs, which are capable of eliminating the tumor through cytotoxic molecules, perforin, and granzymes. T_H1-type help is essential for the generation of effective CTL responses. However, DCs taking up the same MHC class II–restricted TAs can also promote the development of Treg cells (Fig 1). Mechanisms involved in DC-mediated expansion of Treg cells, as opposed to T_H1 (effector) cells or T_H17 cells, are currently not understood, yet Treg cell accumulations at the tumor site and suppression by Treg cell of antitumor specific immunity appear to have adverse effects on the host's ability to eliminate

TABLE IV. Molecularly defined immunoinhibitory factors produced by human tumors*

TNF family ligands	Induce apoptosis through the TNF family receptors
FasL	Fas
TRAIL	TRAIL-R
TNF	TNF-R1
B7-H1 (PD1L)	Binds PD1 and inhibits lymphocyte and DC functions
Cytokines	
TGF-β	Inhibits lymphocyte proliferation and perforin and granzyme mRNA expression; promotes Treg cell expansion
IL-10	Inhibits cytokine production, including that of IL-12; promotes Treg cell expansion
GM-CSF	Promotes expansion of immunosuppressive tumor-associated macrophages; recruits MDSCs
IL-17	Largely produced by CD4$^+$ T cells in the tumor; binds to IL-17 receptor on tumor cells, initiating the IL-6/STAT3 cascade
Enzymes	
Indoleamine-2,3-dioxygenase (IDO)	Inhibits T-cell activation
Arginase I	Metabolizes L-arginine, another amino acid for essential T cell proliferation
iNOS	Produces immunosuppressive nitric oxide
COX2	Produces immunosuppressive PGE_2
Small molecules	
PGE_2	Inhibits leukocyte functions through increased cyclic AMP levels
Epinephrine	Inhibits leukocyte functions through increased cyclic AMP levels
Adenosine	Inhibits leukocyte functions through increased cyclic AMP levels
ROS	Inhibits leukocyte functions through superoxide generation
Viral-related products	
p15E (CKS-17, synthetic peptide)	Inhibits production of type I cytokines, upregulates IL-10 synthesis
EBI-3 (homologue of IL-12 p40)	Inhibits IL-12 production
Tumor-associated gangliosides	Inhibit IL-2–dependent lymphocyte proliferation, induce apoptotic signals, suppress nuclear factor κB activation, interfere with DC generation

FasL, Fas ligand; *TRAIL*, tumor necrosis factor-related apoptosis-inducing ligand.
*This partial listing of tumor-derived immunoinhibitory factors demonstrates the diversity of mechanisms that human tumors are known to have evolved to incapacitate the host immune system.

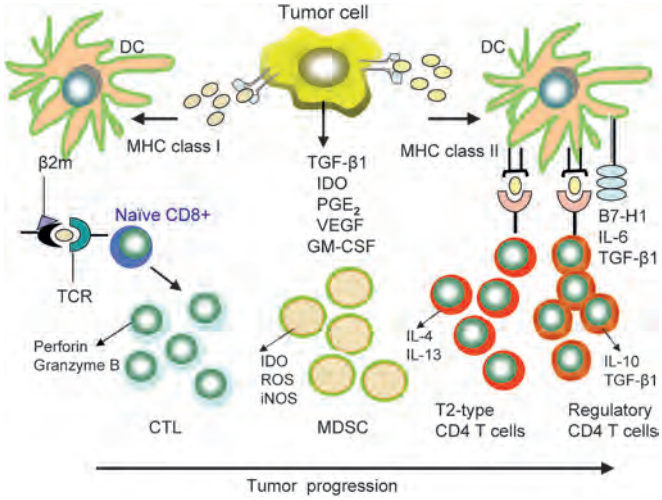

FIG 1. Effects of the tumor on immune cells. In the tumor microenvironment an excess of immunoinhibitory factors favors the generation and expansion of T_H2-type T cells and Treg cells ather than CTLs and T_H1-type effector cells. The downregulation of MHC molecules and defects in the APM components in DCs, as well as the immunosuppressive effects of accumulating MDSCs on DC maturation and function, contributes to the polarization of immune responses toward tolerance and away from immunity. The balance between stimulatory and suppressive responses shifts in favor of suppression as the tumor grows. Immune therapies are expected to shift this balance back to T_H1-type responses, which promote expansion of $CD4^+$ T_H1 cells producing IFN-γ and IL-2, as well as $CD8^+$ CTLs. *IDO*, Indoleamine 2,3-deoxygenase.

TABLE V. Potential strategies for the design of more effective antitumor therapies

Induce and sustain activity and survival of CTLs and of nonspecific antitumor effector cells: passive or active immunotherapy with antibodies, immune cells, or antitumor vaccines
Prevent immune suppression
Inhibit production or activity of tumor-derived suppressive factors
Inhibit generation or functions of Treg cells and MDSCs
Alter tumor microenvironment
Optimize lymphocyte/DC functions in the tumor microenvironment to enhance T_H1-type responses
Combine therapeutic antitumor vaccines with chemotherapy
Treat early disease or in an adjuvant setting

cancer and might influence prognosis.[84] In contrast, accumulations of $CD4^+$ T_H17^+ cells seem to predict a better survival in some cancers but in others correlate with tumor progression.[47] In patients with cancer, cellular interactions between TA-presenting DCs and T cells preferentially lead to expansion and accumulation Treg cells and MDSCs at the tumor site and in the periphery.[52] It appears that tumors have the capability to enhance the maturation of a distinct type of DC that does not promote the generation of TA-specific T_H1 cells but instead is programmed to induce Treg cells and to recruit MDSCs (Fig 1). The proinflammatory cytokines IL-6 and TNF-α produced by these DCs in combination with tumor-derived soluble immunoinhibitory factors appear to be important for shifting the balance of immune response from immunogenic to tolerogenic.

Thus signals delivered to T cells by DCs in the tumor microenvironment determine whether these T cells will develop into Treg or T_H1 cells. These signals might be influenced by (1) the dose and type of TA processed by DCs, (2) the DC maturation status because immature DCs are known to induce tolerance rather than immunity, (3) the expression of costimulatory molecules on DCs, and (4) the effects of cytokines produced by interacting DCs and T cells on the induction of Treg versus T_H1 cells.

At the time human tumors are diagnosed, the balance between immunogenic and tolerogenic signals delivered to immune cells is strongly skewed toward tolerance, mainly because of tumor-induced suppression. Therefore immune therapies administered in the minimal residual disease setting and designed to augment antitumor T_H1-type $CD4^+$ T cells and CTLs are expected to tip the balance in favor of immunostimulation and away from immunosuppression. For this reason, therapeutic antitumor vaccination strategies are considered a promising addition to conventional therapies for cancer. However, complexities of the tumor-induced immune suppression, which engages numerous molecular mechanisms, present a formidable challenge to antitumor therapies, including vaccines. Novel approaches targeting these mechanisms of immune suppression (Table V) are needed to improve the treatment of cancer.

CONCLUSIONS

The existing evidence for dysfunction and death of antitumor effector cells in tumor-bearing hosts introduces a new paradigm for immunotherapy of cancer. Although previous emphasis has been on activation of immune cells and upregulation of their antitumor functions, the current concept is to consider therapies that could block or reverse tumor escape, at the same time protecting immune cells from the influence of immunosuppressive factors present in the tumor microenvironment. These novel therapeutic strategies take advantage of the tremendous progress made recently in our basic understanding of interactions between the tumor and the host immune system. Current insights into these interactions suggest that combinations of conventional cancer therapies with newly designed DC-based vaccines and survival cytokines (eg, IL-2, IL-7, and IL-15) offer therapeutic benefits. Some of the other promising strategies under consideration for improvements in the effects of immune therapies are listed in Table V. It is expected that as molecular mechanisms used by tumors to avoid, bypass, or subvert the immune system of the host are becoming clear, novel and more effective antitumor therapies targeting these mechanisms will emerge in the near future.

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Clinical laboratory assessment of immediate-type hypersensitivity

Robert G. Hamilton, PhD *Baltimore, Md*

Clinical laboratory analyses aid in the diagnosis and management of human allergic (IgE-dependent) diseases. Diagnosis of immediate-type hypersensitivity begins with a thorough clinical history and physical examination. Once symptoms compatible with an allergic disorder have been identified, a skin test, blood test, or both for allergen-specific IgE antibodies provide confirmation of sensitization, which strengthens the diagnosis. Skin testing provides a biologically relevant immediate-type hypersensitivity response with resultant wheal-and-flare reactions within 15 minutes of allergen application. Allergen-specific IgE antibody in serum is quantified by using 3 laboratory-based autoanalyzers (ImmunoCAP, Immulite, and HYTEC-288) and novel microarray and lateral-flow immunoassays. Technologic advances in serologic allergen-specific IgE measurements have involved increased automation, with enhanced reproducibility, greater quantification, lower analytic sensitivity, and component-supplemented extract-based allergen use. *In vivo* provocation tests involving inhalation, ingestion, or injection of allergens serve to clarify discordant history and skin- or blood-based measures of sensitization. Other diagnostic allergy laboratory analyses include total and free serum IgE measurement, precipitating IgG antibodies specific for organic dusts, mast cell tryptase, and indicator allergen analyses to assess indoor environments to promote patient-targeted allergen avoidance programs. A critique is provided on the predictive utility of serologic measures of specific IgE for food allergy and asthma. Reasons for the lack of clinical utility for food-specific IgG/IgG4 measurements in allergy diagnosis are examined. When the specific IgE measures are inconsistent with the clinical history, they should be confirmed by means of repeat and alternative method analysis. Ultimately, the patient's clinical history remains the principal arbiter that determines the final diagnosis of allergic disease. (J Allergy Clin Immunol 2010;125:S284-96.)

Key words: *Diagnosis, skin testing, RAST, IgE antibody, provocation testing*

Abbreviations used

BHR: Basophil histamine release
CI: Chronic urticaria
CLIA-88: Federal Clinical Laboratory Improvement Act of 1988
DBPCFC: Double-blind, placebo-controlled food challenge
ISAC: Immunosorbent Allergen Chip
PWV: *Polistes* species wasp venom
WHO: World Health Organization
YJV: Yellow jacket venom

The clinical laboratory plays an increasing role in the diagnosis and management of allergic disorders (immediate or type 1 hypersensitivity). The clinician begins the diagnostic process with a thorough clinical history and physical examination. Symptoms that suggest a diagnosis of asthma, allergic rhinitis and sinusitis, occupational asthma and allergy, food allergy, drug allergy, or an allergic disease of the skin are matched with suspected relevant allergen exposures. Once the clinician has a high degree of suspicion that the patient has a particular allergic disorder, *in vivo* (skin and provocation tests) and laboratory-based serologic analyses for IgE antibody are performed to strengthen the likelihood that the chosen allergy diagnosis is correct. A definitive diagnosis of allergic disease then permits a number of therapeutic interventions involving avoidance, pharmacotherapy, immunotherapy, or anti-IgE therapy to be instituted. Management of a patient with allergic disorders can also be facilitated with different laboratory analyses. This chapter examines clinical laboratory tests that aid in the diagnosis and management of patients with a disease associated with type 1 hypersensitivity.

IGE PROPERTIES

The reagin in serum that mediates the immediate-type wheal-and-flare reaction was identified as IgE in 1967.[1,2] The properties of human IgE are described in Table I. IgE (approximately 190,000 d) circulates as a monomer at a serum concentration that is highly age dependent. It constitutes approximately 0.0005% of the total serum immunoglobulins in adults.[3] Cord blood levels of IgE remain low (<2 kU/L [<4.8 μg/L]) because IgE does not cross the placental barrier in significant amounts. Mean serum IgE levels progressively increase in healthy children up to 10 to 15 years of age and then decrease from the second through eighth decades of life. By 14 years of age, total serum IgE levels of greater than 333 kU/L (800 μg/L) are considered abnormally increased and strongly associated with and suggestive of atopic disorders, such as allergic rhinitis, extrinsic asthma, and atopic dermatitis.[4,5]

Environmental antigen exposure can occur by means of inhalation, ingestion, or skin and parenteral contact. Once taken up by antigen-presenting cells, processed antigen is presented to helper T cells that secrete a number of cytokines that cause B-cell lymphocytes to proliferate and in some cases produce allergen-specific IgE antibody. IgE binds onto high-affinity Fcε receptors on the surface of a number of cells, particularly mast cells and basophils, creating a state of "sensitization" within the patient.

From the Allergy and Clinical Immunology Division, Department of Medicine, Johns Hopkins University School of Medicine.

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Reprint requests: Robert G. Hamilton, PhD, Room 1A20, Johns Hopkins Asthma and Allergy Center, 5501 Hopkins Bayview Circle, Baltimore, MD 21224. E-mail: rhamilt2@jhmi.edu.

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Subsequent allergen exposure causes mast cell surface–bound IgE antibody to be cross-linked, leading to an increase in intracellular calcium levels and the release of both preformed mediators (eg, histamine and proteases) and newly synthesized lipid-derived mediators (eg, leukotrienes and prostaglandins). Allergic symptoms can subsequently occur as a result of mediator-induced physiologic and anatomic changes. The measurement of allergen-specific IgE antibody will thus be a principal focus of this chapter.

ALLERGENS

Several hundred allergenic proteins, (glycoproteins and lipoproteins), are extracted from well-defined (usually biological) sources, including weed, grass, and tree pollens and animal dander, molds, house dust mites, parasites, insect venoms, occupational allergens (eg, natural rubber latex), drugs, and foods.[6] These allergens elicit IgE antibody production when introduced into an immunocompetent and genetically predisposed host. Individual allergenic proteins can be identified by using IgE antibody–containing human serum in combination with a number of immunochemical assays that separate proteins based on their net charge (isoelectric focusing), size (Western blot analysis), and ability to bind antibody (competitive inhibition immunoassay). A compendium of the known clinically important allergens (together with their scientific names), purified major allergen components, and diagnostic codes is presented elsewhere.[6]

Many important allergenic proteins from dust mites, pollens, animal dander, insects (eg, cockroach and Hymenoptera venoms), molds, and foods have been cloned and sequenced, and recombinant proteins have been expressed during the past decade.[7,8] This has sparked a debate as to whether native allergens possess any unique advantages over their recombinant counterparts as diagnostic reagents and whether crude allergen mixtures should give way to the use of purified allergens in cocktails to detect IgE antibody in the skin and blood. Allergens extracted from natural sources are known to be heterogeneous, often containing many nonallergenic proteins. Moreover, different natural extracts vary in their allergen composition and potency, and they can be contaminated with allergens from other sources. Purified recombinant allergens are attractive because their availability in pure form simplifies reagent preparation and promotes reproducibility and standardization. However, allergic patients are known to respond differently to combinations of isoallergens that are essentially identical except for minor differences in their primary amino acid composition or substituted side chains. Thus a single recombinant allergen that does not represent all the isoallergen forms of that allergen might not be sufficiently "globally diagnostic" to be able to detect all clinically relevant IgE antibodies of that allergen specificity.

Extract-based reagents for both skin test and IgE antibody serology are here to stay for the foreseeable future because of their more comprehensive coverage of the allergenic repertoire of any particular specificity. However, the future use of certain purified recombinant allergens as diagnostic reagents in both *in vivo* and *in vitro* IgE antibody testing holds promise. A purified recombinant or native principal allergen of a particular specificity, such as Bet v 1 from birch pollen, can be a good indicator allergen for detecting sensitization to that specificity. However, it is not sufficiently comprehensive to replace the birch extract–based diagnostic reagent, especially for evaluation of subjects who produce IgE antibody to less predominant allergens that are present in the birch pollen extract. Additionally, the allergenic profile of any given specificity of an allergen-containing reagent, as produced by different manufacturers, is expected to vary in its protein composition, allergenic potency, and immunoreactivity, regardless of extensive cross-validation. One generic rule of allergy diagnostics has evolved from this, namely that despite clearance by regulatory agencies, such as the US Food and Drug Administration, each *in vivo* or *in vitro*-allergen extract–containing reagent should be expected to detect slightly different populations of IgE antibodies. Thus IgE antibody measurements generated with different skin test– or serum-based IgE antibody assay reagents are expected to produce reasonably clinically equivalent but not identical results.[6]

DIAGNOSTIC ALGORITHM FOR ALLERGIC DISEASE

The diagnosis of allergic disease begins with a thorough clinical history and physical examination.[9,10] The signs and symptoms associated with the various allergic disorders are extensively discussed in chapters 8-14. Once the history has been collected, one of several primary confirmatory tests for sensitization can be performed to detect allergen-specific IgE in the skin or blood. Because the history is viewed by many as the arbiter of the diagnostic test's performance, a subject with a positive clinical history for allergic disease and a positive skin or blood test result for IgE is considered to have a true-positive result (Table II). Ideally, all patients with a positive allergy history would have a positive allergen-specific IgE test result, and those with a negative history would have a negative allergen-specific IgE antibody test result. However, more realistically, some patients with allergic disease are classified as having false-negative IgE antibody test results, and others with no evident allergic disease are identified as having positive IgE antibody test results that would be considered false-positive results.

In vivo provocation tests are considered secondary-level confirmatory tests that are available when one needs to adjudicate the correctness of discordant clinical history and results from allergen-specific IgE antibody skin or serologic tests.[9-11] However, provocation tests are more difficult to perform in a reproducible manner than skin or blood tests for IgE antibodies, and they place the patient at some risk for a reaction because they involve a direct allergen challenge. Interpretation of their results can also be difficult because they often involve subjective end points that can be altered by observer and patient bias. In certain cases, such as food allergy, the *in vivo* provocation test (double-blind, placebo-controlled food challenge [DBPCFC]) has become the reference benchmark for identifying type 1 hypersensitivity to foods. The actual *in vivo* provocation test that is useful in the diagnostic workup of a patient ultimately depends on the nature of the disease process that is being investigated (eg, sting challenge for Hymenoptera venom allergy).

The presence of IgE antibodies is necessary but not sufficient for allergic disease expression. Allergen-specific IgE antibody might be detectable in the patient's skin or blood, and the patient might not have had any evident allergic symptoms after allergen exposure. Some health care workers with a positive immediate-type latex skin test result, IgE anti-natural rubber latex blood test result, or both experience no allergic symptoms when they are exposed to highly allergenic powdered latex examination gloves.[12] The relative strengths and limitations of *in vivo* and *in vitro* diagnostic tests and the principal technical reasons for false-negative and false-positive test results are discussed subsequently.

TABLE I. . Biological and chemical properties of human IgE and IgG antibodies

Property	IgE	IgG1	IgG2	IgG3	IgG4
Heavy (H) chain class	ϵ	γ1	γ2	γ3	γ4
H chain molecular weight (d)	70,000	50,000	50,000	60,000	50,000
H Chain carbohydrate %	18	3-4	3-4	3-4	3-4
H Chain no. of oligosaccharides	5	1	1	1	1
Light chain type	K and λ	K and λ	K and λ	K and λ	K and λ
Averaged immunoglobulin light chain K/L ratio		2.4	1.1	1.4	8.0
Molecular weight of secreted form (d)	190,000	150,000	150,000	160,000	150,000
H chain domain no.	5	4	4	4	4
Hinge (amino acids)	None	15	12	62	12
Interchain disulfide bonds per monomer	NA	2	4	11	2
pI range, mean (SD)	NA	8.6 (0.4)	7.4 (0.6)	8.3 (0.7)	7.2 (0.8)
Tail Piece	No	No	No	No	No
Allotypes	Em1	G1 m: a(1), x(2), f(3), z(17)	G2 m: n(23)	G3 m: b1(5), c3(6), b5(10),b0(11) b3(13),b4(14) s(15), t(16), g1(21)c5(24), u(26),v(27), g5(28)	G4 m Gm4a(i) Gm4b(i)
Distribution: % intravascular	50	45	45	45	45
Biological half-life (d)	1-5	21-24	21-24	7-8	21-24
Fractional catabolic rate (% intravascular pool catabolized per day)	71	7	7	17	7
Synthetic rate (mg/kg/d)	0.002	33	33	33	33
Total immunoglobulin in adult serum (%)	0.004	45-53	11-15	0.03-0.06	0.015-0.045
Approximate adult range (age 16-60 y) in serum g/L	0-0.0001 nonatopic subjects	5-12	2-6	0.5-1	0.2-1
Functional valency	2	2	2	2	1-2
Transplacental transfer	0	++	+	++	++
Binding to phagocytic cells		++	+	++	+
Binding to basophils and mast Cells	+++	?	?	?	?
Complement activation classical pathway	0 + alternate pathway	++	+	+++	0

NA, not available; *pI*, isoelectric point.
Modified from Tables I and II in Hamilton RG. Human immunoglobulins. In: O'Gorman MRG, Donnenberg AD, editors. Handbook of human immunology. 2nd ed. Boca Raton (FL): CRC Press; 2008.

DIAGNOSTIC SKIN TESTING

Skin testing is one of 2 primary confirmatory tests for allergen-specific IgE antibody that are used in the diagnosis of human allergic disease. An epicutaneous administration (previously referred to as a prick/puncture) or an intradermal injection can both be used to apply allergen in the form of an extract to the skin.[13]

Epicutaneous skin test

Performance of the epicutaneous skin test involves placing a drop of allergen, often in glycerinated saline, onto the surface of the skin. A variety of single-, dual-, and multiple-point standardized test devices are currently available to introduce the allergen into the epidermis.[13,14] Excess allergen is then removed with gauze or tissue paper, and any immediate reaction (wheal and erythema) is read at 15 to 20 minutes as it reaches a maximum diameter. Separate test sites need to be spaced sufficiently distant from each other to prevent overlapping of any erythema. False-positive results can occur as a result of bleeding or direct skin irritation by some extracts that might contain naturally occurring histamine. Dermographism which is a constitutional whealing tendency in which firm stroking of the skin can cause capillary and arteriolar dilatation and transudation of fluid causing edema, can also lead to a false-positive wheal and erythema and invalidation of the skin test result. False-negative results can occur as a result of prior consumption of antihistamines or other medications. A positive control comprising histamine (1.8 or 10 mg/mL) and a negative control of saline must be applied in parallel with the test allergen extracts to document validity and control for confounding problems associated with antihistamine premedication and dermographism.

Variability of epicutaneous skin test results can occur as a result of several factors.[13,14] These include the subject's biological responsiveness, the skin tester's skill, the general technique (needle and application method) used to perform the puncture, the reagents (stability, vehicle [eg, 50% glycerol], allergen concentration, and

TABLE II. Predictive value of diagnostic tests applied to populations without and with allergic disease

	Positive allergen-specific IgE test result	Negative allergen-specific IgE test result	Totals
Positive clinical history for allergic disease	True-positive allergic test result (TP)	False-negative allergen-specific IgE antibody test result (FN)	TP + FN
Negative clinical history for allergic disease	False-positive allergen-specific IgE antibody test result (FP)	True-negative test result in nonallergic subject (TN)	FP + TN
Totals	TP + FP	FN + TN	TP + FP + TN + FN

FN, Number of patients with allergic disease misclassified by a negative IgE antibody test result; *FP*, number of patients with no allergic disease misclassified by a positive IgE antibody test result; *TN*, number of patients with no allergic disease correctly identified with a negative IgE antibody test result; *TP*, number of patients with allergic disease correctly identified by a positive IgE antibody test result.
Diagnostic sensitivity of an IgE antibody test: Percentage positivity of an IgE antibody test result in patients with allergic disease = TP/[TP + FN] × 100.
Diagnostic specificity of an IgE antibody test: Percentage negativity of an IgE antibody test result in patients with no allergic disease = TN/[TN + FP] × 100.
Positive predictive value of an IgE antibody test: Percentage of patients with a positive IgE antibody test result who have allergic disease = TP/[TP + FP] × 100.
Negative predictive value of an IgE antibody test: Percentage of patients with a negative IgE antibody test result who have no allergic disease = TN/[TN + FN] × 100.
Efficiency of an IgE antibody test: Percentage of patients correctly classified as having allergic disease or not having allergic disease = [TP + TN]/[TP + FP + FN + TN] × 100.
Modified from Table 2C-3 in Galen RS, Peters T Jr. Analytic goals and clinical relevance of laboratory procedures. In: Tietz NW, editor. Textbook of clinical chemistry. Philadelphia: WB Saunders Co; 1986. p, 395-7.

purity), and the method used to delimit, measure, and report skin reactions. In one study dust mite contamination of dog dander extracts was shown to be the cause of false-positive epicutaneous skin test results in patients sensitized only to house dust mites.[15] The inoculum volume is another variable that can contribute to epicutaneous skin test variation. To examine this, Antico et al[16] performed 16 skin puncture tests (8 in each forearm) on 15 healthy volunteers (9 men and 6 women; age, 64 ± 4 years) using 50% glycerol-saline containing radioactive Tc_{99m} and a 1-mm acrylic copolymer, pyramidal-tipped, Morrow Brown needle (Alkaline Corp, Oakhurst, NJ). They measured a mean 16 nL of inoculum volume delivered to the skin (range, 0.42-82.25 nL) using a gamma camera. This high variability was shown to depend primarily on the characteristics of the subjects' skin and the reagents, whereas the skin tester's skill and technique were considered less significant sources of variability. The study concluded that variation in epicutaneous skin test results can only be reduced to certain limits by the standardization of the skin-testing technique and reagents.

Intradermal skin test

Intracutaneous (intradermal) administration of allergen (0.01-0.05 mL) can be accomplished by using a tuberculin syringe with a 26- to 27-gauge needle. A 2- to 3-mm-diameter bleb can be produced by injecting 0.02 mL. The skin test is then read at 15 to 20 minutes, when the wheal and erythema are considered maximal. A number of scoring schemes that have been used for skin testing are presented in Tables III through V. Subcutaneous injection of allergen can lead to a false-negative intradermal skin test result, whereas a minor change in the extract volume only minimally alters the wheal-and-flare diameters. The allergen concentration and the presence of naturally occurring histamine contamination of undialyzed extracts can markedly influence final intradermal skin test results. Rather than a single-dose injection, a skin test titration can be performed that involves the administration of the same volume (eg, 0.02 mL) of 3- or 10-fold serial dilutions of an extract into different sites in the skin. The purpose of skin test titration is to identify the concentration of an extract that produces a defined wheal or erythema diameter (eg, 8-mm wheal). The greater the patient's sensitivity to the allergen extract, the lower the concentration of allergen that is required to induce the predefined wheal or erythema diameter. The intradermal skin test requires approximately 1,000-fold lower concentrations of antigen than the epicutaneous skin test to produce a same-sized skin reaction.[17] Intradermal testing, when done as in clinical practice with an extract concentration of 1:500 or 1:1,000 versus 1:20 wt/vol for epicutaneous skin testing, is a "bigger dose" by approximately 100- to 1,000-fold because of the differential volumes and concentrations.

Adhesive cellulose tape can be applied over the wheal and erythema that has been previously outlined with a ballpoint pen to obtain a permanent record of the skin reaction. The maximal diameter and midpoint perpendicular diameter of the wheal and erythema are averaged. Alternatively, a midpoint diameter might be interpolated from the skin test titration reference curve in which the allergen dose is plotted against the wheal or erythema diameter. Erythema size is sometimes preferred over wheal size because the slope of the flare's regression line is reportedly steeper.[18] A strong relationship exists between the size of the intradermal erythema and the wheal, which is useful when evaluating reactions in dark skin, on which the erythema can be difficult to assess.

DIAGNOSTIC IMMUNOLOGY LABORATORY TESTS

Although the presence of allergen-specific IgE antibody is necessary but not sufficient for clinically manifested allergic disease, it has become the primary clinical laboratory measurement used in the diagnosis of human allergic disease. Most clinical laboratories offer a number of additional serologic tests that can be useful in selected circumstances for the diagnosis or management of patients with type 1 hypersensitivity. These measurements include total serum IgE, the Hymenoptera venom-specific IgE inhibition test, Hymenoptera venom-specific IgG, mast cell tryptase, eosinophil cationic protein, and precipitins for assessing hypersensitivity pneumonitis. Basophil histamine release (BHR), although rarely offered as a clinical test because of the requirement for fresh blood, can be a useful investigational or research tool to clarify discordant diagnostic test results, and thus it will also be examined in this section.

Allergen-specific IgE antibody

Table VI summarizes the analytes that are most commonly analyzed in the diagnostic immunology laboratory during the workup of an allergic patient. Of these, allergen-specific IgE antibody is the most important analyte in the diagnosis of type 1 hypersensitivity reactions. The Phadebas RAST (Pharmacia Diagnostics [currently Phadia], Uppsala, Sweden) was the first

TABLE III. Grading system for epicutaneous skin testing with histamine as a reference*

Grade or class	Wheal size
0	No discernible wheal
1+	<½ Histamine diameter
2+	≥½ Histamine and <histamine diameter
3+	= size of histamine wheal ± 1 mm
4+	>Histamine diameter and <2 × diameter
5+	>2 × Histamine control

*Prick/puncture histamine (1.8-10 mg/mL); intradermal histamine (100 μg/mL).

TABLE IV. Grading system for skin testing with wheal and erythema as criteria

Grade or class	Wheal and erythema size
0	No reaction or reach no different than negative control
1+	Erythema <21 mm
2+	Wheal <3 mm and erythema >21 mm
3+	Wheal >3 mm with surrounding erythema
4+	Wheal with pseudopods and surrounding erythema

Extracted from Sheldon J, Lovell R, Mathews K. A manual of clinical allergy. 2nd ed. Philadelphia: WB Saunders Company; 1967.

TABLE V. Alternative skin test grading system for intradermal skin testing only involving wheal and erythema responses

Grade or class	Wheal size (mm)	Erythema size (mm)
0	<5	<5
+/−	5-10	5-10
1+	5-10	11-20
2+	5-10	21-30
3+	10-15	21-40
4+	>15 with pseudopods	41-50

Extracted from Norman PS. In: Middleton E, Ellis EF, Reed CE, editors. Allergy: principles and practice. 2nd ed. St Louis: Mosby; 1982.

assay for the detection of allergen-specific IgE antibodies.[19] This early allergen-specific IgE antibody assay has evolved with many technologic advancements into 3 present-day autoanalyzer-based, allergen-specific IgE antibody assays that essentially mimic the RAST's solid-phase chemistry.[6] The ImmunoCAP by Phadia (UniCAP100, ImmunoCAP250) uses a cellulose sponge matrix in the form of a small cap as an allergosorbent on which allergen is covalently coupled. The Immulite System from Siemens (Berlin, Germany) uses a biotinylated allergen that is bound to an avidin solid phase. The HYTEC-288 system from Hycor/Agilent Technologies (Santa Clara, Calif) uses a cellulose wafer on which allergen is covalently coupled. All 3 systems are performed on autoanalyzers to maximize precision and minimize the turnaround time. They all use nonisotypically labeled anti-human IgE and are calibrated by means of interpolation of response data from a heterologous total serum IgE calibration curve that has been referenced to the World Health Organization (WHO) total IgE serum standard.[6]

Although convergence or harmonization of these technical factors has led to improved intermethod agreement among reported IgE antibody results, specific IgE antibody levels, as measured with different commercial assays, are still not interchangeable or identical. Differences remain in the specificity of the allergen-containing reagents used in the different assays.[20] Except for single-component drugs (eg, insulin, penicillin, and protamine) and recombinant or native component allergens, the allergen preparations used in IgE antibody assays remain mixtures of proteins that are prepared from biological extracts that differ in their composition between manufacturers because of several factors. The principal variables include the season in which the raw material is collected, the degree of difficulty in identifying a pure source of material, the presence of morphologically similar raw materials that might cross-contaminate, and differences in the extraction and final processing during allergen reagent production by the assay manufacturers. Fortunately, allergen extracts selected for use in allergosorbents undergo extensive quality control and documentation with isoelectrofocusing, SDS-PAGE, crossed immunoelectrophoresis, and immunoblotting methods.

Allergenic potency is assessed by using a soluble antigen inhibition format of the allergen-specific IgE assay. In this assay soluble allergen (typically in an extract) or buffer (sham control) are added to different aliquots of serum before the serum mixture is analyzed in the specific IgE assay. The percentage of inhibition is computed as a semiquantitative estimate of relative allergen potency. There are other issues with stability of allergen extracts during storage: heterogeneity of the human IgE antibody–containing sera used for quality control and different criteria for acceptance of the finished allergen-containing reagent by different manufacturers. Thus allergen-containing reagents from different manufacturers should thus be expected to detect different populations of IgE antibodies for any given allergen specificity.[6]

Several new IgE antibody technologies have emerged to enhance the allergen-specific IgE antibody data that are available to both the clinician and the patient. The microarray chip technology[21] has been commercialized in the form of the ImmunoCAP Immunosorbent Allergen Chip (ISAC) or Immuno Solid phase Allergen Chip (VBC Genomics-Phadia). It currently has 103 native/recombinant component allergens from 43 allergen sources that are dotted in triplicate onto glass slides. Twenty microliters of serum is pipetted onto the chip, and antibodies specific for the allergens attached to the chip surface bind during a 2-hour incubation period. After a buffer wash, bound IgE is detected with a fluorescently labeled anti-IgE. The chip is read in a fluorometer, and fluorescent signal units are interpolated into ISU or ISAC units as semiquantitative estimates of specific IgE antibody in the original serum. The analytic sensitivity of the ISAC varies as a function of the particular allergen specificity and is generally less than that of the ImmunoCAP system when the same allergens are coupled to sponge allergosorbent. This device has been providing clinical data to clinicians in Europe for several years but is not yet cleared by the US Food and Drug Administration for clinical use in the United States.

The unique clinical utility of the microarray system rests in its ability to identify the patient's IgE antibody cross-reactivity among structurally similar allergens from different biological substances. For instance, Bet v 1 from birch tree pollen has structurally similar homologues in the PR10 family that include allergenic proteins from alder tree pollen (Aln g 1), hazelnut pollen (Cor a 1), apple (Mal d 1), peach (Pru p 1), soybean (Gly m 4), peanut (Ara h 8), celery (Apr g 1), carrot (Dau c 1), and kiwi (Act d 8). A primary sensitivity to Bet v 1 might result in oral allergy symptoms after exposure to any of these other structurally similar (cross-reactive) allergenic molecules. The microarray also can assess cross-reactivity among other allergen families, such as the profilins, the lipid transfer proteins, the calcium-binding proteins, the tropomyosins, and the serum albumin family.[6]

TABLE VI. Analytes measured in the diagnostic allergy laboratory

Diagnosis
Allergen-specific IgE
Multiallergen-specific IgE screen (adult and pediatric forms)
Individual allergen specificities
Total serum IgE*
Free IgE (in serum of patients receiving omalizumab)
Precipitating antibodies specific for proteins in organic dusts
Tryptase (α and β; mast cell protease and used as a marker for mast cell–mediated anaphylaxis)
Other tests: Complete blood count and sputum examination for eosinophils and neutrophils
Management
Allergen-specific IgG (Hymenoptera)
Indoor aeroallergen quantitation in surface dust
Der p 1 and 2/Der f 1 and 2 (dust mite, *Dermatophagoides* species)
Fel d 1 (cat, *Felis domesticus*)
Can f 1 (dog, *Canis familiaris*)
Bla g 1/Bla g 2 (cockroach, *Blattela germanica*)
Mus m 1 (mouse, *Mus musculus*)
Rat n 1 (rat, *Rattus norvegicus*)
Cotinine (metabolite of nicotine measured in serum, urine, and sputum and used as a marker of smoke exposure)
Research analytes
IgE-specific autoantibodies
Eosinophil cationic protein
Mediators†,‡
Preformed biogenic amine: histamine
Newly formed: leukotriene C_4, prostaglandin D_2
Proteoglycans†
Heparin
Chondroitin sulfate E
Proteases†
Mast cell chymase
Mast cell carboxypeptidase
Cathepsin G
Fibroblast growth factor†
Cytokines
IFN-γ
TNF-α
IL-4, IL-5, IL-6, and IL-13‡

*Total serum IgE is the only one of these tests listed that is regulated under the CLIA-88.
†Primarily released from mast cells.
‡Primarily released from basophils.

Knowledge of the extent of IgE cross-reactivity among these structurally similar proteins provides unique information to the allergist as support to the clinical history in the diagnosis and management of the allergic patient.

A second trend in IgE antibody serology is the emergence of a point-of-care IgE assay in which a drop of blood from a finger prick is inserted into the sample well of a lateral-flow cassette. The ImmunoCAP Rapid (Phadia) allows antibody to flow with the fluid front across two nitrocellulose strips that have been impregnated with 5 lines each of extract-based aeroallergens (cat dander, *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, Bermuda grass, short ragweed, oak tree, *Alternaria* species, timothy grass, elm tree, and dog dander). If IgE antibody is bound, it is detected with anti-IgE–colloidal gold that subsequently migrates up the same nitrocellulose strips after the addition of developing solution to the appropriate well. Use of the ImmunoCAP Rapid in a study of 215 children (1-14 years old) demonstrated effective (88.4% to 97.6%) correct identification of allergic sensitization in children with recurrent wheezing depending on the final color degree considered on the membrane.[22] This device has received US Food and Drug Administration clearance and is intended for use by primary care physicians who would then (in theory) refer their IgE-positive patients to an allergist for a comprehensive diagnostic workup. An additional point-of-care testing device for detection of 12 aeroallergen- or 12 food allergen–specific IgE antibodies from a finger-stick specimen is the FastCheck System from DST Diagnostiche & Technologies GmbH (Schwerin, Germany) that is available only in Europe.

Utility of quantitative measures of allergen-specific IgE antibody

The importance of quantitative IgE antibody measurements in blood can be illustrated by the results of a prospective study on food allergy.[23] One hundred children who were referred for food allergy evaluation provided sera that was tested for IgE antibodies to egg, milk, peanut, soy, wheat, and fish by using the ImmunoCAP System. Diagnosis of food allergy was established in each child based on a history and an oral food challenge. The results of this study demonstrated that greater than 95% of food allergies were correctly identified by using previously established 95% predictive decision criteria with retrospective data.[24] An IgE antibody level was identified above which there was a defined probability of reacting to a food challenge. Using the ImmunoCAP, IgE antibody levels of egg (6 kUa/L), milk (32 kUa/L), peanut (15 kUa/L), and fish (20 kUa/L) could predict clinical reactivity (positive food challenge results) with greater than 95% certainty. The authors concluded that the need for oral food challenge could be reduced by about 50% by quantitatively measuring food-specific IgE antibody levels in serum and applying these 95% predictive decision criteria.

Quantitative estimates of serum IgE antibody might also facilitate the management of asthmatic patients who have pet epidermal and dust mite aeroallergens as triggers for their disease. By using specific IgE as a continuous variable, the risk of current wheeze and reduced lung function in children was shown to increase significantly with increasing summed measurements of dust mite-, cat-, and dog-specific IgE antibody.[25] These data identified subjects who were not only sensitized but who also could benefit from avoidance through environmental control measures.

Although the ability to quantify the level of select food allergen– and aeroallergen-specific IgE antibody in the blood has shown promise in facilitating the diagnosis and management of allergic disease, one must be careful in interpreting these reported positive, predictive IgE antibody decision points too literally. Using cow's milk–specific IgE as an illustration, Table VII[23,24,26-32] summarizes 8 published studies that report levels of IgE anti-milk in kUa/L, as measured by using the Phadia ImmunoCAP for positive predictive decision points.[24,25,27-32] The level of cow's milk–specific IgE antibody that allows one to predict a positive food challenge result with up to 95% confidence varies widely as a function of the age range and disease state of the children studied, the prevalence of cow's milk allergy in the population, the study design with either open or placebo-controlled food challenges, and the statistical method used to derive the predictive decision point. In addition, patient-dependent bias can occur in which IgE anti–cow's milk measurements alone by

TABLE VII. published positive predictive values of milk-specific IgE testing

Study	Population	Age (y)	No.	Prevalence of cows' milk allergy	Study design	Oral milk challenge	Statistical method for predictive decision point	Positive predictive decision point (%)/specific IgE (kUa/L)
Sampson and Ho[24]	With food allergy	5.2 (average)	196	49%	Retrospective	DBPCFC	2–by-2 tables	95%: 32 90%: 23
Sampson[23]	With suspected food allergy	3.8 (median)	100	66%	Prospective	DBPCFC	2–by-2 tables	95%: 15
Garcia-Ara et al[27]	With suspected cow's milk allergy	0.4 (average)	170	44%	Prospective	Open controlled	2–by-2 tables	95%: 5 90%: 2.5
Garcia-Ara et al[28]	With cow's milk allergy	0.4 (average), start	66	100%	Prospective follow-up	Open controlled	2–by-2 tables	95%: 2.7 for age 1.1-1.5 y 95%: 9 for age 1.6-2 y 95%: 24 for age 2.1-3 y 90%: 1.5 for age 1.1-1.5 y 90%: 6 for age 1.6-2 y 90%: 14 for age 2.1-3 y
Celik-Bilgili et al[29]	With suspected food allergy	1.1 (median)	501	*	Retrospective	DBPCFC or open	Logistic regression	90%: 88.8
van der Gugten et al[30]	With suspected cow's milk allergy??	3.0 (average)	213	44%	Retrospective	DBPCFC	2–by-2 tables logistic regression	95%: 52 90%: 26.8 95%: >100 90%: 66.4
Perry et al[31]	With food allergy, 77% allergic to >1 food	4.8 (median) at first challenge	391	*	Retrospective	Open	2–by-2 tables	50%: 2
Komata et al[32]	With suspected milk and egg allergy	1.3 (median)	969	*	Cross-sectional	Open (majority)	Logistic regression	95%: 5.8 for age <1 y 95%: 38.6 for age = 1 y 95%: 57.3 for age ≥2 y

*Not provided.

means of ImmunoCAP might underestimate by up to 2.4-fold the actual level of specific IgE that is measurable with the 5 milk component allergens (α-lactalbumin, β-lactoglobulin, casein, lactoferrin, and BSA) on individual allergosorbents.[26] Given these concerns with the published positive predictive decision points, one must be careful to interpret the serologic levels of IgE antibody within the context of the patient's clinical history.

Finally, the concentration, specific activity (specific/total IgE ratio), affinity (tightness of binding), and clonality (epitope specificity) of the IgE antibody response have all been shown to affect effector cell activation.[33] Higher levels of basophil activation occurred with higher overall concentrations of serum IgE anti–Der p 2, higher Der p 2–specific IgE/total IgE ratios, broader clonality (specificities or number of recognized epitopes), and higher IgE antibody affinities. Future design of serologic assays for IgE antibody will need to more effectively assess these 4 important humoral immune response parameters in the evaluation of patients for allergic disease.[34]

Performance of IgE antibody assays in the skin and blood

Comparison of the diagnostic performance (Table II) of any 2 *in vivo* tests, serologic tests, or both for allergen-specific IgE from peer-reviewed published data is difficult for several reasons. First, various investigators use different clinical criteria, test criteria, or both to define cases (subjects with disease). Second, study populations might vary widely within their disease category because of differences in the magnitude and frequency of their allergen exposures. Third, IgE antibody assay performance is highly dependent on the criterion that is used to define the positive threshold, which varies among clinical studies, especially for *in vivo* methods.

Table VIII summarizes the relative clinical utility of skin test and serologic assays for the assessment of systemic (venom and drug), food-related, and respiratory-related allergic diseases. Maximal clinical sensitivity is needed for evaluating patients with suspected venom and drug allergies because of the potential for life-threatening systemic reactions. In these cases the graded

TABLE VIII. Relative diagnostic utility of skin test and serologic measures of allergen-specific IgE antibody

	Allergen-specific IgE antibody	Epicutaneous (prick/puncture) skin test	Intradermal skin test
Systemic reactions Venom allergy Drug allergy	Complementary to intradermal skin test	Not sufficient	Preferred
Food allergy	Acceptable	Acceptable	Not needed (false-positive results)
Respiratory allergy	Acceptable	Acceptable	Usually not needed (false-positive results)

intradermal skin test, which errs on the side of false-positivity,[35,36] is preferred because the epicutaneous skin test is not sufficiently sensitive. By using dialyzed venom that removes irritating amines, the sensitivity of the intradermal venom skin test can be further enhanced.[37] When the intradermal skin test results are inconsistent with the clinical history, they should be repeated, and IgE antibody serology should be performed as a complementary test.

For food and respiratory allergy, IgE antibody as detected in the serum by using current autoanalyzer technology and in the skin by using the epicutaneous test are considered equivalent as confirmatory tests in terms of their sensitivity and accuracy.[35,38] The clinical use of intradermal testing with a single injection for foods or aeroallergens is contraindicated. Improved screening of patients for allergic disease can be achieved when epicutaneous skin test and serologic measurements of IgE antibody are used together.[39] Serologic IgE antibody assay results of greater than 0.35 kUa/L and epicutaneous skin test results larger than 3 to 4 mm have been most effectively correlated with the presence of allergic symptoms that are induced in allergen challenge studies. Serology has the advantage with complex allergen extracts, such as those derived from foods and molds, that it uses allergosorbents that have defined expiration dates and are quality controlled by using panels of human sera from subjects who are known to be clinically allergic to the specific target allergen.[6] In deciding which confirmatory diagnostic allergy test to use in clinical practice, the allergist needs to consider the test's relative sensitivity, inherent variability, the relationship between IgE antibody levels and disease expression, patient safety and comfort, timeliness, and cost.[6,36-41]

IgE screening assays

Occasionally patients provide a questionable or negative history for atopic disease or a history from which no one allergen specificity can be pinpointed with a reasonable certainty as the cause of allergic symptoms. The multiallergen IgE antibody screen is a single qualitative serologic assay that evaluates a patient's serum for the presence of IgE antibodies specific for a mixture of approximately 15 principal indoor and outdoor aeroallergens that are believed to account for a large majority of allergic respiratory disease.[6] A pediatric form of the multiallergen screening test can evaluate common food-specific IgE antibodies (eg, milk, egg, peanut, wheat, and soybean) in addition to IgE specific for common weed, grass, and tree pollens; molds; pet epidermal; and dust mite aeroallergens. A negative multiallergen screen result reduces the probability that IgE antibodies are involved in the patient's clinical problems to less than 5%. In a recent study one version of the pediatric multiallergen screen (Phadiatop, Phadia) correctly identified allergic sensitization in 97.6% of 215 children (ages 1-14 years) with recurrent wheezing.[22] These screening assays are possibly most useful in confirming the absence of significant atopic disease in subjects who are suspected of having an intrinsic or non–IgE-mediated respiratory, cutaneous, or gastrointestinal disease process. Such a test can minimize the need for multiple *in vivo* or serologic allergen-specific IgE measurements in patients with a low clinical probability of atopic disease. The use of this screening test in unselected populations is likely to generate many false-positive results because IgE antibody responses are much more frequent than symptomatic disease.

Total serum IgE

Total serum IgE measurement is currently the only diagnostic allergy test that is regulated in the United States under the Clinical Laboratory Improvement Act of 1988 (CLIA-88). These assays are either nephelometric or 2-site (capture and detection antibody), noncompetitive immunometric (labeled antibody) assays. The analytic sensitivity of the total serum IgE assays is 1 to 2 μg/L (1 kU/L is equivalent to 2.4 μg/L IgE). Intermethod agreement of commercially available IgE assays as assessed by using intermethod coefficients of variation are less than 10% for serum IgE levels of greater than 30 kU/L in a proficiency-based study.[42] Calibration of total serum IgE assays to the WHO IgE International Reference Preparation (WHO 75/502) has enhanced worldwide agreement.

The clinical utility of total serum IgE measurements in the diagnosis of allergic disease has always been limited by its age-dependent concentration and the wide overlap in concentrations in serum between atopic and nonatopic populations. The total serum IgE level must therefore be viewed always within the context of its nonatopic age-adjusted reference interval.[6] With the licensing of anti-IgE (Xolair [omalizumab]; Genentech, Inc, South San Francisco, Calif) therapy in 2003, there has been an increase in total IgE measurements because Xolair dosing requires knowledge of the patient's total serum IgE level. The increased use of Xolair has led to concern that some serum specimens are being analyzed for total serum IgE levels while containing Xolair, which can potentially interfere and reduce the assays' accuracy. In a proficiency survey–based study, total serum IgE levels, as measured by using ImmunoCAP, were shown to be minimally reduced (2.4% to 9.0%) by the presence of 50 to 200 molar excess of omalizumab to the level of serum IgE.[43] In contrast, other clinically used total serum assays showed marked reductions from 12.5% to 67.2% ($P < .001$), and the interference increased in proportion to the total serum IgE level in the serum. Counter to claims in the Xolair package insert, total serum IgE can be accurately measured by using the ImmunoCAP assay in the presence of therapeutic levels of Xolair. Clinical assays to measure free IgE or IgE that is not bound with therapeutically administered anti-IgE are in the developmental stage. Free IgE measurements should help the clinician with a problematic Xolair-treated patient to determine whether the dose of anti-IgE should be escalated to obtain greater clinical efficacy.

Proficiency testing for total and allergen-specific IgE antibody assays

The CLIA-88 requires that all federally licensed clinical laboratories participate in an external proficiency survey. One such diagnostic allergy survey is conducted by the College of American Pathologists.[42] The survey involves analysis of 5 or 6 challenge sera every 17 weeks (3 cycles per year) for total serum IgE and IgE antibody levels to 5 allergen specificities and a multiallergen screen. Results are impartially collated, and interlaboratory (intramethod) coefficients of variation are computed, critiqued, and then sent to both participating laboratories and credentialing agencies. Except for the occasional nonatopic serum, interlaboratory/intramethod and intermethod coefficients of variation for total serum IgE are routinely excellent at less than 15%.[42]

Allergen-specific IgE levels historically have been reported by different assays in nonequivalent arbitrary units or classes. Today, the 3 principal assays report allergen-specific IgE levels in more quantitative kUa/L units. Although differences continue to exist in the levels of reported allergen-specific IgE among the various IgE antibody assays, in general, the 3 principal assays correctly identify the IgE-negative (nonsensitized) subjects' sera from sera that are IgE antibody positive (sensitized) for most of the allergen specificities. It is the responsibility of the laboratory to indicate the method they use on their final report to the clinician. It is, however, the responsibility of the referring physician to ensure that the laboratory that performs IgE antibody testing is CLIA-88 certified and that they use a validated assay method and perform successfully on a diagnostic allergy proficiency survey.[6,44]

Venom competitive inhibition IgE antibody assay

One unique competitive inhibition form of the IgE antibody assay has a specific application to patients with Hymenoptera venom sensitivity. Of the medically important Hymenoptera, structural similarity exists between the vespid and *Polistes* species wasp allergens phospholipase A1/B (Ves g I and Pol a I) and hyaluronidase (Ves g II and Pol a II), which leads to IgE antibody cross-reactivity. A serologic venom inhibition assay is used to determine the most appropriate therapeutic composition of venoms for immunotherapy.[45] Patients with venom allergy who have a strong skin test response or high level of serum IgE antibody to yellow jacket venom (YJV) and a weak skin reactivity or low level of serum IgE antibody specific for *Polistes* species wasp venom (PWV) are candidates for this analysis. In the assay a patient's serum that contains YJV- and PWV-specific IgE antibodies is preincubated with soluble YJV (heterologous venom), PWV (homologous venom control), or buffer (no inhibition control). The mixtures are then incubated separately with PWV allergosorbent, and the assay is completed with the final addition of labeled anti-human IgE antibody. The amount of IgE anti-PWV bound to the PWV allergosorbent is measured, and greater than 95% inhibition of IgE anti-PWV binding with the addition of soluble YJV is considered complete cross-inhibition. Sera from 305 patients with Hymenoptera venom allergy with greater than 2 ng/mL of IgE antibody to YJV and PWV were evaluated to determine whether PWV should be included in the venom immunotherapy regimen together with yellow jacket or mixed vespid venom. The venom competitive inhibition assay identified one third (36.4%) of these subjects as having an exclusive YJV sensitivity. These subjects were candidates for exclusion of PWV from their immunotherapy regimen because their IgE anti-PWV was greater than 95% cross-inhibitable with soluble YJV.[45]

Hymenoptera venom–specific IgG

Allergen injections during immunotherapy are known to enhance the production of specific IgG "blocking" antibodies (Table I).[46] As a general rule, quantitative measurements of allergen-specific IgG (or IgG subclass) antibodies in studies of allergic rhinitis have not correlated well with improvement in clinical symptoms of individual patients receiving immunotherapy. However, clinically successful immunotherapy is almost always accompanied by high serum levels of allergen-specific IgG, particularly of the IgG4 subclass. One proposed application of allergen-specific IgG antibody measurements has been as an aid in documenting effective immunotherapy in patients with Hymenoptera venom sensitivity. In a prospective study Hymenoptera venom–specific IgG antibodies were monitored in the serum of 109 patients with venom allergy to examine whether their levels could provide an indication for the relative risk of a systemic reaction after a sting challenge in patients receiving venom immunotherapy.[47] Over a 4-year period, systemic symptoms occurred in 16% of 211 venom sting challenges in the group with less than 3 μg/mL venom-specific IgG antibody. This contrasted with a reaction rate of 1.6% in patients with venom IgG levels of greater than 3 μg/mL. The highest rate of allergic reactions (26%) occurred among patients who had both a venom-specific IgG antibody level of less than 3 μg/mL and less than 4 years of venom immunotherapy. The study concluded that quantitative venom-specific IgG antibody levels can be useful for individualizing the dose and frequency of injections to maximize its protective effects. The clinical utility of venom-specific IgG antibody measurements, however, appears to be restricted to the first 4 years of venom immunotherapy.

Food-specific IgG and IgG4 antibodies

Historically, IgG4 reaginic antibodies were believed to be diagnostic because monoclonal anti-human IgG4 could induce BHR from allergic donors cells.[48] In 1992, this issue was challenged by Lichtenstein et al,[49] who showed no histamine release (<10%) was detected from nonatopic donor cells after incubation with a panel of highly specific International Union of Immunological Societies–documented human IgG subclass–specific mAbs.[50] Moreover, 85% of these same cells released to anti-IgE. In contrast, the study confirmed that 75% of atopic donor basophils released greater than 10% of their histamine to 1 or more of the human anti-IgG subclass–specific mAbs and not only of the IgG4 subclass specificity. After a series of elaborate basophil-based lactic acid stripping and add-back experiments, it was shown that atopic subjects can possess basophil IgE receptor–bound IgG anti-IgE–IgE complexes, and cells from these subjects can be triggered by the addition of anti-IgG mAbs that cross-link the IgE receptors through this complex. This provided a rationale for why the presence or levels of IgG or IgG4 antibodies specific for food antigens have never shown a correlation with the diagnostic results of positive DBPCFCs. This also supports the European Academy of Allergy and Clinical Immunology Task Force recommendation[51] that food-specific IgG and IgG4 antibody responses are not useful diagnostic tools for assessing allergic disease or planning food-elimination diets. Further work on this issue is needed with modern IgG and IgG4 antibody autoanalyzers with sera from non–IgE-mediated food-sensitive subjects to confirm this recommendation and verify that allergen-specific IgG antibody levels are simply a reflection of the extent of a subject's environmental antigen exposure and not a marker for allergen sensitization.[52]

Precipitating IgG antibodies (precipitins)

Extrinsic allergic alveolitis, also referred to as hypersensitivity pneumonitis, is an inflammatory reaction in the lung interstitium and terminal bronchioles induced by chronic exposure to antigenic organic dusts (eg, molds and bird droppings). Although the lung-lesion histology indicates a cell-mediated pathology, most patients with hypersensitivity pneumonitis have high levels of precipitating IgG antibody to the offending antigens in their blood.[53,54] Some clinical laboratories still perform the double-diffusion (Ouchterlony) assay to detect precipitating antibodies to extracts of organic dusts. This involves inserting a crude antigen extract of the organic material (bird fecal material and mold) into one well and a control and the patient's serum (containing antibody) in 2 other closely spaced wells in a porous agarose gel. If diffusion of the antigen and antibody over 2 to 3 days in a moist chamber produces precipitating antibodies with lines of identity to the control antiserum, this can support the diagnosis of hypersensitivity pneumonitis. Precipitating antibodies, or precipitins, can be detected in the sera of nearly all patients who have active hypersensitivity pneumonitis, but they are also present in the sera of as many as 50% of asymptomatic subjects who have been exposed to the relevant organic dusts.[54] Immunoassays for IgG antibody to the appropriate organic dust antigens appear to be too sensitive and are viewed as less diagnostically useful. Precipitin assays are performed to organic dusts containing the thermophilic actinomyces (*Micropolyspora faeni*, *Thermoactinomyces vulgaris*, and *Thermoactinomyces candidus*), multiple antigens from *Aspergillus* species (*Aspergillus fumigatus*, *Aspergillus niger*, and *Aspergillus flavus*), pigeon serum, *Aureobasidum pullulans*, and fecal particles from parakeets and a variety of exotic household birds (eg, cockatiel and blue Amazon).

Mast cell tryptase

Mediators, which include prestored histamine and newly generated vasoactive mediators, are released from activated mast cells into surrounding soft tissue (Table VI). Mast cell tryptase (molecular weight 134,000 d), which is a serine esterase with 4 subunits, each with an enzymatically active site, is also released from an activated cell. When dissociated from heparin, tryptase rapidly degrades into its monomers and loses enzymatic activity. Human basophils contain 300- to 700-fold less tryptase than lung or skin mast cells, and therefore tryptase in serum is considered a marker of systemic mast cell activation.[55] The α-tryptase concentration in blood is a measure of the mast cell number, and it is estimated by subtracting β-tryptase levels from the total serum tryptase concentration. In contrast, β-tryptase levels in blood are considered a measure of mast cell activation.

Healthy nondiseased subjects have serum total tryptase levels that range from 1 to 10 ng/mL (average, 5 ng/mL). If baseline total serum tryptase levels exceed 20 ng/mL, systemic mastocytosis should be suspected. Serum β-tryptase levels of less than 1 ng/mL are observed in nondiseased subjects. β-Tryptase levels of greater than 1 ng/mL indicate mast cell activation. Blood samples should be collected from 0.5 to 4 hours after the initiation of a suspected mast cell–mediated systemic reaction for optimal results.[55,56] Peak β-tryptase levels of greater than 10 ng/mL in a postmortem serum suggest systemic anaphylaxis as one probable cause of death. An insect sting–induced β-tryptase level can peak at greater than 5 ng/mL by 30 to 60 minutes after the sting and then decrease with a biological half-life of approximately 2 hours.[57] Increased postmortem tryptase levels, however, have been observed in the absence of anaphylaxis,[58] thus reducing the utility of postmortem tryptase levels in placing the cause of death for some deceased Hymenoptera-sensitive subjects. Tryptase has also been measured in bronchoalveolar lavage fluid, nasal lavage fluid, tears, and skin chamber fluid; however, there are currently no clinical indications for such measurements.

BHR test

The BHR assay has been used to detect the presence of allergen-specific IgE on surface basophils by means of direct challenge or passive sensitization. When used as an alternative confirmatory diagnostic test for allergen-specific IgE antibody, BHR test results have highly correlated with results from skin testing and bronchoprovocation.[59] In the direct challenge BHR assay, peripheral blood leukocytes are isolated from whole blood by means of dextran sedimentation, washed, and incubated with allergen or anti-human IgE at varying concentrations (eg, 3- to 10-fold dilutions). In the passive sensitization BHR assay, basophils are stripped of their IgE by means of lactic acid elution and then incubated with serum containing IgE antibody and challenged with antigen. In either the direct or passively sensitized BHR assay, mediator release is complete within 30 minutes, and then histamine or leukotriene released into the supernatant is measured. The BHR dose-response curve typically consists of a characteristic bell-shaped curve with a linear ascending portion, which is maximal or peaks at the optimal cross-linking allergen dose, and a descending portion at higher than optimal allergen concentrations. The allergen concentration required to induce 50% histamine release can be used to define the relative sensitivity of the patient's basophils for a given allergen extract. At present, the BHR test is used primarily in research laboratories because of its need for fresh blood. It has been especially useful as an alternative assay for clarifying discrepancies between skin test and serologic IgE antibody test results.

The basophil has been examined as a possible indicator cell for assessing the autoimmune status of patients experiencing a form of chronic urticaria (CU).[60-63] Autoantibodies specific for IgE, FcεR1, or FcεRII can be present in 30% to 50% of patients with CU.[60] One clinical laboratory offers a CU index test in which highly selected donor basophils are incubated with serum from the patient with CU, and released histamine is quantified.[62] Other investigators dispute the validity of this assay and suggest that a primary basophil abnormality, unknown serologic factors, or both affecting basophils in patients with CU might be more clinically relevant to disease pathogenesis than the presence or level of FcεRI/II, IgE-reactive autoantibodies, or both.[63]

As a measure of basophil activation, flow cytometry has been used to quantify the level of basophil surface markers after exposure to allergen (CD63, CD203c), allergen exposure in the presence of IL-3 (CD63), and exposure to other degranulating stimuli, such as N-formyl-methionyl-leucyl-phenylalanine and ionophores. Although whole blood can be used for these analyses, conditions used to lyse contaminating red blood cells can interfere by directly stimulating basophil activation. Controversy remains as to whether an individual surface marker or the panel of activation markers should be analyzed to reflect basophil mediator release and how well the kinetics of change of each marker actually reflect basophil activation. Details of the BHR assay and flow cytometric detection of basophil activation surface markers are presented elsewhere.[64]

IN VIVO DIAGNOSTIC PROVOCATION TESTING

When discordance occurs between the clinical history and primary diagnostic confirmatory test results, one of several provocation tests might be performed.[13] Bronchial and nasal provocation challenges are techniques used to identify a relationship between an inhaled substance and a change in the patient's bronchial or nasal physiology. A DBPCFC is used to evaluate patients who have experienced food-induced gastrointestinal reactions (eg, nausea, colic, vomiting, and diarrhea) that can occur within minutes to hours after the consumption of selected foods. Each of these tests will be briefly discussed.

Bronchoprovocation studies involving the use of methacholine are particularly useful in the diagnosis of difficult cases of asthma.[65] In general, bronchoprovocation involves the administration of either methacholine or histamine by means of a calibrated nebulizer starting at doses of 0.05 to 0.1 mg/mL and doubling the concentration up to 10 to 25 mg/mL. Methacholine is an analog of acetylcholine that directly stimulates bronchial smooth muscle rather than inducing mast cell enzyme and mediator release. Alternatively, allergen extracts can be administered in increasing doses. Allergen, in contrast to methacholine, induces changes in pulmonary function as a direct result of mast cell activation in the lung. The clinical effect of the analyte exposure is monitored with pulmonary function tests after each dose. A positive response is usually defined as the concentration of agonist that results in a decrease in FEV_1 of 20% or more from the preprovocation baseline value, which must be greater than 70% of predicted value for valid interpretation. More extensive details regarding the methods and interpretation of bronchial challenges are presented elsewhere.[13,65]

Nasal provocation involves the controlled administration of buffer (human serum albumin–saline) or increasing concentrations of allergen into a washed nasal passage. A symptom score is collected (eg, number of sneezes induced) and/or the concentration of mast cell mediators or albumin released into nasal lavage fluids after each concentration of allergen indicates the relative sensitivity of a subject to the allergen in question. N-tosyl-L-arginine methyl ester [TAME] esterase and histamine are commonly monitored in nasal lavage fluid. Nasal airway resistance is a less satisfactory end point because of high intrinsic variation. Details of the procedure and applications can be found elsewhere.[66]

The DBPCFC involves the controlled ingestion of frequently eaten foods that are known to contain potent allergens. These foods typically include cow's milk (caseins, β-lactoglobulin, and α-lactalbumin), chicken egg white (ovalbumin, ovomucoid, and ovotransferrin), cereal grains (wheat, rye, barley, and oats), legumes (peanut, soybean, and white bean), fish, and seafood (shrimp, crabs, lobsters, and oysters). The DBPCFC begins with a strict elimination diet for the suspect foods for 7 to 14 days before the challenge. An equal number of randomly alternating food allergen and placebo challenges, starting with 125 to 500 mg of lyophilized food, are then administered to the patient in a fasting state, doubling the dose every 15 to 60 minutes. Clinical reactivity can be ruled out once 10 g of lyophilized food blinded in masking foods (eg, pudding and chili) or capsules is tolerated. Negative DBPCFC results must then be confirmed with an open feeding challenge under observation to rule out possible false-negative challenge results. Serum levels of food-specific IgE antibody can sometimes be used to exclude the need for a food challenge if the levels are sufficiently high to exceed reported 95% confidence limits for a positive food challenge result (Table VII).[23,24] An extensive discussion of the DBPCFC and variables influencing its outcome are presented elsewhere.[67]

INDOOR AEROALLERGEN TESTING

Avoidance by separating the allergic patient from the allergen source is possibly the least expensive and most effective mode of treatment for allergic disease, when it is achievable. Knowledge about the levels of allergen in an environment can support the decision to initiate expensive alterations of their home, school, or workplace to facilitate avoidance of indoor aeroallergens. Some clinical laboratories perform environmental allergen quantification in which an air sample or a surface dust specimen is collected with a vacuum from either the general indoor environment or individual rooms. An inexpensive air-sampling cassette or surface dust collector is attached to a vacuum, and a bulk dust specimen is collected. It is sent to a specially equipped laboratory, where it is processed through a 50-mesh metal sieve to exclude particles larger than 300 μm. Fine dust is then quantitatively extracted (eg, 100 mg per 2 mL of physiologic saline-albumin buffer). Soluble allergens, once extracted, are quantified with mAb-based immunoenzymetric assays or bead-based multiplex assays for dust mite–, pet epidermal–, rodent-, cockroach-, and mold-related indicator allergens. Currently, Der f 1 and 2 and Der p 1 and 2 are allergens that are excreted in fecal particles by dust mites (*D farinae* and *D pteronyssinus*). Fel d 1 and Can f 1 are allergens excreted by the sweat glands of the domestic cat *(Felis domesticus)* and dog *(Canis familiaris)*. Bla g 1/Bla g 2 allergens are released by the German cockroach *(Blatella germanica)*. Mus m 1 and Rat n 1 are allergens excreted into urine by the mouse *(Mus musculus)* and rat *(Rattus norvegicus)*. The level of these indoor allergens serve as "indicators" for environments that are contaminated with higher than desirable levels of allergens for sensitized subjects (especially children with atopic asthma). Indoor evaluation allows allergen-laden environments to be identified and cleaned in an attempt to facilitate avoidance of allergen exposure. Risk levels have been assigned for some of the allergens. Levels of Der p 1 allergen, Der f 1 allergen, or both of greater than 2,000 ng/g fine dust have been associated with an increased risk of allergic symptoms in sensitized subjects, whereas levels of greater than 10,000 ng/g of fine dust have been associated with an increased risk of sensitization. For other allergens, such as cockroach, mouse, and rat allergens, just the presence of detectable allergen can be an indicator of clinically relevant environmental contamination. Further details can be obtained elsewhere.[68]

The kingdom Fungi encompasses yeasts, molds, smuts, and mushrooms, which are plants without leaves, flowers, or roots that reproduce from spores (2-20 μm in diameter and 1-100 mm in length). Molds lack chlorophyll and vascular tissue and range in form from a single cell to a body mass of branched filamentous hyphae that spread into and feed off of dead organic matter or living organisms. Some molds produce allergen-laden spores that are generally invisible to the naked eye and are used in speciation of the mold by means of microscopic, immunologic, and molecular biological techniques.

Sampling for mold is unnecessary in cases in which visible mold growth or musty odors identify mold infestation. Alternatively, a bulk dust can be distributed on a microbiological culture plate containing media and antibiotics or inoculated with a swab or by being placed in a gravity sampler or a suction impactor.

Viable spores are enumerated 24, 48, and 72 hours later by means of macroscopic and microscopic assessment.[69] *Alternaria* and *Aspergillus* species allergens (Alt a 1 and Asp f 1) can be quantified in extracts of surface dust by using mAb-based immunoenzymetric assays; however, their utility is limited to environmental conditions in which molds secrete these allergens. Alternatively, an Environmental Relative Moldiness Index test involves PCR-based DNA analysis for the relative levels of 26 molds associated with water damage and 10 molds not associated with water damage. When levels of these 36 molds were measured by using DNA techniques in dust from 271 homes of asthmatic children, the Environmental Relative Moldiness Index level was more effective than a binary classification of homes as either moldy or nonmoldy based on onsite inspection in predicting the development of respiratory illness (wheeze, rhinitis, or both).[70]

OUTDOOR AEROALLERGEN TESTING

Most major cities across the United States have an aerobiology monitoring station with a collection device on a platform or roof top, typically 1 story off the ground (eg, 13 feet). Ideally it is in an open space distant from trees, which can bias the aeroallergen results. The Rotorod Sampler (Sampling Technologies, St Louis Park, Minn) is one widely used rotating-arm impactor that recovers airborne particles on 2 rapidly moving plastic collector rods.[71,72] It contains a pair of 1.59-mm-wide plastic rods that extend during rotation on a central arm at defined time intervals (eg, 10-60 seconds every 10 minutes). A thin layer of silicon grease that is coated on the leading edge of the rod (edge in the direction of rotation) impacts particles in the air, and they imbed in the grease. Every 24 hours, the rod is removed from the device, stained, and microscopically evaluated by a qualified technician for the number and types of pollens and mold spores (grains or spores per cubic meter of air sampled for the previous 24-hour period). The efficiency of particle collection on the Rotorod decreases with particle size (eg, 7-μm particle: 10% efficiency to 25-μm particle: 100% efficiency).[72] Fungal spores are smaller (diameter = 1 to >100 mm) than pollen grains (diameters = 20-70 μm). Newer devices, such as the Burkard Hirst Trap and Burkard SporeWatch (Burkard Mfg Co. Rickmansworth, Herts, England), are suction impactors that are more effective in detecting mold spores than the rotarod.[73] These devices also have the capacity to collect longitudinal samples over a 7-day period. Although pollen and spore counts are commonly transmitted to local weather stations and newspapers for public use, they are somewhat limited in their use because they describe the levels in the air over the previous 24 hours.

CONCLUSION

A number of analytic measurements are used to promote more accurate diagnosis and better management of allergic subjects. The clinician should remember that all *in vivo* and serologic analyses are subject to inherent variation and potential interference. Thus it is prudent to question the validity of any *in vivo* or laboratory test that is inconsistent with a carefully collected clinical history. One should repeat *in vivo* testing on a different day or perform serologic testing with a new blood specimen, a different laboratory, or both. Alternative tests might seem redundant, but they are useful in confirming observations because different methods (eg, skin test and serologic assays) measure different subsets of the IgE antibody response. Most importantly, let the clinical history drive the diagnosis. Maintain a healthy skepticism about diagnostic test results, and verify the quality control and validity of *in vivo* diagnostic reagents used and the performance standards of serologic assays and the laboratories that perform them.

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Laboratory evaluation of primary immunodeficiencies

João B. Oliveira, MD, PhD, and Thomas A. Fleisher, MD *Bethesda, Md*

Primary immunodeficiencies are congenital disorders caused by defects in different elements of the immune system. Affected patients usually present clinically with recurrent infections, severe infections, or both, as well as autoimmune phenomena that are associated with many of these disorders. Early diagnosis is essential for referral to specialized care centers and the prompt initiation of appropriate therapy. In this article the authors describe a general approach for the investigation of the most common primary immunodeficiencies, outlining the typical clinical symptoms and most appropriate laboratory investigations. (J Allergy Clin Immunol 2010;125:S297-305.)

Key words: *Primary immunodeficiency, laboratory assessment, immunologic diagnosis, immunity*

Abbreviations used

ALPS:	Autoimmune lymphoproliferative syndrome
APECED:	Autoimmune polyendocrinopathy, candidiasis, ectodermal dystrophy syndrome
DHR:	Dihydrorhodamine 123
FOXP3:	Forkhead box protein 3
HLH:	Hemophagocytic lymphohistiocytosis
IFNGR1:	IFN-γ receptor 1 gene
IFNGR2:	IFN-γ receptor 2 gene
IL12RB1:	IL-12 receptor β1 gene
IPEX:	Immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome
IRAK4:	IL-1 receptor–associated kinase 4
LAD:	Leukocyte adhesion deficiency
NEMO:	Nuclear factor κB essential modulator, also called IKK-γ
NK:	Natural killer
PID:	Primary immunodeficiency
SCID:	Severe combined immunodeficiency
STAT:	Signal transducer and activator of transcription
TCR:	T-cell receptor
TLR:	Toll-like receptor
TREC:	T-cell receptor excision circle
XLP:	X-linked lymphoproliferative syndrome

The clinical spectrum of characterized primary immunodeficiencies (PID) has expanded significantly over the past 2 decades, and the underlying genetic basis of the majority of primary immunodeficiencies (PIDs) also has been identified. The accurate diagnosis of patients with PIDs is critical for appropriate therapy and also affords the opportunity to provide appropriate genetic counseling to the patient and his or her family. In virtually all cases the clinical symptoms involve increased susceptibility to infection, and early diagnosis and therapy provides the greatest opportunity to prevent significant disease-associated morbidity. In this setting the laboratory serves as the primary source of diagnostic information used to define the immunologic defect. The optimal use of the laboratory for the diagnosis and characterization of PIDs is the focus of this chapter.

EVALUATING SUSPECTED ANTIBODY DEFICIENCY DISORDERS

When to suspect

The majority of patients with primary antibody deficiencies present with recurrent bacterial infections of the sinopulmonary tract, including recurrent otitis media, sinusitis, and pneumonia (Table I).[1,2] The most commonly isolated organism is *Streptococcus pneumoniae*, but *Haemophilus influenzae* (often untypeable), *Staphylococcus* and *Pseudomonas* species are also seen. Diarrhea affects up to 25% of these patients, often associated with *Giardia lamblia* infection. However, infections with rotavirus, enterovirus, *Campylobacter*, *Salmonella*, and *Shigella* species might also be found.[1] In addition, autoimmune manifestations are seen in up to 25% of these patients, with autoimmune hemolytic anemia and autoimmune thrombocytopenia being most commonly observed. Finally, granulomatous disease involving various organs with particular predilection for the lung might also be present, and in some patients this process can result in significant morbidity.[1]

PIDs that commonly manifest some degree of hypogammaglobulinemia include selective IgA deficiency, common variable immunodeficiency, and congenital agammaglobulinemias (both X-linked and autosomal recessive inheritance, Table II). Less common causes include agammaglobulinemia with thymoma (Good syndrome) and X-linked lymphoproliferative syndrome (XLP).[1] X-linked agammaglobulinemia should be suspected in all male patients with recurrent otitis and even a single episode of pneumonia, even if the family history is negative. This condition also might present with neutropenia and sepsis by *Pseudomonas* or *Staphylococcus*.[3] Occasionally, the ataxia-telangiectasia syndrome manifests with recurrent infections and upper respiratory tract symptoms associated with IgA deficiency before the onset of overt neurologic signs.[4] Concomitant bacterial sinopulmonary and opportunistic infections, including low pathogenic mycobacteria, should raise suspicion of a cellular defect that also affects antibody production, such as nuclear factor κB essential modulator (NEMO; also called IKK-γ) or CD40 ligand (CD154) deficiencies.[5,6] Selected complement deficiency and phagocytic defects might also have a clinical presentation similar to that of antibody deficiency and could be considered for investigation (Table II).

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Reprint requests: Thomas A. Fleisher, MD, Bldg 10 Room 2C306, 10 Center Dr, MSC 1508, Bethesda, MD 20892-1508. E-mail: TFleishe@cc.nih.gov.

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TABLE I. Common pathogens and infection sites according to the underlying immune defect

Affected immunity arm	Typical site of infection	Common pathogens
B cells	Sinopulmonary tract, GI tract, joints, CNS	Pyogenic bacteria: streptococci, staphylococci, *Haemophilus influenzae* Enteroviruses: ECHO, polio *Mycoplasma* species
T cells	Sepsis, lung, GI tract, skin	Viruses: CMV, adenovirus, measles, molluscum Fungi: *Candida* and *Aspergillus* species, *Pneumocystis jiroveci* Pyogenic bacteria Protozoa: *Cryptosporidium* species
Phagocytes	Skin infections, lymphadenitis, liver, lung, bone, GI tract, gingivitis/periodontitis	Bacteria: staphylococci, *Serratia marcescens*, *Burkholderia cepacia*, *Klebsiella* species, *Escherichia coli*, *Salmonella* species, *Proteus* species Fungi: *Candida*, *Aspergillus*, and *Nocardia* species
Complement	Systemic infections, meningitis	Pyogenic bacteria: streptococci, *Haemophilus influenzae*, *Neisseria* species

GI, gastrointestinal; *CNS*, central nervous system; *ECHO*, echovirus; *CMV*, cytomegalovirus.

TABLE II. Differential diagnosis of antibody deficiencies and associated laboratory findings

Primary B-cell disorders
Common variable immunodeficiency: low IgG and IgA levels, variable IgM levels, usually normal B-cell numbers
Selective IgA deficiency: low IgA levels, normal IgG and IgM levels, normal B-cell numbers
Congenital agammaglobulinemia: low IgG, IgA, and IgM levels; undetectable or very low B-cell numbers (<2%)
Specific antibody deficiency: normal IgG, IgA, and IgM levels; normal B-cell numbers; defective antibody response to vaccination
Agammaglobulinemia with thymoma (Good syndrome): low IgG and IgA levels, variable IgM levels, low B-cell numbers
Combined cellular and humoral disorders
Hyper-IgM syndromes: low IgG and IgA levels, normal, low or high IgM levels, normal B-cell numbers
Ectodermal dysplasia with immunodeficiency syndrome (NEMO/IκBα deficiency): variable immunoglobulin levels, normal B-cell numbers
XLP: low IgG and IgA levels, variable IgM levels, typically normal B-cell numbers
Ataxia-telangiectasia syndrome: low IgA levels
Other causes to consider
Drug-induced hypogammaglobulinemia; sickle cell disease with secondary hyposplenism; primary asplenia; immunodeficiency, centromeric instability, facial anomalies syndrome; cystic fibrosis; complement component deficiency; myelodysplasia; chronic lymphocytic leukemia; multiple myeloma; dysmotile cilia syndrome; warts, hypogammaglobulinemia, immunodeficiency and myelokathexis (WHIM) syndrome

Laboratory evaluation

The initial clinical laboratory screening of antibody-mediated immune function can be accomplished by measuring the levels of the major immunoglobulin classes IgG, IgA, IgM, and IgE (Table III). The results must be compared with age-matched reference intervals (normal ranges) that are typically provided as 95% CIs. There are no rigid standards regarding the diagnosis of immunoglobulin deficiency, although an IgG value of less than 3 g/L (300 mg/dL) in an adolescent or adult, as well as values clearly below the age-appropriate reference (95% confidence interval) in a child should trigger further evaluation. An additional and readily available test is quantitation of IgG subclass levels. This test is most useful in evaluating an IgA-deficient patient with significant recurrent bacterial infections. However, in most settings, detection of an IgG subclass deficiency still requires documentation of an abnormality in specific antibody production before initiating therapy, making this test of more limited utility. Measurement of specific antibody responses is useful in confirming defective antibody production and is essential when the total immunoglobulin levels are only modestly decreased (or even normal) in the setting of recurrent bacterial infection. The simplest method is evaluation for spontaneous specific antibodies (eg, anti–blood group antibodies [isohemagglutinins]) and antibodies to previous immunizations or infections. The definitive method to evaluate *in vivo* antibody production involves immunizing a patient with protein antigens (eg, tetanus toxoid) and polysaccharide antigens (eg, Pneumovax, Merck & Co, Inc, Whitehouse Station, NJ) and assessing preimmunization and 3- to 4-week postimmunization antibody levels. Guidelines for normal responses, which are usually provided by the testing laboratory, typically consist of finding at least a 4-fold increase in antibody levels and/or protective antibody levels after immunization. An alternative method to access the humoral immune response that is specifically useful in patients already receiving immunoglobulin replacement therapy involves vaccination with a neoantigen, such as the bacteriophage Phi X174; however, this is only available in some specialized centers.[7]

Additional testing focuses on determining the presence or absence of B cells by using flow cytometry. This is particularly useful as a marker for congenital forms of agammaglobulinemia because this group of disorders typically is characterized by absent or extremely decreased circulating B-cell numbers based on the underlying defects that block B-cell development.[2] More recently, characterization of B-cell subsets, particularly directed at memory and immature B cells, has been put forward as a means of further characterizing patients with common variable immunodeficiency.[8] Studies that test *in vitro* B-cell signaling and immunoglobulin biosynthesis are generally performed only in research centers.

TABLE III. Evaluation of suspected antibody deficiency

Screening tests
Quantitative immunoglobulins
Specific antibody levels
Circulating specific antibody levels to prior vaccines and blood group antigens (isohemagglutinins)
Pre/postimmunization antibody levels
Protein antigens
Carbohydrate antigens
IgG subclasses
Secondary tests
B-cell immunophenotyping
In vitro functional studies
Tests to exclude rare and secondary causes
Thoracic computed tomography to exclude thymoma (particularly useful if patient is >50 years old with low B-cell numbers)
Intracellular flow cytometry or genetic evaluation for BTK (XLA) or SAP/XIAP (XLP)
Genetic evaluation of NEMO to rule out anhydrotic ectodermal dysplasia with immune deficiency
Fecal α_1-antitrypsin, urinary protein, serum albumin, absolute lymphocyte count to exclude gastrointestinal or urinary protein loss or lymphatic loss
HIV testing to exclude AIDS
Complement function (CH50, AP50) to exclude complement deficiency
Karyotype to exclude immunodeficiency, centromeric instability, facial anomalies syndrome
Sweat chloride or genetic evaluation to exclude cystic fibrosis

BTK, Bruton tyrosine kinase; *XLA*, X-linked agammaglobulinemia; *SAP/XIAP*, SLAM-associated protein/X-linked inhibitor of apoptosis.

TABLE IV. Most common T-cell and combined immunodeficiencies and distinctive features

SCID: failure to thrive, chronic diarrhea, oral thrush, recurrent or severe bacterial, viral and/or fungal infections
CD40 and CD40 ligand deficiency: recurrent sinopulmonary and opportunistic infections with low IgG and IgA levels and variable IgM levels
Wiskott-Aldrich syndrome: easy bruising, eczema, recurrent otitis media, diarrhea, thrombocytopenia with small platelets
DiGeorge syndrome: hypoparathyroidism, cardiac malformations, dysmorphic features, variable T- and B-cell defects
Anhydrotic/hypohidrotic ectodermal dysplasia with immunodeficiency (NEMO or IκBα deficiency): recurrent mycobacterial or pyogenic infections, with or without skin, hair, and nail abnormalities; poor fever responses
XLP: hypogammaglobulinemia, persistent or fatal EBV infection
Chronic mucocutaneous candidiasis: recurrent oroesophageal and skin *Candida* species infection

EVALUATING SUSPECTED T-CELL OR COMBINED T- AND B-CELL IMMUNODEFICIENCY DISORDERS

When to suspect

Patients affected by severe combined immunodeficiency (SCID) or other primary conditions with markedly abnormal T-cell function usually manifest failure to thrive and recurrent infections with opportunistic pathogens, such as *Candida albicans* (thrush), *Pneumocystis jiroveci*, or cytomegalovirus very early in life (Table I).[9] Other common findings are chronic diarrhea, recurrent bacterial infections affecting multiple sites, and persistent infections despite adequate conventional treatment. SCID is a pediatric emergency because early diagnosis can dramatically improve the clinical outcome. Skin rashes are common, particularly with specific T-cell disorders, including Omenn and Wiskott-Aldrich syndromes.[10] Other severe cellular or combined defects present with varied clinical symptoms, as listed briefly in Table IV.

Laboratory evaluation

Careful analysis of the white blood cell count and differential is of utmost importance when evaluating patients suspected of cellular immunodeficiency disorders. The absolute lymphocyte count must be compared with age-matched control ranges for proper interpretation. Severe lymphopenia in an infant (<3,000/mm^3) is a critical finding that should prompt immediate immunologic evaluation if confirmed on a repeat test. The caveat in using low T-cell number during infancy as the screen to detect defects in T-cell development is that this would not identify patients with Omenn syndrome. In this disorder normal or increased T-cell numbers are typically found in the face of profound cellular immunodeficiency caused by an oligoclonal expansion of T cells.[10] In addition, circulating T cells might also be seen in the face of a severe cellular immune defect as a result of maternal T-cell engraftment. The maternal T cells will consist of primarily memory $CD45RO^+$ cells (compared with naive $CD45RA^+$ T cells found in a healthy infant) that do not provide host protection.[11] Finally, transfusion of nonirradiated blood products in the setting of a severe cellular immune defect will result in circulating donor T cells that can produce graft-versus-host disease, a potentially fatal process. This scenario emphasizes the need to irradiate any blood product used in an infant with a suspected T-cell deficiency.

HIV infection has to be ruled out in all patients with symptoms of cellular immunodeficiency, and this typically requires testing for the presence of virus (ie, HIV viral load assay) rather than serologic testing for anti-HIV antibody (Table V).

After T-cell screening tests, the next step would be a directed assessment of cellular immunity (Table V). This includes immunophenotyping of T cells by means of flow cytometry together with *in vitro* functional testing (eg, proliferation and cytokine production assays).[12] The immunophenotyping for a patient suspected of having SCID not only helps to establish the diagnosis, but it can also point to the potential underlying genetic defect (Table VI).[12] It is important to carefully review the percentage and absolute numbers for all lymphocyte

TABLE V. Evaluation of suspected T-cell and combined immunodeficiency

Screening tests
HIV testing
Lymphocyte immunophenotyping
Delayed-type hypersensitivity skin testing
Secondary tests
T-cell proliferation (mitogens, alloantigens, recall antigens)
T-cell cytokine production
Flow cytometric evaluation of surface or intracellular proteins, such as CD40 ligand (CD154 on activated T cells), IL-2 receptor γ chain (CD132), MHC class I and II, IL-7 receptor α chain (CD127), CD3 chains, WASP
Enzyme assays: adenosine deaminase, PNP
FISH for 22q11 deletion
TREC numbers
TCR repertoire analysis
Mutation analysis

WASP, Wiskott-Aldrich syndrome protein; *PNP*, purine nucleoside phosphorylase; *FISH*, Fluorescence *in situ* hybridization; *TREC*, T-cell receptor excision circle.

subsets, comparing them with age-appropriate reference ranges. Typically, defects in cytokine signaling molecules result in a $T^-B^+NK^-$ phenotype, whereas mutations in DNA-editing proteins required for T- and B-cell receptor expression are associated with a $T^-B^-NK^+$ phenotype; severe metabolic defects usually are toxic for all lymphocyte types, resulting in a $T^-B^-NK^-$ phenotype (Table VI).

Other useful tests in special circumstances include fluorescence *in situ* hybridization for the 22q11 microdeletion found in the majority of patients with DiGeorge syndrome and specific enzyme assays to evaluate for adenosine deaminase and purine nucleoside phosphorylase (PNP) deficiencies.[13] Evaluation for intracellular Wiskott-Aldrich syndrome protein expression by means of flow cytometry can be performed in selected centers to screen for possible Wiskott-Aldrich syndrome.[14] Direct evaluation of T-cell function, as assessed by the proliferative response to mitogens, recall antigens, and/or alloantigens, is an important part of evaluating cellular immunity. The same sort of culture conditions can also be used to evaluate for cytokine production using the culture supernatant (alternatively, one can evaluate cytoplasmic cytokine expression using flow cytometry).[15]

Quantification of T-cell receptor excision circles (TRECs) and evaluation of the T-cell repertoire can be used for additional evaluation of immune status. TRECs are formed during the normal editing of the T-cell receptor (TCR) genes during T-cell differentiation and maturation within the thymus and persist within the cell as extragenomic circular pieces of DNA. TREC copies are diluted over time as the T cells proliferate after antigen encounter. Therefore naive T cells that have recently emigrated from the thymus will present relatively high TREC levels compared with those of aged, antigen-experienced T cells.[16] TREC evaluation (also $CD4^+CD45RA^+CD31^+$ T cells by flow cytometry) can be used as a diagnostic confirmation of low thymic output that would be found in DiGeorge syndrome or to monitor immune reconstitution after bone marrow transplantation. More recently, the quantification of TRECs on blood derived from the Guthrie card obtained from infants after delivery has been initiated as a neonatal screening tool for SCID (and other significant T-cell defects) in both Wisconsin and Massachusetts.[17] The finding of low TREC levels in neonates should prompt immediate follow-up with immunophenotyping by means of flow cytometry. A recent report from Wisconsin suggests that this test has a very low rate of false-positive or inconclusive results (approximately 0.00009% and 0.0017%, respectively).[18]

TABLE VI. Immunophenotypic findings and associated genetic defects in patients with SCID

Phenotype	Pathway affected and genetic defect(s)
$T^-B^+NK^-$	Cytokine signaling: IL-2 receptor γ, JAK3
$T^-B^-NK^+$	DNA editing: RAG1/2, Artemis, ligase 4, Cernunnos
$T^-B^-NK^-$	Metabolic defects: adenosine deaminase, AK2
$T^-B^+NK^+$	Cytokine signaling: IL-7 receptor α chain
$CD8^+CD4^-B^+NK^+$	Positive selection/signaling: MHC class II, p56lck
$CD4^+CD8^-B^+NK^+$	Signaling: ZAP70

JAK3, Janus kinase 3; *RAG*, recombination-activating gene; *AK2*, adenylate kinase 2; *ZAP70*, zeta-chain associated protein kinase, 70 kD.

Analysis of the T-cell repertoire can be useful in specific clinical situations. The T-cell repertoire in circulating T cells from healthy subjects includes expression of the majority of the 24 TCR Vβ chain families, which can be promptly assessed by flow cytometry.[19] Alternatively, evaluation of TCR Vβ CDR3 region diversity can be performed by PCR and is commonly referred to as spectratyping. The PCR-amplified product from each of these Vβ families normally demonstrates a Gaussian distribution of variously sized PCR products, each differing by 3 nucleotides. In settings in which there is an oligoclonal T-cell population, such as is found in patients with Omenn and atypical DiGeorge syndromes, a very limited number of Vβ families will be represented, with each demonstrating a very distorted (non-Gaussian) distribution.[19]

EVALUATING SUSPECTED PHAGOCYTE DYSFUNCTION SYNDROMES

When to suspect

The clinical features of neutrophil dysfunction (including neutropenia) usually include recurrent bacterial and fungal infections of the skin, lymph nodes, lung, liver, bone, and, in some cases, the periodontal tissue (Table I).[20] The clinical pattern of infection often can help to discriminate the underlying problem. Common phagocyte defects and accompanying laboratory findings are presented in Table VII. Patients with neutropenia and those with leukocyte adhesion deficiency (LAD) tend to have recurrent cellulitis, periodontal disease, otitis media, pneumonia, and rectal or gastrointestinal infections with diminished

TABLE VII. Differential diagnosis of phagocyte defects and associated laboratory findings

Chronic granulomatous disease: defective oxidative burst by means of DHR assay or NBT
Leukocyte adhesion defects
LAD1: low/absent CD18 and CD11 expression by means of flow cytometry; persistent leukocytosis
LAD2: Bombay phenotype; absent CD15 (Sialyl-Lewis X) expression
LAD3: mutation analysis only
Chediak-Higashi syndrome: giant lysosomal inclusion bodies observed on morphologic review of granulocytes (with partial albinism)
Griscelli syndrome type 2: neutropenia without inclusion bodies (with partial albinism)
Severe congenital neutropenia: persistent neutropenia; maturation arrest on bone marrow studies
Cyclic neutropenia: intermittent neutropenia requiring serial measurements
X-linked neutropenia: altered WASP expression by means of flow or mutation analysis
G6PD and MPO deficiency: abnormal functional enzymatic assay
Hyper-IgE syndrome: IgE level >2,000 IU/mL; low T_H17 cell numbers
Other disorders to be considered
Drug-induced neutropenia; autoimmune/alloimmune neutropenia; hypersplenism; chronic mucocutaneous candidiasis; TCII deficiency; hyper-IgM syndrome, XLA; Schwachman-Bodian-Diamond syndrome; warts, hypogammaglobulinemia, immunodeficiency and myelokathexis (WHIM) syndrome

NBT, Nitroblue tetrazolium; *WASP*, Wiskott-Aldrich syndrome protein; *G6PD*, glucose-6-phosphate dehydrogenase; *MPO*, myeloperoxidase; *XLA*, X-linked agammaglobulinemia.

TABLE VIII. Evaluation of suspected phagocyte defects

Absolute neutrophil count and morphologic analysis: congenital neutropenia syndromes and Chediak-Higashi syndrome
Oxidative burst by means of DHR or NBT assays: chronic granulomatous disease; rarely complete G6PD or MPO deficiency
CD18 (also CD11a, CD11b, and CD11c) expression by means of flow cytometry: LAD1
CD15 expression by means of flow cytometry: LAD2
Bombay phenotype: LAD2
Anti-neutrophil antibodies: autoimmune neutropenia
Bone marrow biopsy: exclude defective myeloid production in neutropenia syndromes
Chemotaxis/phagocytosis assays: limited utility

NBT, Nitroblue tetrazolium; *G6PD*, glucose-6-phosphate dehydrogenase; *MPO*, myeloperoxidase.

inflammation and lack of pus formation.[20] Although LAD is accompanied by a persistent granulocytosis, there is effectively a tissue neutropenia caused by the underlying adhesion defect that prevents the directed movement of these phagocytic cells to sites of infection. Delayed umbilical cord separation is commonly seen in patients with LAD; however, LAD is very rare, and most infants whose cords persist for up to 1 month are actually healthy. In patients with cyclic neutropenia, there are short periods of fever, mouth ulcers, and infections recurring at intervals of 18 to 21 days in concert with the decreased neutrophil count. Other more common instances of neutropenia include drug-induced and immune-mediated neutropenia.

In contrast, patients with chronic granulomatous disease have significant problems with liver and bone abscesses, as well as pneumonias with selected organisms, including *Staphylococcus aureus*, *Serratia marcescens*, *Burkholderia cepacia*, and *Nocardia* and *Aspergillus* species.[21] Furthermore, they tend to have a lower frequency of *Escherichia coli* and streptococcal species infections compared with patients with neutropenia or LAD.

Finally, patients with hyper-IgE syndrome present with recurrent skin abscesses and cavitary pneumonias caused by *S aureus* and other pyogenic bacteria and demonstrate chronic mucocutaneous candidiasis.[22] In addition, they typically demonstrate specific nonimmunologic findings, such as coarse facial features, scoliosis, hyperextensible joints, increased risk for bone fractures, and delayed or failed shedding of primary dentition.[23]

Laboratory evaluation

Screening studies directed at the evaluation of neutrophil function should start with a leukocyte count, differential, and morphologic review (Table VIII). The diagnosis of cyclic neutropenia requires multiple absolute neutrophil counts 2 to 3 times a week for at least 4 to 6 weeks.[24] A diagnosis of severe congenital neutropenia (Kostmann syndrome) is made with neutrophil counts of less than 0.5×10^9/L on several occasions.[24] Bone marrow analysis is useful to exclude insufficient production because of neoplasia or other causes and to document other abnormalities, such as the maturation arrest typical of Kostmann syndrome.

If neutropenia and morphologic abnormalities are ruled out, the evaluation should be directed at assays that provide functional information about neutrophils. LAD workup involves flow cytometric assessment of the neutrophil adhesion molecules CD11 and CD18, the expression of which is absent or decreased on neutrophils (and other leukocytes) from patients with LAD1.[25] CD15 (Sialyl-Lewis X) expression is absent on neutrophils from patients with LAD2.[26]

The neutrophil oxidative burst pathway can be screened with either the nitroblue tetrazolium tests or a flow cytometric assay (dihydrorhodamine 123 [DHR]), the results of both of which are abnormal in patients with chronic granulomatous disease, but the latter is a more sensitive test.[27]

The diagnosis of autosomal dominant and sporadic hyper-IgE syndrome has been associated with heterozygous pathogenic mutations in the gene encoding signal transducer and activator of transcription (STAT) 3.[28,29] A consistent feature in this disorder is a very increased IgE level (>2,000 IU/mL), and more recently, low to absent IL-17–producing T cells (T_H17) have been demonstrated.[30]

Finally, evaluation of neutrophil-directed movement (chemotaxis) can be performed *in vivo* by using the Rebuck skin window technique, as well as *in vitro* with a Boyden chamber or a soft agar system. Abnormalities of chemotaxis have been observed after

use of certain pharmacologic agents, as well as in patients with LAD, Chediak-Higashi syndrome, Pelger-Huet anomaly, and juvenile periodontitis. However, chemotactic tests are difficult to perform, very hard to standardize, and available in only a limited number of laboratories.

EVALUATING SUSPECTED NATURAL KILLER AND CYTOTOXIC T-CELL DEFECTS

When to suspect

Deficiency in natural killer (NK) cell function has been described in a limited number of patients with recurrent herpes virus family infections. Another category of NK and cytotoxic T-lymphocyte defects results in an uncontrolled inflammatory response initiated in association with certain specific infections that produces multiple organ damage (hemophagocytic lymphohistiocytosis [HLH]). One of these disorders is XLP, which is usually asymptomatic until the patient has an EBV infection and then leads to an uncontrolled lymphoproliferative disorder that is often fatal without aggressive treatment.[31] Importantly, approximately 30% of patients with XLP present with hypogammaglobulinemia without other symptoms. Bone marrow transplantation is the only long-term cure for XLP.[31]

The clinical manifestations of familial HLH are rather nonspecific, requiring a high suspicion index for early diagnosis.[32] They include persistent fever, hepatosplenomegaly, neurological symptoms (ataxia and seizures), lymphadenopathy, and skin rashes. Diagnosis mandates an immediate therapeutic response and prompt referral for bone marrow transplantation because this is currently the only curative approach. Disorders caused by defective intracellular vesicle trafficking, such as Chediak-Higashi syndrome and Griscelli syndrome type 2, also commonly manifest with a secondary lymphohistiocytic syndrome.[32]

Laboratory evaluation

Testing of NK cell function includes immunophenotyping NK cells by means of flow cytometry and assaying cytotoxicity with standard *in vitro* assays. Patients with XLP1 will demonstrate absent invariant-chain NK T cells in peripheral blood, as measured by $CD3^+V\alpha24^+V\beta11^+$ staining.[31] Additionally, intracellular flow cytometry can be used to evaluate for expression of SAP (SLAM-associated protein) and XIAP (X-linked inhibitor of apoptosis), the proteins defective in XLP1 and XLP2, respectively.[33,34] Absent protein would indicate disease, whereas normal expression could be the result of an abnormal protein that is not distinguished from the normal protein by means of antibody staining. Therefore this screening test would require further investigation directed at cell function when the protein is detected in a patient suspected of having XLP. HLH is commonly associated with cytopenias, including anemia and thrombocytopenia; increased liver function test results; hypofibrinogenemia; and hypertriglyceridemia.[32] High ferritin and circulating soluble CD25 levels are also typical and represent laboratory findings used to establish the diagnosis of HLH.[32] Low intracellular perforin expression, as determined by flow cytometry, can be used to diagnose HLH2, and decreased surface expression of CD107a (LAMP1, lysosomal-associated membrane protein 1) on NK cells after activation can predict the presence of mutations in MUNC13-4 and syntaxin 11.[35,36]

EVALUATING SUSPECTED DEFECTS INVOLVING THE ADAPTIVE-INNATE IMMUNITY INTERFACE

IL-12/23–IFN-γ pathways

An emerging concept in the field of PIDs is that monogenic disorders can cause recurrent severe infections involving 1 or a very restricted range of pathogens.[37] Recently, patients with severe invasive infections caused by low virulence or environmental *Mycobacteria* and *Salmonella* species have been found to harbor defects in genes encoding different components of the IL-12/23–IFN-γ pathway: the IFN-γ receptor 1 gene *(IFNGR1)*, the IFN-γ receptor 2 gene *(IFNGR2)*, the IL-12 receptor β1 gene *(IL12RB1)*, *IL12B*, and *STAT1*.[38] The 2 most prevalent genetic defects among this group involve *IL12RB1* and *IFNGR1*, typically resulting in absent cell-surface protein expression.[39] This can be readily assessed by using flow cytometry with monoclonal reagents specific for these 2 proteins.[25] In addition, there is an autosomal dominant defect affecting *IFNGR1* that results in overexpression of the protein, and this also can be detected with flow cytometry.[40] Screening for other defects in IFN-γ signaling (abnormalities in *IFNGR2* or *STAT1*) can be done by evaluating monocyte STAT1 phosphorylation (by means of flow cytometry or Western blotting) *ex vivo* in response to IFN-γ.[41] Defects in IL-12 production can be tested by evaluating IL-12 production in response to *ex vivo* stimulation of mononuclear cells with LPS and IFN-γ.

Toll-like receptor and NF-κB signaling defects

Recently, recurrent infections involving *S pneumoniae* and *Staphylococcus* species have been associated with defects involving molecules of the Toll-like receptor (TLR) pathway, including IL-1 receptor–associated kinase 4 (IRAK4), MYD88 (myeloid differentiation primary response gene 88), and NEMO.[42-44] One of the distinctive features of patients with IRAK4 and MYD88 mutations is the markedly diminished inflammatory response to infection with little or no fever and acute-phase reactants observed.[45] NEMO deficiency is a more complex X-linked recessive disorder with a wide-ranging clinical phenotype and varied degree of immunologic abnormalities.[5] Finally, susceptibility to herpes simplex encephalitis has been linked to mutations in the genes encoding the receptor, TLR3, and an accessory protein of the TLR pathway, unc-93 homolog (UNC-93B).[46,47] Additional defects in TLR function associated with specific clinical phenotypes are likely to be identified and represent an evolving field in clinical immunology. Currently, the evaluation of TLR function is confined to a limited number of centers that usually screen response by stimulating mononuclear cells with various TLR-specific ligands and measuring cytokine production. This can then be followed by direct sequencing of the suspected mutant gene or genes involved in the specific TLR signaling process. Recently, von Bernuth et al[48] described a simplified assay for the screening of TLR function that is reported to detect functional defects in the signaling process by using whole blood samples. This assay involves stimulation of leukocytes with a series of specific TLR ligands and then evaluating for CD62L shedding from granulocytes by using flow cytometry. In cells with intact TLR signaling pathways, CD62L is promptly shed from the cell surface in contrast to the failure of CD62L shedding in cells from patients with IRAK4 or UNC-93B deficiency. One caveat is that the sample has to be analyzed shortly after obtaining the blood sample to prevent interpretation problems resulting from spontaneous CD62L shedding.

TABLE IX. Main clinical and laboratory findings of immune dysregulation syndromes and causative genes

Disorder	Distinctive clinical findings	Key laboratory findings	Gene(s) involved
ALPS	Lymphadenopathy, splenomegaly, autoimmune hemolytic anemia and/or thrombocytopenia, high risk for lymphomas	↑ $CD3^+\alpha\beta$-TCR-$\alpha\beta^+CD4^-CD8^-$ cells, hypergammaglobulinemia, Coomb positive, ↑ plasma IL-10 levels, ↑ serum vitamin B12 levels, ↑ soluble Fas ligand levels	*FAS*, *FASL*, *CASP8*, *CASP10*, *NRAS*
IPEX	Early-life enteritis, dermatitis, autoimmune endocrinopathy (usually type 1 diabetes)	↑ IgE levels, diminished $FoxP3^+$ CD4 T-cell subpopulation	*FOXP3*
APECED	Adrenal insufficiency, hypothyroidism, chronic mucocutaneous candidiasis	Organ-specific autoantibodies	*AIRE*

FASL, Fas ligand; *CASP8*, caspase 8; *CASP10*, caspase-10, *NRAS*, neuroblastoma *RAS* viral oncogene homolog; *FOXP3*, forkhead box protein 3; *AIRE*, autoimmune regulator.

The identification of this new class of defects has also opened up potentially new therapeutic approaches, including the use of IFN-γ to augment antibiotics in selected patients with recurrent mycobacterial disease. In the case of herpes simplex encephalitis, the findings that patients with UNC-93B and TLR-3 defects have diminished virally induced type 1 interferon production suggests that supplementation of conventional antiviral therapy with IFN-α could be beneficial in terms of decreasing morbidity, but this study has yet to be undertaken.[49]

EVALUATING SUSPECTED COMPLEMENT DISORDERS

When to suspect

The clinical setting in which complement defects should be suspected depends on the site of the defect. Abnormalities in the early components of the classical complement pathway (C1, C4, and C2) typically manifest as systemic lupus erythematosus–like autoimmunity, but recurrent sinopulmonary infections are also seen, especially in C2 deficiency.[50] Defects in C3 produce a clinical phenotype that is indistinguishable from an antibody defect, although this complement deficiency is markedly less frequent than humoral immunodeficiencies.[51] Defects in the late components of complement producing defects in the generation of the membrane attack complex (C5-C9) present with increased susceptibility to infections with *Neisseria* species that might not manifest until adolescence or young adulthood.[51] Clinically, these patients manifest neisserial meningitis, sepsis, or gonococcal arthritis. Alternative complement pathway defects, including properdin, factor B and factor D deficiencies also present with severe neisserial and other bacterial infections. Factor H deficiency is associated with atypical (not associated with diarrhea) hemolytic uremic syndrome or glomerulonephritis and also with secondary C3 deficiency that can result in recurrent pyogenic infections.[51] Finally, C1 esterase inhibitor deficiency causes hereditary angioedema, whereas DAF (decay-accelerating factor) and CD59 defects are seen in patients with paroxysmal nocturnal hemoglobinuria.[51]

Laboratory evaluation

The best screening test for defects in the classical complement pathway is the total hemolytic complement activity (CH50) assay, whereas the AH50 assay screens for defects in the alternative complement pathway. Assuming correct handling of the serum sample (complement components are very labile), a classical complement component deficiency will result in virtual absence of hemolysis on CH50 testing in contrast to the markedly decreased but not absent results seen in diseases like systemic lupus erythematosus. A decreased AH50 test result suggests a deficiency in factor B, factor D, or properdin. A decrease in both CH50 and AH50 test results suggests deficiency in a shared complement component (from C3 to C9).

Selected component immunoassays are available in larger laboratories, whereas specific component functional testing is typically only available in a very limited number of specialized complement laboratories.

EVALUATING SUSPECTED IMMUNE DYSREGULATION DISORDERS

When to suspect

Under this category are included monogenic autoimmune disorders, such as the autoimmune lymphoproliferative syndrome (ALPS); the immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX); and the autoimmune, polyendocrinopathy, candidiasis, ectodermal dystrophy syndrome (APECED; Table IX). Patients with ALPS present early in life with persistent nonmalignant lymphadenopathy and splenomegaly commonly accompanied by immune thrombocytopenia, hemolytic anemia, or both.[52] Organ-specific or vasculitic-type autoimmunity is rarely seen in patients with ALPS. IPEX is an immunologic emergency and typically presents in the neonatal period with severe watery or bloody diarrhea, skin eczema, and type 1 diabetes.[53] An immediate diagnosis is mandatory because these children require aggressive immunosuppression to control the acute symptoms, and bone marrow transplantation is currently the only curative therapy that should be undertaken before islet cells are destroyed, if at all possible. Finally, APECED is characterized by endocrine organ–directed autoimmunity (adrenal insufficiency and hypothyroidism) and chronic mucocutaneous candidiasis.[54] Patients might also have type 1 diabetes, gonadal failure, pernicious anemia, autoimmune hepatitis, and cutaneous manifestations. This is usually not a life-threatening condition, and immunosuppression is usually not required, with specific therapy directed at the endocrine abnormalities.

Laboratory evaluation

The diagnosis of ALPS currently requires the presence of compatible clinical symptoms and the presence of a characteristic T-cell population on immunophenotyping that expresses CD3 and

αβ-TCR but does not express CD4 or CD8 markers, which are referred to as double-negative T cells (Table IX). Determination of this T-cell subpopulation requires the use of antibodies to αβ-TCR because most double-negative T cells in normal samples are $\gamma\delta$-TCR$^+$ and are not relevant for establishing a diagnosis of ALPS. Normal ranges for αβ double-negative T cells should be established for each laboratory. At the National Institutes of Health, more than 1% of the total lymphocyte population is considered abnormal in adults. Other supporting features include hypergammaglobulinemia and increased plasma IL-10, vitamin B12, and soluble Fas ligand levels (J.B.O. and T.A.F., unpublished observations).[55] In addition, for a diagnosis of certainty, one must demonstrate defective lymphocyte apoptosis *in vitro* or the presence of a mutation on *FAS*, *FASL* (FAS ligand), *CASP8* (caspase-8), *CASP10* (caspase-10), or *NRAS* (neuroblastoma RAS viral oncogene homolog).[56-61]

Screening for IPEX is based on demonstrating absent or diminished population of forkhead box protein 3 (Foxp3)–expressing CD4 T cells in the peripheral blood, as assessed by intracellular flow cytometry. Another common laboratory finding is a marked increase in IgE levels. The gold standard for diagnosis is the demonstration of mutations on the *FOXP3* gene. However, in approximately 50% of patients with clinical findings compatible with IPEX, no mutation is demonstrated (Troy Torgerson, personal communication). Diagnosis of APECED in the setting of a clinically consistent phenotype currently requires sequencing of the *AIRE* (autoimmune regulator) gene.

CONCLUSION

Laboratory testing serves as the critical approach necessary for evaluating immune function in the setting of a patient with a history of recurrent infections, unusual infections, or both. The appropriate and directed use of immune function testing provides not only critical diagnostic information but also directs decisions regarding the most appropriate therapy. The latter is crucial to limit disease-associated morbidity. The use of the laboratory in evaluating the immune system should not follow a shotgun approach but rather should be a focused evaluation using specific testing in an orderly process based on the clinical history.

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Allergen immunotherapy

Anthony J. Frew, MD, FRCP *Brighton, United Kingdom*

Specific immunotherapy (SIT) involves the administration of allergen extracts to achieve clinical tolerance of those allergens that cause symptoms in patients with allergic conditions. Immunotherapy is effective in patients with mild forms of allergic disease and also in those who do not respond well to standard drug therapy. Most SIT is given by means of injection, but there is increasing interest in performing SIT through the sublingual route. SIT remains the treatment of choice for patients with systemic allergic reactions to wasp and bee stings and should be considered as an option in patients with allergic rhinitis, asthma, or both. SIT can modify the course of allergic disease by reducing the risk of new allergic sensitizations and inhibiting the development of clinical asthma in children treated for allergic rhinitis. The precise mechanisms responsible for the beneficial effects of SIT remain a matter of research and debate. An effect on regulatory T cells seems most probable and is associated with switching of allergen-specific B cells toward IgG4 production. Few direct comparisons of SIT and drug therapy have been made. Existing data suggest that the effects of SIT take longer to develop, but once established, SIT achieves long-lasting relief of allergic symptoms, whereas the benefits of drugs only last as long as they are continued. (J Allergy Clin Immunol 2010;125:S306-13.)

Key words: *Immunotherapy, immunomodulation, rhinitis, asthma, T cell, B cell, IgE, IgG, sublingual*

In allergen specific immunotherapy (SIT) allergen extracts are given to patients with allergic conditions to modify or abolish their symptoms. The process is specific in that SIT targets those allergens identified by the patient and physician as responsible for symptoms. Although the precise mechanisms involved remain uncertain, there is a substantial body of clinical evidence and practice to support the use of SIT. Before deciding to use SIT, the patient's condition needs to be carefully assessed, with particular regard to allergic triggers. In addition, because the course of treatment is lengthy and relatively expensive, there must also be an assessment of the risks and costs compared with those of symptomatic treatment with antihistamines and topical corticosteroids.

Abbreviations used
EPD: Enzyme-potentiated desensitization
SIT: Specific immunotherapy
SLIT: Sublingual immunotherapy
VIT: Venom immunotherapy

Immunotherapy was first developed at St Mary's Hospital London at the end of the 19th century,[1] and many of the basic principles described by Noon and Freeman remain valid today. However, over the years, SIT has evolved in different ways in different centers and in different countries, leading to varied treatment regimens and distinct philosophic approaches to the therapy. Indeed, much of the early literature on SIT is striking for its clinical empiricism and the lack of the type of objective evidence that would be required if this technique were to be introduced nowadays. Unfortunately, this has allowed critics to level charges of unscientific practice against allergists, even though the same point could be made about a whole range of medical practice. In recent years, clinical trials conducted according to modern principles have confirmed the effectiveness of SIT and have validated several of the alternative regimens that have been tried over the years. However, there is still a range of clinical practice and a variety of strongly held opinion about the best way to perform SIT. In particular, American allergists tend to treat for all sensitivities identified as clinically relevant on skin testing using mixtures of extracts prepared from bulk vials, whereas in Europe patients are normally only treated with a single allergen, which is supplied direct from the manufacturer. Mixed allergen extracts are available and used in some parts of Europe but only as custom mixes from manufacturers. Another difference in clinical practice is that allergen extracts used in the United States are prepared in the allergist's office, whereas those used in Europe are usually supplied by the manufacturer in their final form. European extracts are dialyzed to remove low-molecular-weight components and standardized according to their ability to elicit a wheal. In the United States extracts might not be dialyzed; although ragweed and cat extracts are standardized in terms of major allergen content, most extracts are standardized by their ability to elicit erythema rather than wheal. However, at the end of the day, the basic aims and principles of SIT are similar worldwide: the differences are in the details.

Typically, patients receive a course of injections, starting with a very low dose of allergen and building up gradually until a plateau or maintenance dose is achieved. Maintenance injections are then given at 4- to 6-week intervals for 3 to 5 years. The updosing phase is generally given as a series of weekly injections, but several alternative induction regimens have been tried, some giving several doses on each day and then waiting a week before giving a further series of injections (cluster protocol), whereas others give the whole series of incremental injections in a single day (rush protocol). The main drawback to the rush protocol is the risk of adverse reactions, which are much more common than in conventional or cluster protocols. On the other hand, full

From the Department of Respiratory Medicine, Brighton & Sussex Medical School, Brighton, United Kingdom.

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Reprint requests: Anthony J. Frew, MD, FRCP, Department of Respiratory Medicine, Brighton General Hospital, Brighton, United Kingdom BN2 3EW. E-mail: anthony.frew@bsuh.nhs.uk.

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protection against anaphylaxis induced by Hymenoptera stings can be attained in a few days compared with the 3 months required with the conventional regimen.

MECHANISMS OF IMMUNOTHERAPY

The primary reason for studying the mechanisms of SIT is to seek out the element or elements that are biologically important and hence devise new forms of immunotherapy that might improve efficacy, increase safety margins, shorten treatment courses, or achieve more durable results. Several mechanisms have been proposed to explain the beneficial effects of immunotherapy (Table I). Whether administered by means of injection or sublingually, SIT induces changes in T-cell and antibody responses. The challenge for clinical scientists has been to work out which of the observed changes drive the clinical benefit and which are just epiphenomena. Allergen-specific IgE levels increase temporarily during the initial phase of SIT but fall back to pretreatment levels during maintenance therapy.[2] The immediate wheal-and-flare response to skin testing usually reduces during the initial phases of SIT, but this effect is relatively small compared with the degree of clinical benefit. In contrast, the late-phase response to skin testing is virtually abolished after successful SIT. Similar patterns are observed for late-phase responses in the nose and airways.[3] SIT also induces allergen-specific IgG antibodies, particularly antibodies of the IgG4 subclass. At one time, it was believed that these antibodies might intercept the allergenic particles at the mucosal surface and "block" the allergic response. Current opinion is against this, partly because the increase in IgG levels follows rather than precedes the onset of clinical benefit and partly because many mast cells are on the mucosal surfaces and therefore meet allergen before antibodies can interpose themselves. Moreover, there is a poor correlation between the amount of allergen-specific IgG and clinical protection. In most studies the IgG level correlates better with the dose of allergen that has been given rather than with the degree of protection achieved. That said, there has been a recent resurgence of interest in a possible inhibitory role of specific IgG antibodies in grass pollen immunotherapy.[4] In particular, the time course of this effect raises the possibility of specific IgG antibodies interfering with IgE-dependent cytokine secretion from mast cells or facilitated antigen presentation to T cells.

SIT also induces changes in allergen-specific T-cell responses. In nasal and skin allergen challenge models, successful SIT is accompanied by a reduction in T-cell and eosinophil recruitment in response to allergen. In parallel, there is a shift in the balance of T_H1 and T_H2 cytokine expression in the allergen-challenged site. T_H2 cytokine expression is not affected, but there is an increased proportion of T cells expressing the T_H1 cytokines IL-2, IFN-γ, and IL-12.[5-7] After venom SIT, there is induction of allergen-specific $CD4^+$ regulatory T cells that express CD25, forkhead box protein 3, and IL-10, as well as a shift in T_H1/T_H2 balance.[8,9] Similar findings have also been reported after SIT with inhalant allergens.[10] IL-10 has a complex series of actions on the immune response, including downregulation of T cells and induction of allergen-specific IgG4 antibodies, which probably explains the IgG4 response to SIT. If the IL-10 effect on T cells is what matters, then the IgG4 response should perhaps be viewed as a surrogate marker of IL-10 induction rather than the beneficial mechanism of SIT.[11] Overall, it is clear that SIT has a modulatory effect on allergen-specific T cells, and it seems that this is why clinical and late-phase responses are attenuated without suppression of allergen-specific antibody levels or immediate allergic responses.

TABLE I. Possible mechanisms of immunotherapy

Reduction in specific IgE levels (long-term)
Induction of IgG (blocking) antibodies
Reduced recruitment of effector cells
Altered T-cell cytokine balance (shift to T_H1 from T_H2)
T-cell anergy
Induction of regulatory T cells

CLINICAL INDICATIONS

SIT for venom hypersensitivity

Anaphylaxis to Hymenoptera venom is relatively rare but can be fatal. Venom-specific IgE antibodies are found in 30% to 40% of all adults for a few months after a sting, but these usually disappear in a few months. This response is related to the total serum IgE level and the patient's IgE response to inhalant allergens. Some unlucky subjects react more vigorously with high concentrations of venom-specific antibodies, which can persist for many years without further exposure to stings. This group of patients are at risk of anaphylaxis to subsequent stings, and a small number die from anaphylaxis each year. Precise figures are hard to come by, but a figure of at least 40 deaths per year in the United States has been cited. Additional sting-related deaths may have occured in persons reported to have died of unknown cause.[12]

The purpose of venom immunotherapy (VIT) is 2-fold: to reduce the risk of fatality and to improve the patient's quality of life by allowing him or her to go out and work or play without worrying about the possibility of a serious allergic reaction. Given the relatively small number of fatalities, the main effect of VIT is on a person's quality of life. The decision to proceed with VIT is based on a careful assessment of the patient, as well as an understanding of the natural history of venom allergy.[13] Patients who have experienced systemic symptoms after a sting are at much greater risk of anaphylaxis on subsequent stings compared with patients who have only had large local reactions. The frequency of systemic reactions to stings in children and adults with a history of large local reactions is about 5% to 10%, whereas the risk in patients with a previous systemic reaction is between 30% and 70%. In general, children are less at risk of repeated systemic reactions, as are those with a history of milder reactions. With time, the risk of a systemic reaction decreases: by 10 years after a previous systemic response, the risk is about 15% compared with the general population's risk of 2% to 3%. Occupational and geographic factors that might affect the likelihood of future stings should also be considered. Bee stings are much more common in beekeepers, their families, and their neighbors. For most persons, wasp stings are sporadic, but they are an occupational hazard for bakers, greengrocers, gardeners, tree surgeons, for example. Other factors to consider are the potential risks of emergency treatment with epinephrine and the various medical contraindications to SIT (see below).

Desensitization with venom accelerates the rate at which the risk decreases and rapidly provides protection against field and laboratory stings. After completing VIT, there is a residual risk of systemic reactions of approximately 10%, but when reactions do occur to stings after VIT, they are typically mild. Patients who

receive VIT should be supplied with antiallergic medication for use in the event of a sting during or after therapy. Some allergists recommend providing injectable epinephrine during therapy, but this is not generally considered necessary once the patient has reached the maintenance dose of SIT.

SIT for allergic rhinitis

SIT is a useful treatment for allergic rhinitis, especially when the range of allergens responsible is narrow. As with all forms of SIT, it is important to select patients appropriately. The allergic basis of the rhinitis should be carefully assessed based on both history and skin or blood test results, and other causes of nasal symptoms should be excluded. Direct challenge tests to assess nasal sensitivity to allergen are not used in routine clinical practice but might be useful for assessing effectiveness in clinical trials. The most difficult group to assess are patients with persistent nonseasonal rhinitis, especially those who have small positive skin test responses to house dust mite or other perennial allergens. In this group it can be extremely difficult to determine whether the patient's symptoms are truly due to allergy or whether they have nonallergic rhinitis and just happen to be sensitized to an allergen that is not clinically relevant. This difficulty in determining clinical relevance contributes to the reported lower degree of efficacy in SIT trials with perennial allergens compared with SIT for seasonal allergies.

The effectiveness of SIT in patients with intermittent (seasonal) allergic rhinitis has been confirmed in many trials with grass, ragweed, and birch pollen extracts.[14] Importantly, SIT has been shown to be effective even in patients with severe seasonal rhinitis caused by grass pollen that is resistant to conventional drug therapy.[15] Importantly, this study showed that patients with multiple allergic sensitizations responded at least as well as those who were monosensitized to grass pollen.

The benefits of 1 year's treatment wear off quickly,[16] but there are good data showing that 3 years' therapy provides lasting benefit.[17] Less well-controlled data show that the effects of SIT can persist for many years after discontinuing therapy.[18] This contrasts with conventional drugs, the effects of which wear off very soon after discontinuing therapy. The benefits of SIT for perennial rhinitis are less than those for seasonal rhinitis. In part, this reflects the difficulty in determining the extent to which allergy is responsible for perennial symptoms. Sensitization to house dust mite is common and does not always cause symptoms. Conversely, there are other causes of perennial rhinitis, including vasomotor instability, infection, and aspirin sensitivity. Nevertheless, clinical trials have shown a definite benefit in appropriately selected subjects. Clearer evidence has been obtained in patients with rhinitis caused by pet allergy. Several studies have shown a marked improvement in tolerance of cat exposure after SIT, which was confirmed both on challenge tests and simulated natural exposure.[19]

As with any therapy, the risks and cost-effectiveness of SIT need to be assessed on a case-by-case basis. Current drug therapy for rhinitis can be very effective, but a significant minority of patients have suboptimal control of their symptoms.[20] Some patients with rhinitis experience nosebleeds from intranasal steroids or excessive drowsiness from their antihistamines; others find pharmacotherapy inconvenient or ineffective. Moreover, we are now more aware of the adverse effects of rhinitis on quality of life. SIT offers a useful option for these patients, as well as a logical approach to dealing with the underlying problem.

SIT for asthma

Immunotherapy has been widely used to treat allergic asthma, although the introduction of effective inhaled therapies has changed the general pattern of asthma care. Concern over adverse reactions, including a small number of fatalities, has led some countries (eg, the United Kingdom) to restrict the use of SIT for asthma treatment, although asthma remains a common indication for SIT in many parts of North America and continental Europe.[21,22]

Current drug therapies for asthma aim to suppress airways inflammation and relieve bronchospasm. None of these treatments are curative, and asthma recurs rapidly on ceasing treatment. Allergen avoidance helps in some patients with allergic asthma, but although extreme forms of allergen avoidance (eg, admission to the hospital and sending children to holiday homes at altitude) can improve asthma control, there is only limited evidence for benefit with the degree of allergen avoidance that can be achieved in suburban homes. There is thus the scope for improving asthma care and for identifying allergen-specific therapies. SIT offers the possibility of deviating the immune response away from the allergic pattern and toward a more protective or less damaging response. However, SIT remains controversial as a treatment for asthma because of the potential side effects.

The efficacy of SIT in adult asthma has been assessed in many trials over the last 65 years. The results of these studies have often been difficult to interpret, either because poor-quality allergen extracts were used or because of poor study design. Many trials were not placebo controlled; they were either open or single blind, and in most cases, only small numbers of patients were treated. A recently updated meta-analysis[22] identified 75 articles published between 1954 and 2001. Thirty-six of these were for mite allergy, 20 for pollen allergy, 10 for animal dander allergy, 2 for mold allergy, and 1 for latex allergy, and 6 used combinations of allergens. Concealment of allocation was clearly adequate in only 15 trials. A wide variety of different measurements were made, which makes it difficult to comment on the overall effectiveness of SIT. Symptom scores improved in the treated groups; it was necessary to treat 4 patients to prevent 1 from experiencing symptom exacerbation and to treat 5 patients to prevent 1 from needing an increase in medication use. SIT reduced the airways response to inhalation of specific allergen and also improved nonspecific bronchial reactivity.

Three double-blind, placebo-controlled studies have found that SIT has a beneficial effect in patients with grass pollen–induced asthma, as assessed by a reduction in asthma symptom and treatment scores. Active treatment led to a 60% to 75% reduction in symptom scores compared with those seen in placebo-treated patients. An important study of SIT for ragweed allergy found that patients who received active injections had an improvement in peak flow rates during the pollen season, as well as reduced hay fever symptoms and reduced sensitivity to laboratory challenge with ragweed pollen extracts.[23] In addition, the active group required much less antiasthma medication. However, the parallel economic analysis indicated that the cost savings in asthma drugs was less than the costs of SIT.

In asthmatic patients sensitive to cats, SIT reduces both the early asthmatic response to inhaled allergen and responses to simulated natural exposure in a "cat room." Interestingly, there was no protection against allergen-induced increases in

nonspecific bronchial hyperresponsiveness, despite the clear delay in onset of symptoms and an overall reduction in symptoms and peak flow recordings after exposure to cats. Others have found reductions in both specific and nonspecific bronchial reactivity after SIT for cat allergy (measured by using inhalation challenges with cat extract and histamine, respectively).[24]

The main drawback in using SIT to treat asthma is the risk of serious adverse reactions. The vast majority of fatal reactions to SIT have occurred in patients with asthma, and although asthma is not an absolute contraindication, it is clear that patients with unstable asthma should not be offered SIT, and caution should be exercised in anyone with an increased level of asthma symptoms or transiently reduced peak flow rates.

Comparison of SIT with other types of treatment for asthma

The majority of clinical trials of SIT for patients with asthma have compared SIT either with untreated historical control subjects or with a matched placebo-treated group. To date, the effectiveness of SIT in patients with asthma has rarely been compared with conventional management (avoidance measures and inhaled or oral antiasthma drugs). One recent study assessed SIT in asthmatic children receiving conventional drug therapy and found no additional benefit in patients who were already receiving optimal drug therapy.[25] There were some significant flaws in the design of this study, and further work of this type is urgently needed.

Effects on natural history of allergic disease

Children often start with a limited range of allergic sensitivities and progress over time to have IgE against a wider range of inhaled allergens. Treatment with SIT might limit this tendency to acquire new sensitizations,[26] although the clinical benefit of this preventive effect is not clear. A proportion of patients with allergic rhinitis develop asthma each year. This annual rate of progression has been estimated at 5% in college students,[27] but this is perhaps surprisingly an area of considerable ignorance. A number of long-term epidemiologic studies are now in progress under the auspices of the International Study of Asthma and Allergies in Childhood, and these should eventually shed light on the rate of progression at different ages and the extent of regional and international variation. It has been suggested that SIT might modify the natural history of asthma in children who are known to be atopic but have not yet developed asthma. Only limited data are available to support this proposition. In the key study a group of 205 children aged 6 to 14 years without previously diagnosed asthma were treated with SIT for birch or grass pollen allergy in an open randomized design. Three years after completing treatment, 45% of the untreated group had asthma, whereas only 26% of the treated group had asthma. These results have been sustained out to 7 years after completing therapy. Thus 4 children had to be treated to prevent 1 case of asthma, which makes this an extremely effective therapy.[28] SIT might also modify the progression of established asthma. An early open study with uncharacterized mixed allergen extracts supported this view, with about 70% of treated children losing their asthma after 4 years' therapy compared with about 19% of untreated control subjects, a result that was sustained up to the age of 16 years. The proportion of children whose asthma was severe at age 16 years was also much lower in the treated group.[29] By modern standards, this study was not well designed, and it needs repeating with modern SIT extracts in an up-to-date trial design.

In contrast, there is no current evidence that SIT influences the evolution of established asthma in adults. Studies that have investigated withdrawal of therapy have found rapid recurrence of asthma symptoms, although rhinitis symptoms seem to show much more sustained relief after SIT.[30]

Thus SIT is a valid but controversial treatment for asthma. Although it seems entirely logical to try to treat allergic disorders by specifically suppressing the immune response to the triggering agents, the critical issue is whether SIT in its present form is the best option for managing patients with asthma. To assess this properly would require comparisons of best current SIT versus best current drug therapy, with robust end points including symptoms, objective measures of lung function, evaluation of cost/benefit ratios, safety, and quality of life. *In vitro* and *in vivo* measures, such as skin test responses or allergen-specific IgG4 measurements, are not sufficiently specific or sensitive to serve as surrogates for clinical efficacy. To date, there have been relatively few well-controlled studies of SIT in asthmatic subjects, but there is increasing evidence that SIT is beneficial in patients with mite-induced and pollen-induced asthma. The clinical efficacy of SIT in adult asthmatic patients sensitive to cats or molds is less certain, and no comparative studies with conventional treatment have been performed. Further clinical trials are indicated, particularly in patients with mild-to-moderate childhood asthma and also in patients with atopic disease who have not yet had asthma but are at high risk of progression to asthma.

Safety of SIT

The most obvious risk of SIT is that of provoking a systemic allergic reaction. In the United Kingdom between 1957 and 1986, 26 fatal reactions caused by SIT were reported to the Committee on Safety of Medicines.[31] The indication for SIT was documented in 17 of the fatal cases, 16 of whom were in patients receiving SIT to treat their asthma. Similarly, in the American Academy of Allergy, Asthma & Immunology inquiry into SIT-associated deaths, asthma appeared to be the cause of death in most of the fatal cases.[32,33] In those cases in which asthma was not cited as a contributory factor, asthma status was not documented, whereas bronchospasm was a feature of the clinical course of the fatal anaphylactic reactions. The incidence of systemic reactions in patients receiving SIT for asthma varies between series and has been reported to range from 5% to 35%. The central issue in using safety as an end point is that we have to accept that all treatments carry risks. Where differential risks exist between therapies, a more risky therapy can only be justified if that therapy offers substantial additional benefit over the safer therapy. The science of assessing risk/benefit ratios is still in its infancy, and we have to recognize that even when faced with the same facts, different patients and agencies can come to widely varying risk assessments. However, where possible, we should take steps to minimize the risks.

Separately, there is some concern about the use of immunomodulatory treatments in patients with autoimmune disorders, immunodeficiency syndromes, or malignant disease. Although there is no hard evidence that SIT is actually harmful to these patients, some clinicians feel uncomfortable about manipulating the immune system in such patients, not least because of the risk that spontaneous and unrelated variations in the autoimmune

disorder or cancer might be blamed on SIT. However, provided the risks and benefits are weighed and discussed with the patient, SIT can be administered where the risk/benefit ratio is considered to be in favor of treatment. Other medical contraindications to SIT include the coexistence of significant cardiac disease that might be exacerbated by any adverse reactions to SIT. β-Blockers are also contraindicated in patients receiving SIT. Although they do not increase the risk of adverse reactions, they will prevent the patient from responding to the epinephrine that might be needed to treat adverse reactions to SIT. Where the indication for SIT is strong, alternatives to β-blockers should be used so that the SIT can be given safely. Some clinics advise avoiding angiotensin-converting enzyme inhibitors because they can accentuate angioedema (angiotensin receptor antagonists [sartans] do not share this property).

Alternative forms of immunotherapy

Alternative allergy practice covers 3 principal themes: the use of unconventional diagnostic tests to seek causative agents for diseases that everyone agrees are allergic in origin; the use of unconventional therapies to treat allergic disease; and the diagnosis and therapy of diseases that are not conventionally considered to involve allergic mechanisms. Alternative immunotherapy regimens fall into the second of these categories, but the other 2 areas fall outside the scope of this review.

Unconventional forms of immunotherapy include the use of topical immunotherapy, enzyme-potentiated desensitization (EPD), and homeopathic desensitization.

Topical immunotherapy. High-dose topical immunotherapy regimens were used in the first half of the 20th century but subsequently fell into disrepute. The last 20 years have seen a revival of interest in sublingual immunotherapy (SLIT). The precise mechanisms by which sublingual SIT works remain unclear. In mice locally administered allergen is taken up by mucosal dendritic cells and then presented to T cells together with IL-12, biasing the response toward a T_H1 profile and away from the pro-IgE T_H2 profile. It is less clear whether this mechanism can suppress established allergic responses. In contrast, the immunologic response to SLIT in human studies has been relatively modest. Some changes have been found in skin sensitivity, but most studies have not found any change in systemic parameters, such as specific IgE, specific IgG, or T-cell cytokine balance.

A body of evidence has accumulated from well-conducted clinical trials indicating that SLIT can be effective, with up to 30% to 40% reductions in symptom scores and rescue medication use in patients with seasonal allergic rhinitis.[34] Treatment regimens typically involve a rapid build-up phase followed by treatment given either daily or 3 times per week with rapidly dissolving tablets containing allergen extracts. Some preparations are supplied in liquid form, with a calibrated dropper. A recent meta-analysis of SLIT found 22 studies in which 979 patients received active therapy.[34] Although many of these studies were small and inconclusive, the combined results indicate that SLIT is indeed effective, with an estimated power of about two thirds that seen in comparable studies of injected SIT. Local side effects were common but well tolerated.

In the grass pollen tablet trials about half the patients experienced some local irritation with the first dose. This was minor and generally did not require a reduction in subsequent doses. About half of those with initial side effects had lost these by the eighth day of treatment; only 1 in 25 of all patients had continuing local side effects after 3 months treatment.[35] Systemic side effects were relatively rare, and none of the side effects were judged to be life-threatening. For perennial allergens, less trials data are available,[36] and only limited data are available in children, although the most recent studies have been encouraging.[37,38] Other forms of topical immunotherapy (oral and nasal) have limited efficacy but are associated with high levels of side effects.

SLIT is now being used routinely in some parts of Europe (especially Italy and France), but often the doses and regimens being prescribed are different from those used in the clinical trials. As performed in the published trials, SLIT involves giving 20 to 400 times the total dose that would be given in a course of injected SIT. There is no evidence that giving smaller doses sublingually has any clinical effect. Overall, SLIT is likely to widen the scope of SIT and bring in additional prescribers. As with all forms of immunotherapy, patient selection will be the key to ensuring that therapy is targeted to those who are likely to benefit from it.

Some areas of uncertainty remain. For example, the optimum duration and durability of therapy have not been defined. Recent clinical trials have confirmed that the benefits of SLIT persist for the first year after discontinuing treatment, but if they do persist, for how long do they persist? Based on experience with injected SIT, manufacturers recommend that SLIT should be continued for 3 years, although most clinical trials were short-term (6-12 months). For seasonal allergens, most open-label use in clinical practice has been intermittent, starting 2 to 3 months before the season and stopping at the end of the season. However, the manufacturer of the only licensed product recommends starting 4 months before the first grass pollen season and continuing throughout the year for 3 years. This has major implications for direct costs and cost-effectiveness,[39] and some supporting data would be welcome.

The relative efficacy of SLIT and injected SIT has not been determined. The only published comparative studies were far too small to produce meaningful results.[40,41] Based on the effect size seen in the meta-analyses,[14,34] it seems likely that SLIT has between 60% and 100% of the efficacy of injected SIT, although it is difficult to make a true comparison.

EPD. In EPD very small doses of allergens are given together with the enzyme β-glucuronidase. The allergen doses are approximately 0.1% of the doses used in conventional SIT, and side effects are apparently not encountered. The theory behind EPD is that the β-glucuronidase enables the allergen to gain access to the immune system more efficiently than is possible with conventional SIT. No convincing evidence has been published to support the efficacy of EPD.

Homeopathic desensitization. A detailed discussion of the principles underlying homeopathy lies outside the scope of this chapter. However, homeopathy espouses the concept that diseases can be treated with very small doses of substances that cause similar symptoms. Some homeopathic remedies are mimics of the disorder, whereas others use the actual material that triggers the disorder. Thus homeopathic remedies for hay fever bear some superficial similarity to SIT. A systematic review of homeopathy has concluded that homeopathy did appear to offer some benefit in patients with hay fever and cited trials of homeopathy in hay fever as an example of good practice in homeopathic research.[42] However, a more recent, carefully controlled study of homoeopathy for house dust mite allergy found no evidence of any benefit in patients with asthma.[43]

TABLE II. Possible new technologies for immunotherapy

Recombinant allergens
Hypoallergenic allergens (bioengineered recombinant molecules)
T-cell peptide vaccines
T_H1 immunostimulants (eg, mycobacteria and CpG)
Allergen-immunostimulant complexes
Anti-IgE

FUTURE DIRECTIONS

There is scope to improve conventional SIT (Table II). Possible avenues include the use of recombinant allergens, which would improve standardization of allergen vaccines and might allow fine tuning of vaccines for patients with unusual patterns of reactivity. Most allergic patients react to the same components of an allergen extract, the so-called major allergens, which are defined as those allergens recognized by more than 50% of sera from a pool of patients with clinically significant allergy to the material in question. However, not all patients recognize all major allergens, and some patients only recognize allergens that are not recognized by the majority of allergic patient sera. This latter group might not respond to standard extracts but might be better treated with a combination of allergens to which they are sensitive. Now that recombinant allergens for SIT are available, the range of sensitivities can be better characterized, and this might lead to patient-tailored vaccine products. Thus far, clinical trials have confirmed the efficacy of recombinant allergen cocktails but have not yet shown superiority to conventional vaccines.[44]

Novel forms of allergenic molecules can be created; for example, a recombinant trimer consisting of 3 covalently linked copies of the major birch pollen allergen Bet v 1 has been made. This trimer is much less allergenic, even though it contains the same B-cell and T-cell epitopes as the native molecule and induces T_H1 cytokine release and IgG antibodies analogous to the antibody response to standard SIT.[45] Folding variants and other modifications of the physical structure might also improve the safety of SIT.[46]

Because the epitopes recognized by IgE molecules are usually 3-dimensional, whereas T-cell epitopes are short linear peptide fragments of the antigen, it should be possible to use peptide fragments of allergens to modulate T cells without risking anaphylaxis. Two distinct approaches have been tested. Either large doses of natural sequence peptides are given, deceiving the T cell into high-dose tolerance,[47] or else an altered peptide ligand can be given. Both approaches require consideration of the MHC type of the subject undergoing treatment. By means of sequential alteration of *Dermatophagoides pteronyssinus* peptides, it is possible to suppress proliferation of T-cell clones recognizing native *D pteronyssinus* peptides, as well as suppressing their expression of CD40 ligand and their production of IL-4, IL-5, and IFN-γ. These anergic T cells do not provide help for B cells in class switching to IgE, and importantly, this anergy cannot be reversed by providing exogenous IL-4.[48]

In an animal model intranasal application of genetically produced hypoallergenic fragments of Bet v 1 produced mucosal tolerance, with significant reduction of IgE and IgG1 antibody responses, as well as reduced cytokine production *in vitro* (IL-5, IFN-γ, and IL-10). These reduced immunologic responses were accompanied by inhibition of the cutaneous and airway responses that were seen with the complete Bet v 1 allergen. The mechanisms of immunosuppression seemed to be different for the allergen fragments and the whole molecule in that tolerance induced with the whole Bet v 1 molecule was transferable with spleen cells, whereas that induced by the fragments was not.[49]

From epidemiologic and experimental studies, we know that vaccination with mycobacteria has antiallergic properties. In Japan early vaccination with BCG was associated with a substantial reduction in the risk of allergy,[50] although similar associations were not evident in Sweden.[51] In an animal model it has been shown that administration of BCG before or during sensitization to ovalbumin reduces the degree of airway eosinophilia that follows subsequent challenge with ovalbumin. This effect is not mediated through any direct effect on IgE production or blood eosinophil numbers but is mediated through IFN-γ and can be reversed by exogenous IL-5.[52]

Two new approaches using DNA vaccines are also undergoing serious consideration. The first of these is a general approach, using CpG oligodeoxynucleotides that mimic bacterial DNA and stimulate T_H1-type cytokine responses. In a murine model of asthma, preadministration of CpG oligodeoxynucleotides prevented both airways eosinophilia and bronchial hyperresponsiveness.[53] Moreover, these effects were sustained for at least 6 weeks after CpG oligodeoxynucleotide administration.[54] An alternative approach is to couple CpG oligodeoxynucleotides to the allergenic protein, which enhances immunogenicity in terms of eliciting a T_H1-type response to the allergen but reduces its allergenicity[55] and stimulates T_H1 cytokine expression in cultured human PBMCs.[56] Initial clinical trials confirmed that the hybrid vaccine elicits a T_H1-pattern response,[57] but subsequent trials have been inconclusive. A contrasting approach is to use allergen-specific naked DNA sequences as vaccines. This technology is still in its infancy, but preliminary data suggest that administering naked DNA leads to production of allergens from within the airways epithelial cells.[58,59] Because of the different handling pathways for endogenous and exogenous allergens, it seems that the endogenously produced allergen elicits a T_H1-type response, and if this can be reproduced in allergic human subjects, it is hoped that this might overcome the existing T_H2-pattern response and eliminate the allergy. However, the potential for generating a powerful T_H1-type response to ubiquitous agents means that this approach will require careful evaluation in animal models before it can be pursued in human subjects.

CONCLUSIONS

SIT has been used for more than a century and is clinically effective in patients with rhinitis or asthma whose symptoms are clearly driven by allergic triggers. Perhaps surprisingly, we are still unsure exactly how SIT works, but we do know that SIT induces regulatory T cells that dampen the response to allergen exposure in sensitized subjects. When used in appropriately selected patients, SIT is effective and safe, but care is needed to recognize and treat adverse reactions. As well as careful patient selection, appropriate training of allergists and SIT clinic support staff is essential. Future directions in SIT will include the development of better standardized vaccines and the use of recombinant allergens, both of which should improve the safety profile of SIT. In parallel, the development of allergen-independent immunomodulatory therapies might allow more general approaches to be developed, which would be particularly advantageous for those patients who are sensitized to multiple allergens.

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Immunomodulator therapy: Monoclonal antibodies, fusion proteins, cytokines, and immunoglobulins

Susan J. Lee, MD,[a,b] Javier Chinen, MD, PhD,[c] and Arthur Kavanaugh, MD[a] *San Diego, Calif, and Houston, Tex*

The immune system consists of a diverse array of immunocompetent cells and inflammatory mediators that exist in complex networks. These components interact through cascades and feedback circuits, maintaining physiologic inflammation (eg, tissue repair) and immunosurveillance. In various autoimmune and allergic diseases, a foreign antigen or autoantigen might upset this fine balance, leading to dysregulated immunity, persistent inflammation, and ultimately pathologic sequelae. In recent years, there has been tremendous progress delineating the specific components of the immune system that contribute to various aspects of normal immunity and specific disease states. With this greater understanding of pathogenesis coupled with advances in biotechnology, many immunomodulatory agents commonly called "biologic agents" have been introduced into the clinic for the treatment of various conditions, including immune globulins and cytokines. The 2 most common classes of approved biologic agents are mAbs and fusion proteins with exquisite specificity. These agents have the potential both to optimize outcomes through more thorough modulation of specific parts of the dysregulated immune response and to minimize toxicity compared with less specific methods of immunosuppression. (J Allergy Clin Immunol 2010;125:S314-23.)

Key words: *Monoclonal antibodies, fusion proteins, immunoglobulins, cytokines, autoimmunity*

Abbreviations used

AS: Ankylosing spondylitis
CHF: Congestive heart failure
CTLA-4: Cytotoxic T lymphocyte–associated antigen 4
DMARD: Disease-modifying antirheumatic drug
FDA: US Food and Drug Administration
ICAM: Intercellular adhesion molecule
IL-1Ra: IL-1 receptor antagonist
IVIG: Intravenous immunoglobulin
LFA: Lymphocyte function–associated antigen
MS: Multiple sclerosis
PML: Progressive multifocal leukoencephalopathy
PsA: Psoriatic arthritis
RA: Rheumatoid arthritis
SCIG: Subcutaneous immunoglobulin
SLE: Systemic lupus erythematosus

Biologic agents can work through several mechanisms. The simplest would be inhibition of the function of a target molecule by binding to it, thereby preventing ligation with its counter-receptor and downstream effects. Potential targets include (1) lineage- or activation status–specific molecules on B cells, T cells, and other immunocompetent cells; (2) soluble inflammatory mediators, such as cytokines, chemokines, complement proteins, enzymes, and immunoglobulin molecules; and (3) surface receptors for these mediators. Biologic agents can alter cell populations by engaging effector functions, including the complement cascade and antibody-dependent cellular cytotoxicity; of note, many mAbs and fusion proteins possess functional IgG Fc pieces. Cell depletion can also be induced by apoptosis subsequent to ligation of appropriate targets. Small-molecular-weight immunomodulators, such as glucocorticoids, are reviewed in Chapter 16.

MONOCLONAL ANTIBODIES

Monoclonal antibodies to human targets can be generated either in other species, such as mice, or through recombinant engineering (Fig 1). With chimeric mAbs, the variable region of a murine mAb is fused to the Fc piece of a human IgG molecule. The resulting construct is approximately one quarter murine. For humanized mAbs, only the complementarity determining regions from the original murine mAb are retained, resulting in a construct that is approximately 95% human. There are a number of approaches to create human mAbs to human targets, including immunizing human/severe combined immunodeficient murine chimeras, using EBV-transformed human B cells, and repertoire cloning, in which target antigen is used to capture human complementarity determining regions generated from vast human cDNA libraries, with the mAb then generated from there. Proteins such as mAbs can have residues of polyethylene glycol added. This process, called pegylation, enhances the half-life of the native protein by reducing its renal and cellular clearance after administration. Although even fully human proteins can be immunogenic, in general, the more human a construct, the less immunogenic. Pegylation might further reduce antigenicity and immunogenicity of the native protein. Immunogenicity can develop to molecules with amino acid sequences identical to human sequences related to factors such as differences in patterns of glycosylation. In addition, immunogenicity to mAbs can be anti-idiotypic. Other factors affecting immunogenicity include route of administration (intravenous vs subcutaneous), treatment paradigm (continuous vs intermittent), and concurrent use of immunosuppressive therapy.

Standard nomenclature for mAbs identifies their source with the last 4 or 5 letters: -omab, murine; -ximab, chimeric; -zumab,

From [a]the Division of Rheumatology, Allergy, and Immunology, the University of California, San Diego; [b]the Veterans Affairs San Diego Healthcare System, San Diego; and [c]the Allergy and Immunology Section, Department of Pediatrics, Baylor College of Medicine, Houston.

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Reprint requests: Arthur Kavanaugh, MD, the University of California, San Diego, 9500 Gilman Dr, Mail Code 0943, La Jolla, CA 92093-0943. E-mail: akavanaugh@ucsd.edu.

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humanized; and -umab, human (Fig 1). The middle part of the name reflects the disease indication for which the mAb was initially intended: -lim- for immune and inflammatory diseases, -cir- for cardiovascular disorders, and -tu- for tumors or neoplastic conditions. The first 3 or 4 letters can be chosen by the sponsor/developer. A number of mAbs have been approved for human use; this chapter will focus on several key mAbs used in the treatment of autoimmune conditions.

FUSION RECEPTORS

Fusion proteins are typically composed of the extracellular domains of native transmembrane proteins, such as cell-surface receptors, linked to another molecule. In most cases the linker that has been used has been the Fc portion of human immunoglobulin, which enhances the pharmacokinetic properties of the construct. The Fc portion of the fusion receptor can be engineered to be functional or not. As their primary mechanism of action, fusion receptors competitively inhibit the binding of a ligand to its specific counterreceptor and thereby prevent downstream effects.

AGENTS THAT INHIBIT PROINFLAMMATORY CYTOKINES

In patients with autoimmune diseases, imbalances in the cytokine cascade can help the initiation and propagation of the immune driven inflammation. In several inflammatory arthritides, including rheumatoid arthritis (RA), psoriatic arthritis (PsA), and ankylosing spondylitis (AS), the proinflammatory cytokine TNF-α has been shown to play a central role in inflammatory reactions and has proved to be an especially attractive target for biologic agents. Among its sundry activities, TNF-α activates various cell types, promotes accumulation of immunocompetent cells at sites of inflammation by means of activation of the vascular endothelium and upregulation of adhesion molecules, and stimulates synthesis of other proinflammatory cytokines (eg, IL-1, IL-6, and GM-CSF), chemokines (eg, IL-8), and other mediators. IL-1 also stimulates production of other proinflammatory cytokines, angiogenic factors, and endothelial adhesion molecules. Both TNF-α and IL-1 mediate bone and cartilage destruction through activation of osteoclasts (eg, receptor activator for nuclear factor κB ligand and macrophage colony-stimulating factor) and macrophages to release destructive mediators (eg matrix metalloproteinases, collagenase, and prostaglandins). IL-6 is a regulatory cytokine involved in T- and B-cell activation, osteoclast differentiation/activation, and other activities relevant to the pathogenesis of RA. Other immunomodulatory cytokines considered of significance in the treatment of infectious diseases and malignancies include interferon type I (α and β), IFN-γ, IL-2, and IL-7.

TNF inhibitors: Therapeutic uses

There are 5 currently available TNF inhibitors: infliximab, a chimeric anti–TNF-α mAb initially approved in 1998; etanercept, a recombinant soluble p75 TNF receptor (CD120b)–IgG Fc fusion protein initially approved in 1998; adalimumab, a human anti–TNF-α mAb initially approved in 2002; certolizumab pegol, a pegylated Fab′ fragment of a human anti–TNF-α antibody initially approved in 2008; and golimumab, a human anti–TNF-α mAb initially approved in 2009 (Table I). Although not all 5 TNF inhibitors are approved for the following conditions, TNF inhibitors are most commonly used for the treatment of RA, PsA, AS, Crohn disease, juvenile idiopathic arthritis, and psoriasis.

All 5 TNF inhibitors have been shown to substantially improve the signs and symptoms of disease, functional status, and quality of life and slow radiographic progression in patients with established RA.[1-8] Several studies have demonstrated an even greater clinical and radiographic response and the probability of disease remission among patients with early RA.[9-11] Interestingly, the inhibition of radiographic progression of disease seemed to be dissociated from clinical efficacy, as measured with the typically used composite scoring measures, such as the American College of Rheumatology 20% improvement criteria. Thus some patients who did not achieve an American College of Rheumatology 20% improvement criteria response still experienced inhibition of radiographic damage.[2,12] Although they can be administered as monotherapy, all TNF inhibitors appeared to be more effective when used in combination with disease-modifying antirheumatic drugs (DMARDs), commonly methotrexate. Combination therapy with methotrexate has beneficial pharmacokinetic effects for some TNF inhibitors in addition to clinical synergy for the treatment of RA.

Etanercept and adalimumab have been approved for the treatment of juvenile idiopathic arthritis.[13,14] Children who received TNF inhibitors either with or without methotrexate had better clinical outcomes, as measured by using the American College of Rheumatology Pediatric 30% (ACR Pedi 30) response, which represents a 30% or greater improvement in the signs and symptoms of juvenile idiopathic arthritis.

PsA is characterized by the association of inflammatory arthritis with skin psoriasis. The treatment of patients with PsA requires consideration of peripheral arthritis, axial arthritis, skin and nail involvement, dactylitis, and enthesitis. TNF-α levels are notably increased in biopsy samples of skin and synovial tissues from patients with PsA, providing a rationale for the use of TNF inhibitors in the treatment of PsA and psoriasis. TNF inhibitors have been shown to be highly effective in improving the signs and symptoms of arthritis and increasing functional status and quality of life among patients with PsA. Similar to the effect seen in patients with RA, TNF inhibitors also attenuated the progression of radiographic joint damage.[15-18] Moreover, dramatic improvements in the symptoms of skin psoriasis were achieved, as were improvements in the extra-articular involvement characteristics of PsA, such as dactylitis and enthesitis. Improvement in skin psoriasis with TNF inhibitor therapy has likewise been noted in patients without arthritis. Although improvements in joints and skin often occur in parallel, there might be discordance between dermatologic and articular outcomes in individual patients, suggesting potential heterogeneity to pathophysiologic mechanisms underlying different clinical manifestations.

Until the advent of TNF inhibitors, nonsteroidal anti-inflammatory drugs were the only agents shown to alleviate axial symptoms related to AS. In recent years, TNF inhibitors have demonstrated their ability to substantially decrease signs and symptoms of spinal inflammation.[19-23] Paralleling data from patients with RA, TNF inhibitors provided rapid clinical improvement, often as early as 2 weeks. Patients with increased acute-phase reactants at study entry or with evidence for spinal inflammation on magnetic resonance imaging tended to respond more favorably to TNF inhibitors. Because methotrexate is not an effective therapy for spinal inflammation in patients with AS, it has not been used in studies of the TNF inhibitors. A goal in

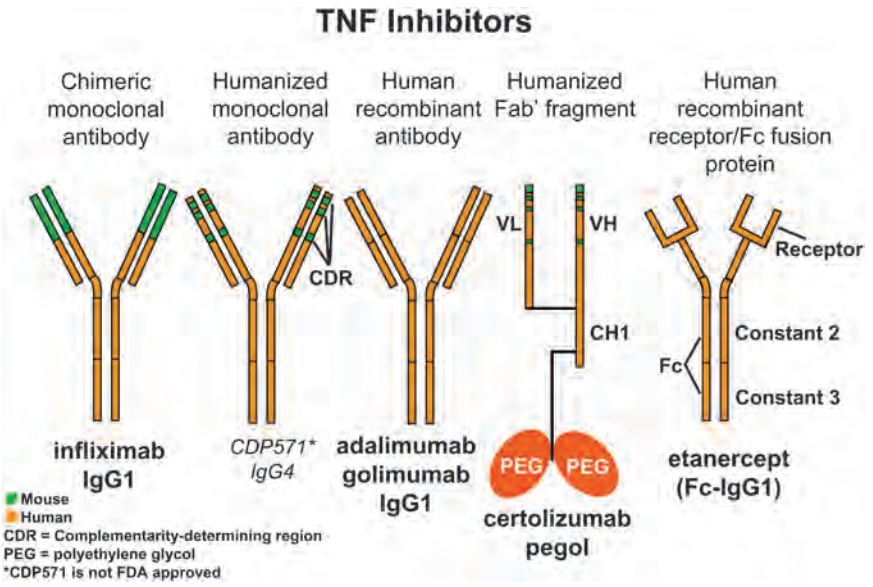

FIG 1. Structure and nomenclature of TNF inhibitors.

TABLE I. Characteristics of biologic agents: Dosing, half-life, and indications

Agent	Typical adult dosing	Mode of delivery	Half-life
Cytokine inhibitors			
Etanercept	25 mg biweekly or 50 mg every week	SQ	4-5 d
Infliximab	3-10 mg/kg q4-8 wk	IV	8-9.5 d
Adalimumab	40 mg every other week	SQ	12-14 d
Golimumab	50 mg every month	SQ	19-27 d
Certolizumab	200-400 mg every 2-4 wk	SQ	12-14 d
Anakinra	100 mg every day	SQ	4-6 h
Rilonacept	320 mg then 160 mg every week	SQ	6-8 d
Tocilizumab	4-8 mg/kg every 4 weeks	IV	12 d
T-cell modulators			
Abatacept	RA: 10 mg/kg every 4 weeks	IV	14.7 d
Alefacept	15 mg IM every week × 12 wk	IM	270 h
B-cell modulators			
Rituximab	1,000 mg every 2 wk × 2 doses	IV	60-170 h
Adhesion cell modulators			
Natalizumab	300 mg every 4 wk	IV	11 d

SQ, Subcutaneous; *IV*, intravenous; *IM*, intramuscular.

treating AS would be to stop the progression of spinal ankylosis. Despite their ability to attenuate spinal inflammation on a sensitive imaging modality, such as magnetic resonance imaging, TNF inhibitors have not seemed to be able to affect radiographic progression when compared with historical control of TNF inhibitor–naive patients with AS.[24]

Levels of TNF-α are increased in the mucosa of inflamed intestines and thought to exert deleterious effects relevant to the pathophysiology of inflammatory bowel disease (Crohn disease and ulcerative colitis). Treatments with TNF inhibitor mAbs have shown improvement in both clinical and endoscopic luminal fistulas and bowel mucosal inflammation associated with Crohn disease.[25-30] To date, etanercept has not been shown to be effective in inflammatory bowel disease.[29] Initially, treatment of Crohn disease with TNF inhibitors was reserved for the most severe, refractory fistulizing disease as a single course. After the success in this group of patients, repeated treatments and more chronic dosing regimens are being used. Intermittent use of infliximab, which is commonly used in the treatment of Crohn disease, has been associated with a greater propensity for the development of antibodies to infliximab and can be attenuated by the concomitant use of immunosuppressive agents, such as corticosteroids, azathioprine, methotrexate, and 6-mercaptopurine.[31] The use of infliximab in combination with immunosuppressive agents (eg, methotrexate and azathioprine) has been shown to enhance efficacy and decrease immunogenicity.[27] TNF inhibitor mAbs are also being studied and used in the treatment of ulcerative colitis.

Several studies, mostly anecdotal and in patients with RA, have demonstrated that switching from one TNF inhibitor to another can be effective and restore clinical response in patients who have lost therapeutic efficacy with the first.[32] Although the success of TNF inhibitors in these autoimmune conditions has been remarkable, it is worth noting that almost uniformly, treatment failed to induce long-term treatment-free remission or immunologic tolerance. Thus maintenance of clinical response required continuous therapy. Also, TNF inhibitors have not proved effective in other

conditions, including several wherein there was pathophysiologic evidence for a role for this cytokine in the disease process. Among autoimmune conditions, TNF inhibitor therapy has been notably ineffective to date in patients with Sjögren syndrome and several forms of vasculitis, including Wegener granulomatosis and polymyalgia rheumatica/temporal arteritis. With regard to congestive heart failure (CHF), data from animal models of ischemic cardiomyopathy implicated TNF as a key mediator of deteriorating cardiac function and hence an attractive target. However, TNF inhibitors have failed to improve symptoms in patients with CHF in clinical trials and sometimes resulted in worsened clinical outcome. Although limited, there were studies on TNF inhibitors that showed negative results in patients with multiple sclerosis (MS), along with anecdotal reports of the development or worsening of demyelinating symptoms among patients with RA treated with these agents. TNF inhibitors are still being actively investigated in a variety of other diseases.

TNF inhibitors: Safety considerations

In general, TNF inhibitors have been well tolerated in clinical trials. *In vitro* studies suggested that TNF inhibitors selectively decrease proinflammatory cytokine levels while preserving both the humoral and cell-mediated arms of the immune response. However, a number of relevant safety issues regarding the use of TNF inhibitors have emerged in postmarketing pharmacovigilance assessments.[33,34] Adverse events associated with TNF inhibitors can be broadly classified as target/class related or agent related. Target-related adverse events include those potentially attributable to the immunosuppression inherent in blocking a key component of the immune system, such as an inflammatory cytokine; this would include increased susceptibility to infections and malignancies. In addition, specific inhibition of TNF might predispose patients to increased susceptibility to tuberculosis, autoantibody production, hepatotoxicity, demyelinating disease, and clinical worsening of CHF. Agent-related adverse events, such as allergic reactions and antigenicity, are idiosyncratic reactions that relate to the particular agent used.

Safety data from clinical trials and registries have shown a small but consistent increase in infections among TNF inhibitor–treated patients compared with those treated with DMARDs, most commonly methotrexate. Generally, the risk of serious infections was not substantially greater, with relative risks ranging from 0 to 2. The risk of infection with TNF inhibitors increased significantly when combined with other biologic agents. For example, combination therapy with the TNF inhibitor etanercept and the IL-1 receptor antagonist (IL-1Ra) anakinra resulted in a higher rate of serious infections in patients with RA, despite the failure to achieve any additive clinical benefit. Data, particularly from pharmacovigilance, have noted a number of opportunistic infections (eg, listeriosis, histoplasmosis, and coccidioidomycosis) among those patients treated with TNF inhibitors. Because of the increased baseline risk of infection among patients with RA, without a control group, it is difficult to ascertain the excess infection risk specifically attributable to TNF inhibitors in these patients. Another potential sequela of immunosuppression is malignancy. With a few notable exceptions, the bulk of the data to date do not support an increased risk of solid tumors related to TNF inhibitor therapy. However, greater numbers of hematologic malignancies, particularly non-Hodgkin lymphoma, have been observed in some registries. Complicating the assessment of the risk attributable to therapy is the increased baseline risk of lymphoma among patients with RA, especially among those with higher disease activity. This introduces bias toward observing cases among patients treated with TNF inhibitors as the most severe, and patients with active RA are often the most common type of patients treated. The relative effect of dose and duration of therapy and host factors, such as comorbidities, relevant genetic polymorphisms, and concomitant medications, on the risk of infections and malignancy remains incompletely defined. Because of potential immunosuppression, vaccination with live vaccines is not recommended while patients are receiving TNF inhibitors.

In addition, inhibition of TNF might predispose patients to a variety of untoward effects that seem to be specific to inhibition of the TNF molecule. There are a fair amount of animal and *ex vivo* data supporting the important role played by TNF in controlling tuberculosis. In contrast to typical presentation of acute tuberculosis as pneumonia, about half of the cases of tuberculosis related to TNF inhibitors presented as extrapulmonary or disseminated tuberculosis. The majority of these tuberculosis cases appear to be reactivation of latent tuberculosis, with infection occurring within the first few months of therapy; however, newly acquired cases have been well described. The incidence of cases might be greater with the mAb TNF inhibitors than with the fusion protein inhibitor. Fortunately, screening for latent tuberculosis before initiating TNF inhibitor therapy has been an effective strategy, with a reduction in incidence of new tuberculosis cases by approximately 85%. Latent tuberculosis can be screened by using either a tuberculin skin test with purified protein derivative or *ex vivo* tests that quantify IFN-γ release from sensitized lymphocytes in blood incubated with tuberculosis antigens. Treatment with TNF inhibitors has also been associated with development of autoantibodies. Although the mechanism of this is unknown, it does not appear to result from inhibition of TNF itself, perhaps through induction of apoptosis. The autoantibodies typically generated include the antinuclear antibody (which develops in about half of patients with RA treated with TNF inhibitors), antibodies to double-stranded DNA (which develop in approximately 10% to 15% of patients treated with TNF inhibitors), and anticardiolipin antibodies. Although rare, progression to a lupus-like illness can occur in patients treated with TNF inhibitors. Also, mild-to-moderate increases in liver function test results (generally <3 times the upper limit of normal) have been observed with TNF inhibitors. Many of these cases were confounded by concomitant use of potentially hepatotoxic drugs and underlying medical conditions. However, in light of the occurrence of liver failure of unidentifiable cause in several cases, clinicians should be aware of these rare events and consider monitoring liver function tests. Lastly, several cases of MS and other demyelinating conditions have been identified among patients treated with TNF inhibitors, although the true effect of TNF inhibitors on the development of MS remains undefined.

Despite their shared ability to inhibit TNF, there are some notable differences between the 5 approved TNF inhibitors. Infliximab, adalimumab, golimumab, and certolizumab are IgG1 mAbs that are specific for TNF-α; etanercept is a fusion protein of the type II TNF receptor and binds both TNF-α and lymphotoxin α (also known as TNF-β). The clinical relevance of this distinction is unknown. In addition, the binding characteristics of the mAbs and the fusion protein differ slightly. Although all agents bind soluble TNF with high affinity, the mAbs have slightly

higher affinity for membrane-bound TNF, presumably related to the physical constraints of the binding domains of the soluble TNF receptor, compared with that of mAbs. Whether these differences might account for the variability in efficacy and safety remains to be seen. The successful introduction of certolizumab pegol would suggest that the ultimate mechanism of action of TNF inhibitors does not appear to require Fc fragment–related activities.

IL-1 inhibitors: Anakinra and rilonacept

IL-1 is synthesized as an inactive precursor. On cleavage by IL-1β–converting enzyme, it activates a variety of cells that can then release mediators destructive to bone and cartilage. In the RA synovium, although there is an increase in the naturally occurring IL-1Ra that prevents the binding of IL-1 to its receptor, the levels are apparently insufficient to counteract the effects of IL-1.

Anakinra, approved in 2001 for the treatment of RA, is a recombinant IL-1ra that differs from the endogenous IL-1Ra by a single amino acid addition at the amino terminus (Table I). Compared with the TNF inhibitors, the clinical responses achieved by anakinra are generally more modest; this, combined with cost and the need for daily injections, has led to its relatively infrequent use in the treatment of RA. However, it has been gaining renewed interest and has been shown to be effective in the treatment of cryopyrin-associated periodic syndromes, including familial cold autoinflammatory syndrome and Muckle-Wells syndrome.[35] These rare autosomal dominant disorders, characterized by a gain-of-function mutation in the cryopyrin gene (CIASI, NLRP3), are associated with oversecretion of IL-1β, rash, arthralgia, and fever. Rilonacept (previously known as IL-1–Trap), which was approved in 2008 for the treatment of cryopyrin-associated periodic syndromes, is a fusion protein comprised of the extracellular domain of the IL-1 accessory protein and IL-1 receptor type 1 attached to the Fc portion of IgG1. Rilonacept binds to IL-1α and IL-1β with high affinity (Table I) and was generally well tolerated, with injection site responses being the most common adverse events.[36] Physicians should remain vigilant about infections with any IL-1 inhibitor. Studies evaluating the role of anakinra and rilonacept in other diseases associated with IL-1 oversecretion, such as chronic gout and adult-onset Still disease, are ongoing, with promising early results.

IL-6 inhibitors: Tocilizumab

Tocilizumab is a humanized anti–IL-6 receptor mAb that binds to both soluble and membrane-bound IL-6 receptor (Table I). Tocilizumab has been shown to improve the signs and symptoms of disease and functional status and slow radiographic progression in patients with RA. The clinical improvement was rapid and evident within the first 2 weeks of treatment.[37-40] Although it can be administered as monotherapy, tocilizumab appears to be more effective when used in combination with methotrexate.

In clinical studies tocilizumab was associated with a slightly higher rate of infections, mainly respiratory and gastrointestinal tract infections. Transient decreases in neutrophil counts, increases in serum lipid levels (total cholesterol, high-density lipoprotein, and low-density lipoprotein), and increases in liver function test results have been observed with tocilizumab. The potential long-term implications of these laboratory abnormalities have not been fully defined.

CYTOKINES

IFN-α

IFN-α is produced by the cells of the immune system in response to the presence of double-stranded RNA viruses, inducing cell activation of macrophages and natural killer cells and enhancing antigen presentation. Both IFN-α2a and IFN-α2b have been used therapeutically with similar results. IFN-α is used in combination with ribavirin in the treatment of hepatitis C viral infection,[41] reducing viremia and providing protection against the development of chronic liver disease and cryoglobulin-associated vasculitis. Side effects can be significant, with up to 68% of patients presenting with psychiatric symptoms, such as depression, irritability, and insomnia. It has also been used to improve survival in patients with advanced renal cancer, although with a modest increase of 2.6 months, achieving a median survival of 11 months.[42] Other uses are in the management of melanoma, hepatitis B infection, and systemic vasculitis.

IFN-β

IFN-β is produced in fibroblasts and is 45% identical to IFN-α, sharing similar antiviral activity against double-stranded RNA viruses. Clinically, it has been used in the treatment of MS because of its additional anti-inflammatory effect.[43] IFN-β slows progression of disease, reducing the percentage of patients with disability from 35% to 22% after 2 years of treatment. Common adverse effects are depression and suicidal ideation, flu-like symptoms, and increase of liver enzyme levels.

IFN-γ

IFN-γ is produced by leukocytes to induce macrophage activation and increase oxidative burst. It is clinically used to enhance immunity in patients with chronic granulomatous disease, in which it has been shown to help by reducing the frequency of infections up to 67% when used in combination with antibacterial and antifungal prophylaxis.[44] IFN-γ is administered subcutaneously at 50 $\mu g/m^2$ 3 times a week. Potential side effects include fever, hypotension, and flu-like symptoms. In patients with congenital osteopetrosis, IFN-γ slows disease progression. It is also used on a trial basis in some patients with the rare occurrence of deficiency of the IFN-γ/IL-12 axis caused by a deficiency of either of these cytokines, expecting that the administration of this cytokine would reduce the patient's susceptibility to severe mycobacterial disease.

IL-2

Recombinant IL-2 has been approved by the US Food and Drug Administration (FDA) for the treatment of metastatic renal cancer[42] and malignant melanoma.[45] IL-2 promotes the activation of T cells and natural killer cells, enhancing their antitumor activity. It also induces the differentiation of regulatory T cells, which are of significance to the control of inflammatory responses. The administration of IL-2 to patients with HIV[46] has resulted in an increase in $CD4^+$ T-cell counts and, when used in combination with highly active anti-retroviral treatment drugs, did not increase HIV viremia and reduced the occurrence of AIDS-defining infections. Side effects are dose related and include hypotension, flu-like symptoms, behavioral changes, and renal impairment.

IL-7

Because of its biologic activity in the homeostasis of T cells, which includes the expansion of naive and memory T cells in the setting of lymphopenia, IL-7 has been suggested as an adjuvant in the treatment of HIV infection and in lymphopenia after chemotherapy. Reports of its administration in HIV-infected patients showed less significant side effects than IL-2 treatment, with sustained dose-dependent expansion of T cells.[47]

AGENTS THAT INHIBIT T CELLS

There is a large body of evidence suggesting autoreactive T cells, especially $CD4^+$ T_H1 T cells, serve a key role in orchestrating the immune-driven inflammatory responses in patients with autoimmune diseases, such as RA, Crohn disease, PsA, and psoriasis. Productive $CD4^+$ T-cell responses require 2 signals: binding of specific antigen-associated MHC class II molecule to the T-cell receptor complex and a second signal from costimulatory molecules. If T cells do not receive the second signal, then tolerance or ignorance of the antigen ensues, and a productive immune response is not generated. Among the most important costimulatory molecules is CD28, which binds CD80 and CD86. CD28 and its natural inhibitor, cytotoxic T lymphocyte–associated antigen 4 (CTLA-4; CD152), are present on T cells and bind to CD80 and CD86 on antigen-presenting cells. CD28 ligation results in stimulation of T cells, whereas CTLA-4 serves an inhibitory role. CTLA-4, which binds CD80 and CD86 with substantially higher affinity than CD28, inhibits the stimulatory effects of CD28 by competitively binding to CD80 and CD86.

Daclizumab and basiliximab

These 2 therapeutic antibodies are directed against CD25, the protein α component of the IL-2 receptor.[48] Their therapeutic effect is the block of IL-2 binding in T and B cells, inhibiting their activation and the development of an immune response and inducing anergy. They are indicated for the prevention of organ transplant rejection, particularly kidney grafts, and have been suggested for the management of autoimmune disorders. For this purpose, the humanized antibody daclizumab is in phase II trials for the treatment of MS and has been shown to decrease the frequency of relapses. These agents induce a state of immunosuppression, which results in an increased frequency of urinary tract infections and respiratory tract infections; however, opportunistic infections have not been observed. Other side effects are paresthesias, transient increased in liver enzyme and bilirubin levels, and skin rash.

Abatacept

Abatacept, approved in 2005 for the treatment of RA, is a soluble protein consisting of the extracellular domain of CTLA-4 linked to the Fc portion of IgG1 (Table I). Abatacept has been shown to improve the signs and symptoms of disease, functional status, and quality of life and slow radiographic progression in patients with RA.[49,50] Abatacept was well tolerated in clinical trials, with a slight increase in the incidence of infections, especially among those with underlying chronic obstructive pulmonary disease. In one study abatacept appeared to have efficacy comparable with that of a TNF inhibitor in patients with RA receiving methotrexate.[51] As with other biologic agents, live vaccines should be avoided when receiving abatacept. A safety study assessing the combination of abatacept and TNF inhibitor therapy observed a higher incidence of serious adverse effects, including infections, at 1-year follow-up compared with that seen in those receiving monotherapy.[52] Given similar findings of increased infections with TNF inhibitors and IL-1ra combination therapy, combination therapy with abatacept and other biologic agents is also discouraged.

Alefacept

Alefacept, approved in 2003 for the treatment of chronic plaque psoriasis, is a fusion protein of a soluble form of the extracellular domain of lymphocyte function–associated antigen (LFA) 3 attached to the Fc portion of an IgG1 molecule. It binds $CD2^+$ T cells and is thought to improve symptoms of psoriasis by inducing memory T-cell apoptosis, inhibiting inflammatory gene expression, and preventing T-cell migration into psoriatic plaques. The interaction of LFA-3 on antigen-presenting cells and CD2 on T cells is thought to be important in T-cell activation and in the development of cells into memory T cells. Alefacept, either as monotherapy or in combination with other psoriasis therapy (eg, methotrexate), has been shown to be effective for skin psoriasis.[53,54] T-cell depletion related to therapy did not correlate or predict the response rate during treatment or follow-up. Despite its effectiveness in the treatment of psoriasis, alefacept appears to be only modestly effective for PsA.[55] With the availability and effectiveness of TNF-I in the treatment of PsA, alefacept is therefore rarely used for the treatment of PsA.

Anti-p40 agents

Another approach to modulating the function of T cells in autoimmune and inflammatory diseases targets cytokines relevant to the development of certain T-cell subsets. IL-12, a cytokine central to the development of T_H1 T cells, and IL-23, a cytokine that helps sustain T_H17 T cells, share a common p40 subunit.[56] Agents that target the p40 subunit, including the human mAb ustekinumab and ABT874, might be expected to attenuate inflammatory processes driven by T_H1 and T_H17 T cells. These therapies are under investigation in a variety of autoimmune diseases, and ustekinumab has received regulatory approval in several countries for the treatment of psoriasis. In patients with psoriasis, ustekinumab therapy induced a substantial improvement, as measured by the psoriasis area and severity index.[57] The extent of improvement appeared to perhaps even have been larger than that achieved with TNF inhibitors, which are themselves highly effective in patients with psoriasis. The same agent has also been studied in patients with PsA and been found to have some efficacy in that condition.[58] Interestingly, the duration of clinical benefit after a few injections is prolonged and appears to far exceed the pharmacokinetic profile of the drug.

AGENTS THAT INHIBIT B CELLS

Recent data suggest that B cells might contribute significantly to the initiation and perpetuation of the immune response in various autoimmune diseases, including RA and systemic lupus erythematosus (SLE). Not only can B cells produce potentially pathologic autoantibodies (eg, rheumatoid factor and antinuclear antibody) and proinflammatory cytokines, but they can also present

antigens to T cells and provide costimulatory signals essential for T-cell activation, clonal expansion, and effector function.

CD20 inhibitor: Rituximab

Rituximab is a chimeric IgG1 mAb directed against the B-lymphocyte surface antigen CD20. It was initially approved in 1997 for the treatment of $CD20^+$ B-cell non-Hodgkin lymphoma and later for the treatment of RA in 2006. CD20 is a cell-surface molecule restricted to the surface of pre-B through activated mature B cells. Rituximab is thought to induce lysis of $CD20^+$ B cells through several mechanisms, including complement activation, antibody-dependent cell-mediated cytotoxicity, and induction of apoptosis. Depletion of B cells can last up to 9 months or longer after a single course of therapy. Rituximab has been shown to improve the signs and symptoms of disease, functional status, and quality of life and slow radiographic progression of disease in patients with RA.[59,60] Although rituximab can be used alone or in combination with DMARDs, the combination therapy yielded better clinical outcomes. Also, patients who are seropositive for rheumatoid factor had greater clinical response compared with rheumatoid factor–seronegative patients. In smaller studies rituximab has shown promising results in the treatment of other autoimmune diseases, such as SLE, primary Sjögren syndrome, idiopathic thrombocytopenic purpura, chronic inflammatory demyelinating polyneuropathy, and vasculitis. Additional trials are underway that should answer questions regarding dosing, treatment intervals, safety, and tolerability in these conditions.

Despite the potential for immunodeficiency related to depletion of mature B cells, no significant increases in infections, either serious or opportunistic, were reported in patients with RA and non-Hodgkin lymphoma treated with rituximab. The overall levels of serum immunoglobulin generally remain stable during treatment. This could be related to preserved function of plasma cells, which lack CD20 and are therefore not depleted by rituximab. However, if rituximab is used as a recurrent or maintenance therapy for autoimmune conditions, this might become more of a safety concern because plasma cells are not replenished by memory B cells. Thus far, some patients have undergone more than 4 cycles of rituximab without increased risk of adverse events.[61] Other notable adverse effects include rare neutropenia, reactivation of hepatitis B, and progressive multifocal leukoencephalopathy (PML). Three cases of PML in patients receiving rituximab for non–FDA-approved conditions, mainly SLE, have been reported.[62] The exact role of rituximab in the development of PML remains unknown given its rare occurrence, but it highlights the importance of pharmacovigilance and potential unforeseen long-term adverse effects related to biologic agents. Although treatment has overall been well tolerated, infusions have been associated with hypersensitivity reactions, Stevens-Johnson syndrome, and type III serum sickness–like illness and cytokine release syndrome. The infusion reactions are more common during the first infusion and might occur more in patients with lymphoma than in those with RA.[33,34] Lastly, given the potential for suboptimal response, vaccinations should be administered before rituximab, if possible.

Anti-IgE antibody: Omalizumab

This antibody was developed to aid in the management of severe asthma with an allergic component. Omalizumab binds IgE with high affinity, considerably reducing levels of free IgE and inhibiting its interaction with the IgE receptor. The clinical improvement correlated well with the measurement of biologic markers.[63] Its administration to patients with severe asthma with low to moderately increased serum IgE levels results in a 26% decrease in the asthma exacerbation rate and a 50% decrease in severe exacerbations and emergency department visits, as well as a reduction in systemic corticosteroid use.[64] It has also been shown to be useful to reduce symptoms in patients with corticosteroid-resistant chronic urticaria.

AGENTS THAT INHIBIT CELL ADHESION, MIGRATION, OR BOTH

Activated T lymphocytes must migrate to sites of inflammation and lymph tissue to exert their diverse effects. The entry of lymphocytes into specific sites occurs through several specific interactions between the adhesion molecules on lymphocytes, including the integrins and their ligands on endothelial cells. Particularly important for lymphocyte migration and homing are LFA-1 and its counterreceptors, intercellular adhesion molecule (ICAM) 1 and ICAM-2, and very late antigen-4 and its counterreceptor, vascular cell adhesion molecule 1.

Integrin inhibitors: Natalizumab

Natalizumab, approved in 2004 for the treatment of MS, is a recombinant humanized IgG4 mAb directed against the α_4 subunit of $\alpha_4\beta_1$; it also binds to and inhibits the function of the $\alpha_4\beta_7$ integrins, the ligand of which is mucosal addressin cell adhesion molecule 1. $\alpha_4\beta_1$ integrin, an adhesion molecule present on leukocytes, has been implicated in the pathogenesis of MS by facilitating migration of lymphocytes into the site of disease. In addition to blocking the migration of lymphocytes into the central nervous system and intestinal parenchyma, natalizumab induces T-cell apoptosis and anergy and prevents T-cell binding to osteopontin and fibronectin, thereby attenuating T cell–mediated inflammation. In 2 large clinical trials, natalizumab, either alone or in combination with IFN-β-1a was associated with significantly lower relapse rates and disability and fewer new MS lesions on magnetic resonance imaging.[65] However, shortly after FDA approval, natalizumab was temporarily withdrawn from the market after 3 cases of PML were reported. Similar to rituximab, the exact role of natalizumab in the development of PML remains unknown.

CD11a inhibitor: Efalizumab

Efalizumab, approved in 2003 for the treatment of psoriasis, is a humanized IgG1 mAb directed against the cell adhesion molecule CD11a. CD11a is an α subunit of the LFA-1 molecule on T cells that binds to ICAM-1 on antigen-presenting cells and endothelial cells. In addition to inhibiting activation of T cells, efalizumab also blocks trafficking of lymphocytes into the skin by blocking LFA-1/ICAM-1 interaction. Efalizumab has been shown to provide greater improvement in symptoms of skin psoriasis after 3 months of therapy, with a continued increase in response if therapy was continued for another 3-month cycle.[66] However, the development of PML among several patients treated with efalizumab led to its withdrawal in 2009.

TABLE II. Clinical use of human immunoglobulin preparations

Primary immunodeficiency diseases (that result in defect in antibody responses)
Secondary immunodeficiency conditions (with impaired antibody responses)
HIV infection
B-cell leukemia
Use of chemotherapy or radiotherapy
Autoimmune syndromes
Hematologic: ITP, autoimmune hemolytic anemia
Rheumatologic: RA, vasculitis, Kawasaki disease, uveitis, SLE
Endocrinologic: Autoimmune diabetes mellitus, Graves ophthalmopathy
Neurologic: Guillain-Barré syndrome, chronic inflammatory demyelinating polyneuropathy, myasthenia gravis, dermatomyositis
Dermatologic: TEN, Steven-Johnson syndrome
Infectious diseases
CMV
Rotavirus
Parvovirus B19

ITP, Immune thrombocytopenic purpura; *TEN*, toxic epidermal necrolysis; *CMV*, cytomegalovirus.

IMMUNOGLOBULINS

Therapeutic use

Immunoglobulin concentrates derived from human plasma have been used since the 1940s and were used initially in the management of viral diseases, such as hepatitis. Bruton published the first report of the use of immunoglobulins to treat a patient with an immune defect who presented with agammaglobulinemia and frequent infections. This resulted in the increase of the gammaglobulin fraction in the patient's serum and a reduction in the number of infections. Currently, human immunoglobulin preparations are derived from pooled plasma of up to 10,000 individual donors per batch of immunoglobulin products, introducing safety concerns regarding the transmission of blood-borne infectious diseases. This is addressed by means of donor screening for infectious diseases and by introducing in the manufacturing process several steps to remove viral particles. There are 2 forms of administration: subcutaneous immunoglobulin (SCIG) and intravenous immunoglobulin (IVIG).[67]

In addition to its use as antibody replacement, IVIG preparations are indicated as an immunomodulator in many inflammatory conditions, such as idiopathic thrombocytopenic purpura (Table II). Only 6 of these indications are approved by the FDA: the treatment of primary immunodeficiencies, HIV infection, Kawasaki disease, and immune thrombocytopenic purpura and the prevention of infections in B-cell leukemias and in patients undergoing bone marrow transplantation.[68] The anti-inflammatory properties of immunoglobulins have been attributed to different mechanisms, including those mediated by neutralization of autoantibodies and anti-idiotypic antibodies and neutralization of toxins and T-cell superantigens and those mediated by the modulation of the Fc receptors in the cells of the immune system. More recently, Anthony et al[69] showed that the anti-inflammatory activity of immune globulins can be explained by the action of Fc fragments containing sialic acid on macrophages inducing the expression of the inhibitory FcγIII receptor. Both human and murine recombinant sialylated Fc proteins were able to suppress inflammation in a murine model of arthritis. This finding might lead to the development of a therapeutic anti-inflammatory agent that is not derived from human plasma and that reduces safety concerns and availability shortages.

Dosage and adverse reactions

In the United States several IVIG preparations and 1 SCIG preparation are commercially available.[68] They differ in their method of purification, osmolality and IgG concentration, IgA and sodium contents, stabilizer (to prevent IgG aggregation; eg, glycine, sucrose, or maltose), and pH; however, they are administered similarly, except when adverse reactions occur, such as idiosyncratic reactions in a particular patient or if suspected hypersensitivity to IgA leads to the use of those products with undetectable IgA. Patients with diabetes mellitus should avoid products containing sugar molecules as stabilizers. IVIG is used as an anti-inflammatory agent at 1 to 2 g/kg in 1 dose or divided in 2 daily doses. The IVIG dose used for replacement in patients with antibody deficiencies is 400 to 600 mg/kg administered every 3 to 4 weeks to maintain a trough IgG level of at least 500 mg/mL and reduce the frequency of infections. Because of increased immunoglobulin catabolism or protein loss, some patients might require even higher doses, which need to be optimized to each patient, also taking into consideration the clinical assessment. Because of the volume limitations for subcutaneous administration, SCIG is not used for inflammatory disorders and is recommended to be administered weekly for immune deficiencies, with doses that correspond to the IVIG dose mentioned above (approximately 100 mg/kg/wk). No differences of efficacy to prevent infections have been found in clinical trials comparing IVIG and SCIG. The weekly subcutaneous administration of immunoglobulins provides a tighter range of serum IgG levels, which is of advantage for patients who experience side effects associated with peak IgG concentrations. Although side effects associated with SCIG infusions are at the infusion site, IVIG side effects are not common, although they can be severe, including back pain, fever, hypotension, thrombosis, headaches, and skin rashes. Premedication with antihistaminic agents, nonsteroidal anti-inflammatory drugs, and corticosteroids and hydration with normal saline are common measures used to prevent these symptoms. Serious adverse effects, such as aseptic meningitis, seizures, anaphylaxis, pulmonary edema, and thrombosis, have been rarely reported. Therefore it is recommended that IVIG be administered with medical monitoring for early detection and management of these possible events.

FUTURE DIRECTIONS

The factors that drove the initial introduction of the biologic agents—a clinical need for better outcomes, greater delineation of pathophysiology allowing definition of various targets, and progress in biotechnology allowing development of agents—will

no doubt continue to fuel progress in this area. It can be expected that additional mAbs and fusion receptors, both directed at existing targets and against novel targets, will continue to be developed and brought to the clinic. Along with the number of agents, it is anticipated that the conditions for which these agents are used will also expand. For existing biologic agents, a number of questions remain as to the optimum treatment paradigms (eg, sequence of biologic agents) and most appropriate patient populations for their use; this will be germane for newer agents as well. As always, the balance between achieving higher levels of efficacy, with disease remission being the ultimate goal, need to be balanced against safety considerations. For macromolecules, such as mAbs and soluble receptors, there is the potential for optimizing their characteristics, including ease of use, immunogenicity, and cost. For certain targets, it is possible that small-molecule inhibitors might be developed that can address some of these issues. However, because these molecules can be anticipated to have pharmacokinetic, mechanistic, and other important differences from their macromolecular counterparts, this might translate into variable safety and efficacy. Therefore newer agents of a different class, even those whose putative target is the same as existing therapies, need to be assessed with the same rigor as the currently available agents.

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Transplantation immunology: Solid organ and bone marrow

Javier Chinen, MD, PhD,[a] and Rebecca H. Buckley, MD[b] *Houston, Tex, and Durham, NC*

Development of the field of organ and tissue transplantation has accelerated remarkably since the human MHC was discovered in 1967. Matching of donor and recipient for MHC antigens has been shown to have a significant positive effect on graft acceptance. The roles of the different components of the immune system involved in the tolerance or rejection of grafts and in graft-versus-host disease have been clarified. These components include antibodies, antigen-presenting cells, helper and cytotoxic T-cell subsets, immune cell-surface molecules, signaling mechanisms, and cytokines. The development of pharmacologic and biological agents that interfere with the alloimmune response has had a crucial role in the success of organ transplantation. Combinations of these agents work synergistically, leading to lower doses of immunosuppressive drugs and reduced toxicity. Reports of significant numbers of successful solid-organ transplantations include those of the kidneys, liver, heart, and lung. The use of bone marrow transplantation for hematologic diseases, particularly hematologic malignancies and primary immunodeficiencies, has become the treatment of choice in many of these conditions. Other sources of hematopoietic stem cells are also being used, and diverse immunosuppressive drug regimens of reduced intensity are being proposed to circumvent the mortality associated with the toxicity of these drugs. Gene therapy to correct inherited diseases by means of infusion of gene-modified autologous hematopoietic stem cells has shown efficacy in 2 forms of severe combined immunodeficiency, providing an alternative to allogeneic tissue transplantation. (J Allergy Clin Immunol 2010;125:S324-35.)

Key words: *Bone marrow transplantation, solid-organ transplantation, graft rejection, graft-versus-host disease*

Abbreviations used
ADA: Adenosine deaminase
ALG: Antilymphocyte globulin
APC: Antigen-presenting cell
ATG: Antithymocyte globulin
CGD: Chronic granulomatous disease
GVHD: Graft-versus-host disease
IL-2R: IL-2 receptor
SCID: Severe combined immunodeficiency

Efforts to transplant organs or tissues from one human subject to another had been unsuccessful for many decades until the discovery of the human MHC in 1967.[1] Identification of this genetic region launched the field of clinical organ and tissue transplantation. In 1968, the World Health Organization Nomenclature Committee designated that the leukocyte antigens controlled by the closely linked genes of the human MHC be named HLA (for human leukocyte antigen). This chapter reviews general immunologic concepts that have supported the success of human organ and tissue transplantation and summarizes current medical progress in the field of transplantation medicine.

TRANSPLANTATION ANTIGENS

MHC

Histocompatibility antigens are tissue cell-surface antigens capable of inducing an immune response in a genetically dissimilar (allogeneic) recipient, resulting in the rejection of the tissues or cells bearing those antigens. The genes that encode these antigens reside in the MHC region on the short arm of human chromosome 6 (Fig 1). The HLA complex contains more than 200 genes, more than 40 of which encode leukocyte antigens.[2,3] These genes and their encoded cell-surface and soluble protein products are divided into 3 classes (I, II, and III) on the basis of their tissue distribution, structure, and function.[3-5] MHC class I and II genes encode codominantly expressed HLA cell-surface antigens, and class III genes encode several components of the complement system, all of which share important roles in immune function.

Class I MHC antigens are present on all nucleated cells and are each composed of a 45-kd α heavy chain encoded by genes of the HLA-A, HLA-B, or HLA-C loci on chromosome 6 and associated noncovalently with a 12-kd protein, β_2-microglobulin, encoded by a gene on chromosome 15 (Fig 2).[3] MHC class II antigens have a more limited tissue distribution and are expressed only on B lymphocytes, activated T lymphocytes, monocytes, macrophages, Langerhans cells, dendritic cells, endothelium, and epithelial cells.[5] Each is a heterodimer composed of noncovalently associated α and β chains of approximately 230 amino acids encoded by genes of the HLA-D region (Fig 2). On cells expressing both class I and class II HLA antigens, there are 3 class I antigens and 3 or more (usually 4) class II heterodimers.

Class III genes are located between the HLA-B and HLA-D loci and determine the structure of 3 components of the complement system: C2, C4, and factor B.[3,4] HLA antigens are inherited in a Mendelian dominant manner. Because of the closeness of the different loci of the MHC and the resultant low crossover frequency, however, HLA genes are almost always inherited

From [a]the Department of Pediatrics, Allergy/Immunology, Baylor College of Medicine, Houston, and [b]the Department of Pediatrics, Allergy/Immunology, Duke University Medical Center, Durham.

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Reprint requests: Rebecca H. Buckley, MD, Duke University Medical Center, Department of Pediatrics, Allergy/Immunology, 362 Jones Building (Campus Box 2898), Durham, NC 27710-0001. E-mail: BUCKL003@mc.duke.edu.

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together. To date, 3756 different class I and II HLA gene alleles have been identified.[2] The fixed combination of these genetic determinants present in 1 chromosome of a subject is referred to as a haplotype. Chromosome 6 is an autosome, and therefore all subjects have 2 HLA haplotypes (1 for each chromosome), and there are only 4 possible combinations of haplotypes among the offspring of any 2 parents. Thus there is a 25% probability that biological siblings will have identical HLA alleles.

The ABO system

ABO incompatibility does not cause stimulation in mixed leukocyte cultures, indicating that ABO compatibility is of much less importance than HLA compatibility in graft survival. However, ABO incompatibility can result in hyperacute rejection of primarily vascularized grafts, such as those of the kidney and heart.[6] This is thought to occur because (1) ABO blood group antigens are highly expressed on kidney and cardiac grafts, particularly those from patients who are blood group A or B antigen secretors, and (2) preformed naturally occurring antibodies to blood group substances are present in mismatched recipients. Advances in immunosuppressive therapies to prevent immune rejection of the graft have more recently allowed performance of organ transplantations across the ABO barrier.[7]

Donor-recipient HLA matching

Two laboratory methods are used to pair donors and recipients for transplantation. The first matching method involves the determination of HLA antigens on donor and recipient leukocytes by using either serologic or DNA-typing methods. The second method is functional and involves the measurement of the response of immunocompetent cells from the recipient to antigens present on donor cells (and *vice versa* for bone marrow transplantation). Results of both methods are generally consistent with each other. Disparities that are serologically detected are referred to as antigen mismatches, whereas differences that can be identified only by DNA-based typing are called allele mismatches. Because these methods take considerable time to perform, results are not known in time for some solid-organ transplantations, such as lung transplantations, which are performed based on immediate organ availability. Since 2000, the National Donor Matching Program performs HLA typing of donor volunteers exclusively using a DNA-based method, the PCR single-strand oligonucleotide probe. Currently, approximately 60% of volunteer donors on the National Donor Matching Program Registry had their HLA types determined by using this method. Efforts continue to improve the efficiency of HLA typing and to reduce the costs of the assays.[8]

Donor-recipient serologic cross-matching

Serologic cross-matching is of particular importance to the success of primarily vascularized grafts, such as those of the kidney and heart. Serum from the prospective recipient is tested against cells from the potential donor for the presence of antibodies to red blood cell or HLA antigens. The presence of such antibodies correlates with hyperacute renal graft rejection.[6] For this reason, a positive serologic cross-match result has been considered a contraindication to renal transplantation, although therapeutic strategies, such as the use of plasmapheresis, are proposed when the mismatch cannot be avoided.[7]

Usefulness of HLA typing in clinical organ and tissue transplantation

Although typing for intrafamilial transplants of all types is clearly of great value, the usefulness of HLA typing in cadaveric kidney grafting has been a point of controversy since cyclosporine became available.[9] Although short-term survival rates did not appear to be that different for closely or poorly matched cadaveric kidneys, the degree of HLA matching does correlate with long-term survival.[10] Until 1980, only HLA-identical siblings could be used as bone marrow donors because both graft rejection and lethal graft-versus-host disease (GVHD) were common complications if this was not the case.[11] Fortunately, the development during the past 3 decades of techniques to rigorously deplete postthymic T cells from donor marrow has permitted numerous successful half-HLA-matched marrow transplantations with no or minimal GVHD.[12,13]

MECHANISMS OF GRAFT REJECTION

Role of alloimmune antibodies

The strongest evidence for a role for antibodies in graft rejection is the hyperacute rejection of primarily vascularized organs, such as the kidney and heart. High titers of antidonor antibodies can be demonstrated in recipients presenting with these reactions.[6] These antibodies combine with HLA antigens on endothelial cells, with subsequent complement fixation and accumulation of polymorphonuclear cells. Endothelial damage then occurs, probably as a result of enzymes released from polymorphonuclear leukocytes; platelets then accumulate, thrombi develop, and the result is renal cortical necrosis or myocardial infarction.[14]

Leukocytes and cytokines in graft rejection

Allograft rejection results from the coordinated activation of alloreactive T cells and antigen-presenting cells (APCs). Although acute rejection is a T cell–dependent process, the destruction of the allograft results from a broad array of effector immune mechanisms. Cell-cell interactions and the release by primed T_H cells of multiple types of cytokines (IL-2, IL-4, IL-5, IL-7, IL-10, IL-15, TNF-α, and IFN-γ) recruit not only immunocompetent donor-specific $CD4^+$ T cells, $CD8^+$ cytotoxic T cells, and antibody-forming B cells but also nonspecific inflammatory cells, which constitute the majority of cells infiltrating an allograft.[15] Other cells specific to the transplanted organ might play a role in the balance of tolerance and rejection, such as the Kupffer cells and the sinusoidal epithelial cells in the liver.[16]

Stimulation of $CD4^+$ T cells through their antigen receptors is not sufficient to initiate T-cell activation unless costimulation is provided by interaction of other ligand-receptor pairs present on the surfaces of T cells and APCs during the encounter. Some of these interactive pairs include the T-cell surface molecule CD2 and its ligand CD58 on APCs, CD11a/CD18-CD54, CD5-CD72, CD40 ligand–CD40, and CD28–CD80 or CD86. $CD4^+$ T-cell anergy or tolerance induction occurs when the T-cell receptor interacts with the APC unless signals are provided through 1 or more of these receptor-ligand interactions (particularly through CD40 ligand–CD40 and CD28–CD80 or CD86) or by cytokines (eg, IL-1 and IL-6 from the APC). Thus T-cell accessory proteins and their ligands on APCs are target molecules for antirejection therapy.[17,18] If costimulation does occur, the $CD4^+$ T cell becomes activated, which leads to stable

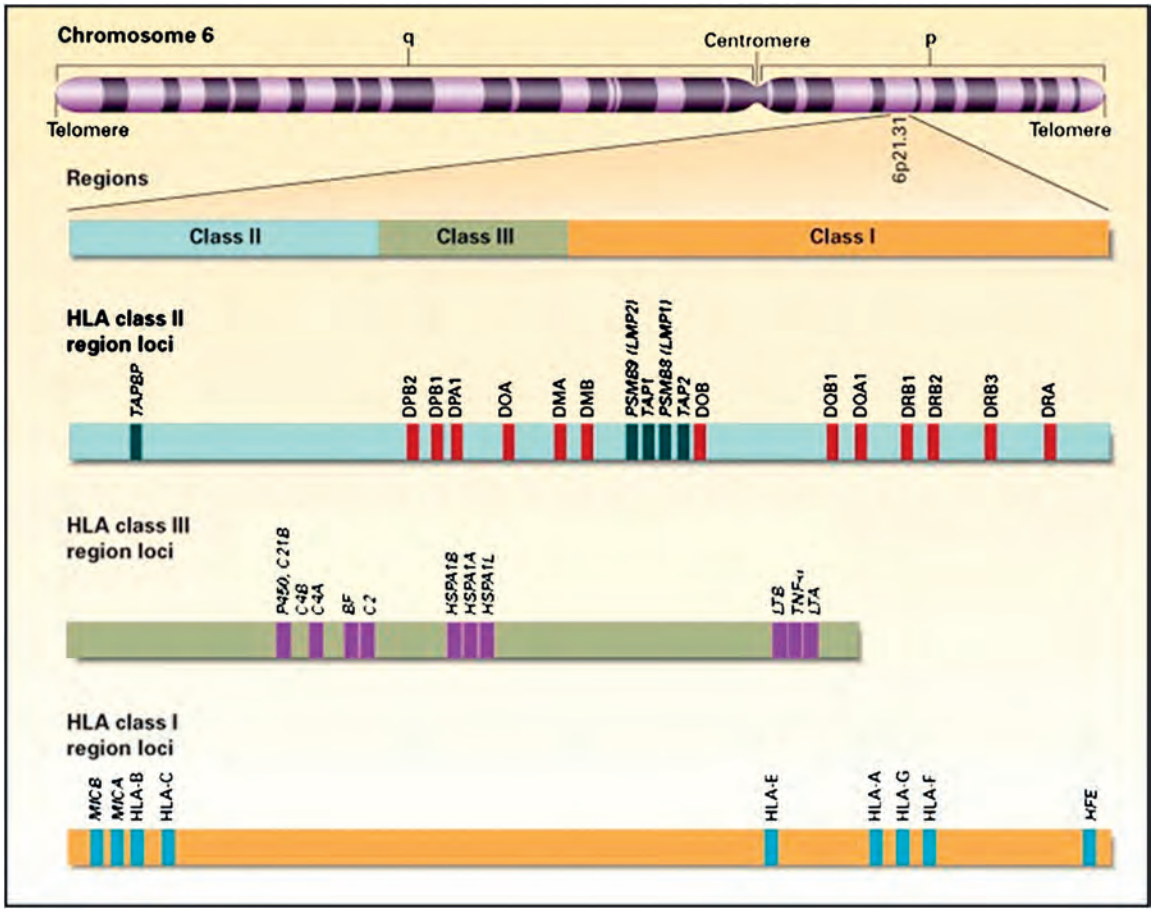

FIG 1. Location and organization of the HLA complex on chromosome 6. *BF*, Complement factor B; *C2*, complement component 2; *C4A*, complement component 4A; *C4B*, complement component 4B; *LTA*, lymphotoxin A; *LTB*, lymphotoxin B; *TAP1*, transporter of antigenic peptides 1; *TAP2*, transporter of antigenic peptides 2. Reprinted with permission from Klein and Sato.[3]

transcription of genes important in T-cell activation. $CD8^+$ T cells recognize antigenic peptides displayed on MHC class I molecules and represent a major cytotoxic effector lymphocyte population in graft rejection. Donor class I molecules on donor APCs in the graft directly activate cytotoxic effector lymphocytes. However, CD8 activation also requires a costimulatory second signal, as well as an IL-2 signal. Activated $CD8^+$ T cells proliferate and mature into specific alloreactive clones capable of releasing granzyme (serine esterase), perforin, and toxic cytokines, such as TNF-α. More recently, the identification of T_H17 effector cells (proinflammatory) and regulatory T cells (downregulators of immune activation) has improved our understanding of the development of graft tolerance or rejection.[19] Stimulation of the B cell by antigen occurs through its antigen receptor (surface immunoglobulin), but costimulation is also required for B-cell activation. This costimulation can be provided by cytokines released by T cells or through many of the same T-cell protein–ligand pairs important in T-cell–APC costimulation because these ligands are also present on B cells. B-cell contribution to the immune rejection of organ transplants is not limited to the production of alloimmune antibodies but also involves antigen presentation and the secretion of proinflammatory cytokines.[20]

Once T-cell activation has occurred, autocrine T-cell proliferation continues as a consequence of the expression of the IL-2 receptor (IL-2R). Interaction of IL-2 with its receptor triggers the activation of protein tyrosine kinases and phosphatidylinositol 3–kinase, resulting in translocation into the cytosol of an IL-2R–bound serine-threonine kinase, Raf-1. This in turn leads to the expression of several DNA-binding proteins, such as c-Jun, c-Fos, and c-Myc, and to progression of the cell cycle. The consequence of all of these events is the development of graft-specific, infiltrating cytotoxic T cells. Cytokines from the T cells also activate macrophages and other inflammatory leukocytes and cause upregulation of HLA molecules on graft cells. The activated T cells also stimulate B cells to produce anti-graft antibodies. Ultimately, if not recognized and managed, all these cellular and humoral factors constitute the rejection process that destroys the graft.

IMMUNOSUPPRESSION

More information on immunosuppresion regimens can be found in Table I.

Currently, there is no method that will suppress the host's immune response to antigens of the graft and at the same time maintain other immune responses. Nonspecific immunosuppressive agents are needed to prevent rejection of the transplanted organ, which can occur even though HLA-matched donors are

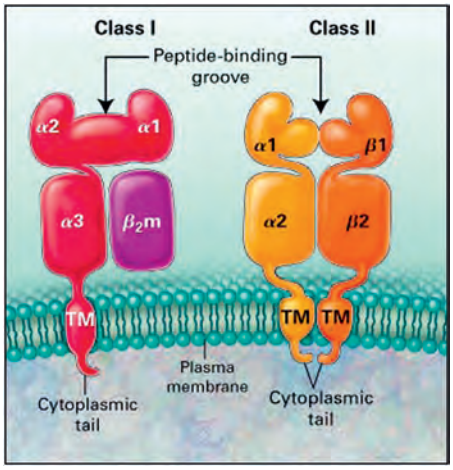

FIG 2. Structures of HLA class I and II molecules. β_2-Microglobulin *(β_2m)* is the light chain of the class I molecule. *TM,* Transmembrane component. Reprinted with permission from Klein and Sato.[3]

used. The development of immunosuppressive strategies during the past 4 decades reflects enormous progress in understanding the cellular and molecular mechanisms that mediate allograft rejection.[21] The success of transplantation between unrelated donors and recipients can be attributed to implementation of these strategies. These agents depress both specific and nonspecific immunity, and they render the recipient more susceptible to both infection and malignancy. Indeed, infection is the most important cause of transplant-recipient death. Thus all patients must have the immunosuppressive regimen fine tuned to prevent rejection yet minimize the risk of infection: too high a dose, and infection supervenes; too small a dose, and the graft is rejected.

The immunosuppressive agents initially used in most transplant centers for nearly 2 decades were corticosteroids, azathioprine, and cyclosporine. Several new agents have been introduced during the past few years: mycophenolate mofetil, which has a similar but more effective mode of action to that of azathioprine; tacrolimus, which has a mode of action and side effects similar to those of cyclosporine; and sirolimus, which blocks IL-2–induced T-cell cycle progression.

Immunosuppressive agents can be categorized by whether they (1) interrupt lymphocyte cell division, (2) deplete lymphocytes, (3) interfere with lymphocyte maturational events, (4) interfere with immune cell costimulation, (5) modulate ischemia–reperfusion injury, or (6) facilitate induction of tolerance.[22] They can also be grouped into those used for induction therapy, for prophylaxis against rejection, for reversal of acute rejection episodes, and for maintenance of immunosuppression.

mAbs to lymphocytes and to cytokine receptors

Antibodies from animals immunized with human lymphoid cells are useful agents for induction therapy, as well as for reversal of acute rejection episodes.[23] They consist of the IgG fraction of serum from horses or rabbits immunized with either human lymphocytes (antilymphocyte globulin [ALG]) or thymocytes (antithymocyte globulin [ATG; thymoglobulin]) or of mAbs (murine or humanized) to T-cell surface antigens (eg, CD3 [OKT3]). In general, ALG, ATG, and OKT3 decrease the onset, severity, and number of rejection episodes. Prevention of graft rejection has also been approached by inhibiting cytokines from interacting with their receptors. Chimeric or humanized murine anti–IL-2R α chain antibodies (daclizumab and basiliximab) have been developed for clinical use. The advantage of these mAbs to the IL-2R α chain is that such molecules are present only on activated T cells; therefore the main effect is on T cells possibly activated by graft antigens.

Calcineurin inhibitors

The main action of calcineurin inhibitors (cyclosporine and tacrolimus) is that they prevent the synthesis of IL-2 and other cytokines that might be produced by T cells activated by allografts.[21] Through its hydrophobicity, cyclosporine enters cell membranes to gain access to and bind to the cytoplasmic isomerase protein cyclophilin. The complex then inhibits calcineurin, an intracellular phosphatase critical for the translocation of signals from the T-cell receptor to the nucleus. In this manner it blocks transcription of the *IL2* gene. In addition, it also blocks the synthesis of other cytokines and thereby interferes with activated $CD4^+$ helper T-cell function. As a consequence, T-cell proliferation and differentiation of precursor cytotoxic lymphocytes are blocked. Tacrolimus binds to a cytoplasmic isomerase protein in the same way that cyclosporine does, but it binds to a different one, the FK-binding protein.[24] The complex formed inhibits calcineurin to prevent T-cell receptor signal transduction to the cell nucleus, blocking cell activation. Tacrolimus thus inhibits synthesis of IL-2, IL-3, IFN-γ, and other cytokines; it was found to be 100 times more potent than cyclosporine as an immunosuppressive agent.[24]

Cytokine receptor signal transduction inhibitors

Sirolimus (Rapamune; Wyeth, Madison, NJ) has a structure similar to tacrolimus, and its activity is also dependent on its binding to the FK-binding protein. However, the complex formed does not inhibit calcineurin but instead prevents the phosphorylation of the p70S6 kinase. This action blocks signal transduction from many cell-surface cytokine receptors, including the IL-2, IL-4, IL-15, and IL-10 receptors. Both *in vitro* and *in vivo* studies have shown a synergistic effect of sirolimus with cyclosporine, as would be expected because sirolimus prevents cytokine receptor signaling and cyclosporine inhibits cytokine production. In addition, sirolimus selectively preserves the development of regulatory T cells.[25] No agent is the perfect nonspecific immunosuppressive drug. Anti-lymphocyte antibodies (including anti-CD3, anti-CD6, and anti-CD52 antibodies), nucleoside synthesis inhibitors, steroids, cyclosporine (or tacrolimus), anti–IL-2R α chain (anti-CD25), and sirolimus all affect allorecognition and antigen-driven T-cell proliferation at different points in the T-cell activation process. Thus the combined use of several of these types of agents provides a synergistic effect rather than a merely additive effect.

SOLID-ORGAN TRANSPLANTATION

The explosive growth of transplantation since the discovery of HLA in 1967 is attested to by the fact that, according to the Global Database on Donation and Transplantation gathering data from 97 countries, in 2007 around 100,000 solid-organ transplantations were performed per year worldwide: 68,250 are kidney transplantations (45% from living donors), 19,850 are liver transplantations (14% from living donors), 5,179 are heart transplantations, 3,245 are lung transplantations, and 2,797 are pancreas transplantations.[26]

TABLE I. Immunosuppresion regimens

Immunosuppression regimen	Immunologic target	Specific use	Major adverse effects
Radiation, anti-metabolite agents	Hematopoietic stem cells, leukocytes	BMT	Cytopenias, opportunistic infections, diarrhea, alopecia, veno-occlusive disease, long-term organ damage: endocrine abnormalities, growth delay, hypodontia, cognitive delay, sterility
Calcineurin inhibitors, anti-lymphocyte antibodies, anti-cytokine antibodies, anti-metabolite agents, and corticosteroids	Lymphocytes	In solid-organ transplantation and BMT: prevention and treatment of graft rejection and GVHD	Opportunistic infections, lymphopenia, renal dysfunction, seizures, hypertrichosis, hypertension, gastritis, osteoporosis, cataracts, growth delay

BMT, Bone marrow transplantation.

Kidney transplantation

Despite major improvements in dialysis techniques, renal transplantation remains the treatment of choice for end-stage renal disease in patients of nearly all ages.[27] Estimates of new cases of end-stage renal disease are at 300 cases per million persons annually, with an increasing trend.[27] For adults and most children, the renal transplantation operation has become standardized. The earlier practice of removing the patient's diseased kidneys 2 to 3 weeks before transplantation has not been carried out routinely in recent years, except for patients with hypertension or infection, and nephrectomy is now performed at the time of transplantation.

Immunosuppressive regimens. Until cyclosporine became available in the early 1980s, most centers used a combination of azathioprine (Imuran; Prometheus Laboratories, Inc, San Diego, Calif) and prednisone to prevent graft rejection. Beginning in 1983, many centers began to use cyclosporine (in lieu of azathioprine) with lower doses of prednisone for immunosuppression.[27,28] Cyclosporine has been given in varying doses at different centers but has generally been given intravenously during or just after transplantation and on the day after. It is then subsequently administered orally and gradually tapered, depending on signs of toxicity or rejection and blood levels. Trough blood levels are periodically monitored, and doses are adjusted to maintain levels of greater than 200 ng/mL. Prednisone is given on the day of transplantation and gradually reduced during the course of 12 weeks. In many centers the induction agents consist of one of the anti–IL-2R α chain antibodies, daclizumab or basiliximab, along with steroids, mycophenolate mofetil (instead of azathioprine), and tacrolimus (instead of cyclosporine). Some transplantation surgeons are combining plasmapheresis, intravenous immunoglobulin, and immunosuppressive drugs for patients who are highly sensitized and have high titers of alloantibodies.[29,30] Acute rejection episodes are treated with intravenous pulses of high-dose methylprednisolone. Among the most useful agents have been ALG for 5 days, ATG for 5 days, and OKT3 for 1 to 14 days. Another anti-lymphocyte mAb, anti-CD52 or alemtuzumab, has also been used successfully, although with differences in the incidence of opportunistic infections.[31,32]

Rejection. Rejection is the most common problem during the 3 months immediately after kidney grafting.[27] Except for hyperacute rejection, most such episodes can be partially or completely reversed by one of the previously described immunosuppressive agents. Rejection episodes are classified as follows (Table II).

Hyperacute rejection occurs within the first 48 hours after the anastomosis takes place in recipients with preformed anti-leukocyte antibodies. It is characterized by fever and anuria. The binding of cytotoxic antibodies to the vascular endothelium activates complement, with subsequent aggregation of neutrophils and platelets, resulting in thrombosis. This is an irreversible event, and the only treatment option is immediate graft removal.

Accelerated rejection occurs on the third to fifth day after transplantation. It is accompanied by fever, graft swelling, oliguria, and tenderness. It is thought to be mediated by non–complement-fixing antibodies to antigens present in the donor kidney. Histopathologically, it is characterized by vascular disruption with hemorrhage. The most effective treatments are anti-lymphocyte reagents, with or without plasmapheresis; these have a success rate of about 60% in reversing this process.

Acute rejection, the most common form, is due to a primary allogeneic response occurring within the first 6 to 90 days after transplantation. It is mediated by both T cells and antibodies, which cause tubulitis and vasculitis, respectively. High-dose pulses of steroids and anti-lymphocyte reagents are effective in reversing the T-cell response about 80% to 90% of the time, but anti-lymphocyte antibodies only reverse the vasculitis about 60% of the time.

Chronic rejection occurs when the tenuous graft tolerance is disturbed 2 or more months after transplantation. It is characterized by marked proteinuria, occasional hematuria, hypertension, and the nephritic syndrome. The primary mediator of this type of rejection is antibody. A kidney biopsy is usually necessary to distinguish rejection from cyclosporine or tacrolimus nephrotoxicity. This process is usually treatment resistant, although progression might be slowed by immunosuppressive regimens.

Efficacy. Renal grafts from HLA-identical sibling donors have a 10-year survival of about 74%. Those transplants from "6 HLA antigen–matched" cadavers have currently a 1-year survival of 95%. The estimated graft survival has slowly improved over time, and the most recent data, from the 1998-1999 cohort, is estimated at 11.6 years, according to national statistics. Grafts from living donors have a higher estimated lifespan of 15 years.[27,33]

Liver and intestinal transplantation

Liver transplantation had its inception in 1963, when the diseased liver of a 3-year-old child with extrahepatic biliary

TABLE II. Solid-organ rejection patterns: Renal rejection as an example

Type	Time after transplantation	Signs and symptoms	Rapidity of onset	Immune component	Pathologic findings	Treatment	Success rate (%)
Hyperacute	<24 h	Fever, anuria	Hours	Antibody and complement	Polymorphonuclear neutrophil deposition and thrombosis	None	0
Accelerated	3-5 d	Fever, graft swelling, oliguria, tenderness	1 d	Non–complement-fixing antibody	Vascular disruption hemorrhage	ALG, ATG, anti-CD3	60
Acute	6-90 d	Oliguria, salt retention, graft swelling, tenderness, sometimes fever	Days to weeks	T cells and antibody	Tubulitis, endovasculitis	Steroids, ALG, ATG, anti-CD3	60-90
Chronic	>60 d	Edema, hypertension, proteinuria, occasional hematuria	Months to years	Antibody	Vascular onion skinning	None	0

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atresia was replaced.[34] Although that patient died, subsequent successes have established liver transplantation as the standard therapy for advanced chronic liver disease.[35] Since 1983, the 1-year survival rates have increased from 25% to 78%, depending on the age and health of the recipient, the underlying condition, and various clinical considerations.

Liver transplantation is indicated for chronic end-stage liver disease, fulminant acute liver failure, and cancer limited to the liver.[36] As with renal transplantation, combined therapy targeting several facets of the potential rejection process is used for liver transplantation.

Anti–IL-2R α chain antibodies are given intravenously on the day of transplantation, followed by tacrolimus, which is given intravenously initially and orally thereafter and then by mycophenolate mofetil and steroids tapered slowly over a year. Survival has increased by 20% in the last 2 decades with tacrolimus-based immunosuppression.[37]

Although this improvement might be the result of several factors, retransplantation as a result of acute or chronic rejection has not occurred in patients treated with tacrolimus. Similar to all solid-organ transplantation, lack of suitable donors is a major problem for liver transplantation. Since 1988, this organ shortage has been approached by partial hepatectomies of living related donors. Donor safety is much greater with use of the left lateral segment.[38]

Intestinal transplantation is offered to patients who have intestinal failure (caused by short-bowel syndrome, mucosal disease, motility disorders, and tumors) and who present with severe complications of parenteral nutrition, such as cholestatic liver disease and recurrent loss of central venous access.[39] Advances in surgical techniques, control of immune rejection, and treatment of infections have improved the outcomes over time. In 2008, 185 intestinal transplantations were performed in the United States. The 1-year rate of patient survival has increased from 57% in 1997 to 80% in 2005 and to 90% if the data are limited to centers that perform the largest number of transplantations.

Heart, heart-lung, and lung transplantation

The various forms of cardiomyopathy are the most common indications for heart transplantation, followed by congenital heart disease. Approximately 25% of heart transplant recipients are infants.[22] Immunosuppressive regimens for heart transplantation are similar in many respects to those already described for renal and hepatic grafts. Usually an anti–IL-2R α chain mAb is given for induction therapy on the day of transplantation, along with high-dose intravenous methylprednisolone. Prednisone is given after the operation and maintained orally until it is discontinued after the first normal findings from an endomyocardial biopsy. Tacrolimus is then begun as the primary immunosuppressive agent with or without mycophenolate.[22]

Since the introduction of cyclosporine 26 years ago, the results of cardiac transplantation have improved greatly. The International Heart Transplantation Registry has shown a 4-year survival of 71% for patients receiving cyclosporine- or tacrolimus-based triple immunosuppression therapy. Survival is influenced by the age of the recipient; patients younger than 40 years have a better survival.[40] Lung transplantation has been performed for the following major diagnostic categories: cystic fibrosis, pulmonary vascular disease, bronchiolitis obliterans, pulmonary alveolar proteinosis, and pulmonary fibrosis, with 4-year survival at approximately 50%.[22,41]

BONE MARROW TRANSPLANTATION

Since 1955, more than 240,000 bone marrow transplantations have been performed worldwide at 450 centers in 47 countries for the treatment of more than 50 different fatal diseases (Table III).[42] Most of these transplantations have been done by reinfusing stored autologous marrow cells collected before the patient receives intensive chemotherapy or irradiation. Annually, 25,000 to 35,000 autologous transplantations are performed compared with approximately 15,000 allogeneic transplantations. Certain unique problems distinguish bone marrow transplantation from transplantation of solid organs, such as the kidney, liver, and heart. The first problem is that immunocompetent cells, both in the recipient and in the donor marrow or blood, have the potential to reject each other, resulting in graft rejection on the one hand and GVHD on the other.[43] The second concern is that successful unfractionated marrow grafting usually requires strict donor and recipient MHC class II antigen compatibility to minimize such reactions. Finally, except for patients with severe combined immunodeficiency (SCID), complete DiGeorge anomaly, or identical twin donors, even HLA-identical recipients must be pretreated with cytotoxic and myeloablative agents to prevent graft rejection.[43] Diseases treated successfully by allogeneic bone marrow transplantation include radiation injury, primary

TABLE III. Conditions treated with hematopoietic stem cell transplantation

Leukemias	Acute lymphoblastic leukemia Acute myelogenous leukemia Chronic lymphocytic leukemia Chronic myelogenous leukemia
Lymphomas	Non-Hodgkin lymphoma Hodgkin disease
Plasma cell disorders	Multiple myeloma and related disorders
Solid-organ neoplasias	Breast cancer, ovarian cancer, melanoma neuroblastoma, lung cancer, sarcoma
Myelodysplastic syndromes	
Severe aplastic anemia	
Autoimmune diseases	Multiple sclerosis, systemic sclerosis, systemic lupus erythematosus
Inherited erythrocyte abnormalities	Sickle cell disease, thalassemia
Inherited metabolic diseases	Mucopolysaccharidosis type I, adrenoleukodystrophy, osteopetrosis
Primary immunodeficiencies	SCID Wiskott-Aldrich syndrome CGD Leukocyte adhesion deficiency CD40 ligand deficiency X-linked lymphoproliferative disease Hemophagocytic lymphohistiocytosis

immunodeficiencies, hemoglobinopathies, aplastic anemia, multiple myeloma, leukemia, neuroblastoma, non-Hodgkin lymphoma, inborn errors of metabolism, and certain autoimmune diseases.[44] In addition, autologous marrow transplantation has been used after lethal irradiation or chemotherapy in the treatment of patients with some hematologic malignancies, solid tumors, or breast cancer, as well as for the treatment of several autoimmune diseases.[45]

Other sources of hematopoietic stem cells for transplantation

Bone marrow is not the only source of hematopoietic stem cells. These cells are capable of reconstituting all blood cell lineages and can also be obtained from peripheral blood or cord blood. Peripheral blood–derived hematopoietic stem cells are retrieved after the donor receives granulocyte colony-stimulating factor, usually at 5 to 10 μg/kg/d for 5 days, to allow mobilization of the hematopoietic stem cells. These are then collected by means of leukapheresis, and the stem cells are positively selected by using affinity columns containing antibodies to the cell-surface markers CD34 or CD133, both of which are suggested to have the highest specificity for pluripotential hematopoiesis.[46] Cord blood is increasingly being used because of its availability and simplicity of procurement and the potential of a lower severity of GVHD without full HLA matching.[47] The number of cells in cord blood units is a limiting factor that is currently being addressed by using more than 1 donor's cord blood.

Clinical features of GVHD

Acute GVHD begins 6 or more days after transplantation (or after transfusion in the case of nonirradiated blood products).[48] Signs of GVHD include fever, a morbilliform erythematous rash, and severe diarrhea.[49] The rash becomes progressively confluent and might involve the entire body surface; it is both pruritic and painful and eventually leads to marked exfoliation. Eosinophilia and lymphocytosis develop, followed shortly by hepatosplenomegaly, exfoliative dermatitis, protein-losing enteropathy, bone marrow aplasia, generalized edema, increased susceptibility to infection, and death.[50] Skin biopsy specimens reveal basal vacuolar degeneration or necrosis, spongiosis, single-cell dyskeratosis, eosinophilic necrosis of epidermal cells, and a dermal perivascular round cell infiltration. Similar necrotic changes can occur in the liver, intestinal tract, and eventually most other tissues.

Treatment of GVHD

Many regimens have been used to mitigate GVHD in both HLA-incompatible and HLA-compatible bone marrow transplants. In MHC-compatible bone marrow transplants into patients with SCID or complete DiGeorge anomaly, it is not usually necessary to give immunosuppressive agents to prevent or mitigate the mild GVHD that might occur, although occasionally steroids are used to treat more severe forms of this condition. For unfractionated, HLA-identical marrow transplants into all patients for whom pretransplantation chemotherapy is given to prevent rejection, however, it is necessary to use prophylaxis against GVHD. Patients are usually given a combination of methotrexate, corticosteroids, and a calcineurin inhibitor daily for 6 months.[51-53] When GVHD becomes established, it is extremely difficult to treat. Antithymocyte serum, steroids, cyclosporine, tacrolimus, anti–IL-2R α chain antibodies, anti–TNF-α inhibitors, mycophenolate mofetil, and murine mAbs to human T-cell surface antigens have ameliorated some cases, but the course has been inexorably fatal in many patients similarly treated.[54-56] The best approach to GVHD reactions is prevention, and by far the best preventive approach is the removal of all postthymic T cells from the donor marrow or blood.

HLA-identical bone marrow transplantation for patients with SCID

The only adequate therapy for patients with severe forms of cellular immunodeficiency is immunologic reconstitution by means of transplantation of immunocompetent hematopoietic stem cells. Until 1980, only HLA-identical unfractionated bone marrow could be used for this purpose because of the lethal GVHD that ensued if mismatched donors were used.[57] In most cases, both T-cell and B-cell immunity have been reconstituted by such fully matched transplants, with evidence of function detected very soon after unfractionated marrow transplantation.[58] Analysis of the genetic origins of the immune cells in the engrafted patients has revealed that although the T cells are all of donor origin, the B cells are often those of the recipient.[12] Initially, it was considered that bone marrow was effective in conferring immunity in patients with SCID because it provided normal stem cells, but it is apparent from later experience with T cell–depleted marrow[59] that the early restoration of immune function after unfractionated HLA-identical marrow transplantation is caused by adoptive transfer of mature T and B cells in the donor marrow. Unfortunately, because of the lack of HLA-identical related donors, unfractionated bone marrow transplantation has not been possible for more than 85% of the immunodeficient patients who could have benefited. As a consequence, before the year 1982, most such patients died with severe infections.

HLA-haploidentical bone marrow transplantation for patients with SCID

The fact that totally HLA-disparate fetal liver cells could correct the immune defect in a few such patients without causing GVHD gave hope that HLA-disparate marrow stem cells could do the same if all donor postthymic T cells could be removed. Early success in T-cell depletion was achieved in experimental animals by treating donor marrow or spleen cells with anti–T-cell antiserum or agglutinating the unwanted cells with plant lectins.[60] The remaining immature marrow or splenic non-T cells restored lymphohematopoietic function to lethally irradiated MHC-disparate recipients without lethal GVHD. This approach was applied to human subjects in the early 1980s and has been highly successful in infants with SCID.[12,59-63]

The time to development of immune function after haploidentical stem cell grafting is quite different from that after unfractionated HLA-identical marrow grafting. Lymphocytes with mature T-cell phenotypes and functions fail to increase significantly until 3 to 4 months after transplantation; normal T-cell function is reached between 4 and 7 months.[59] B-cell function develops much more slowly, averaging 2 to 2.5 years for normalization; many do not have B-cell function developed, despite normal T-cell function.[12,13] Genetic analyses of the lymphocytes from such chimeric patients have revealed all T cells to be genetically from donor origin, whereas the B cells and APCs almost always remain those of the recipient.[61,62] These observations indicate that the thymic microenvironment of most infants with SCID is capable of differentiating half-matched normal stem cells to mature and functioning T lymphocytes that can cooperate effectively with host B cells for antibody production. Thus the genetic defect in SCID does not compromise the function of the thymus.

Efficacy of bone marrow transplantation in patients with immunodeficiency diseases

Although precise figures are not available, during the past 40 years, more than 1,200 patients worldwide with different forms of genetically determined immunodeficiency have been given bone marrow transplants in attempts to correct their underlying immune defects. Possibly because of earlier diagnosis before untreatable opportunistic infections develop, the results have improved considerably during the last 2 decades.[62-67] As would be expected, survival outcomes of HLA-matched related transplants have been superior to those of HLA-haploidentical or HLA-identical unrelated transplants in several series of patients treated in specialized centers worldwide.

SCID. Bone marrow transplantation has been more widely applied and more successful in infants with SCID than any other primary immunodeficiency. The use of pretransplantation myelosuppressive or myeloablative conditioning is advocated by some investigators to prevent graft rejection, but because infants with SCID lack T cells, there should be no need to use pretransplantation chemotherapy. The largest multicenter report of patients with SCID who received bone marrow transplantation was a European collaborative study from 1968 to 1999, including 153 patients receiving an HLA-matched related (from parent or sibling) transplant, with a survival rate of 77%, and 294 patients receiving a haploidentical HLA-matched transplant, with a survival of 54%.[63] Twenty-eight patients received an HLA-matched unrelated donor transplant, with a survival rate of 63%. These outcomes have improved in the last decade, likely because of progress in early diagnosis and medical care, specifically in the availability of newer antibacterial and antiviral agents, as well as immunosuppressive drugs for the control and prophylaxis of GVHD. In addition, difference in the use of myeloablative and rejection prophylaxis regimens with their inherent toxicity is a variable that affects the survival rate. The largest series of patients with SCID receiving bone marrow transplantation in the United States reported 161 patients who did not receive pretransplantation conditioning.[62,68] Sixteen of them received an HLA-matched related donor transplant, with 100% survival. The others received a haploidentical HLA-matched related donor transplant, with a long-term (up to 26 years) survival rate of 77%. Nevertheless, this is a major accomplishment because SCID is 100% fatal without marrow transplantation or, in the case of adenosine deaminase (ADA)–deficient SCIDs, enzyme replacement therapy. Of note, those who underwent transplantation earlier than 3.5 months of age had a survival of 94%, possibly reflecting the influence of opportunistic infections as determinants of transplantation success. These studies and others have shown that such transplants can provide normal numbers of T cells and normalize T-cell function in all known molecular types of SCID. Thus there appears to be no survival advantage in performing such transplantations *in utero*[69,70] as opposed to performing them soon after birth. *In utero* transplantations carry the risks associated with the invasive procedure that involves accessing the fetus and the difficulty of monitoring the possible development of GVHD during gestation.

Other primary immunodeficiencies. The second largest group of patients with immunodeficiency given bone marrow transplants since 1968 are those with the Wiskott-Aldrich syndrome.[71,72] In a report from the International Bone Marrow Transplant Registry, 170 patients with Wiskott-Aldrich syndrome had undergone transplantation, and the 5-year probability of survival for all subjects was 70% (95% CI, 63% to 77%). Probabilities differed according to donor type: 87% (95% CI, 74% to 93%) with HLA-identical sibling donors, 52% (95% CI, 37% to 65%) with other related donors, and 71% (95% CI, 58% to 80%) with matched unrelated donors ($P = .0006$). Boys who had received a matched unrelated donor transplant before 5 years of age had survivals similar to those receiving HLA-identical sibling transplants. Of note, the incidence of autoimmunity in these patients after bone marrow transplantation is up to 20%.[72]

Patients with combined immunodeficiencies characterized by less severe T-cell defects than those seen in patients with SCID, such as ZAP70 deficiency, constitute the third largest group of patients given bone marrow transplants. Forty-five patients with Omenn syndrome were reported as having received marrow transplants, and 23 (51%) were alive at the time of the report.[61] Fourteen (54%) of 26 patients with the bare lymphocyte syndrome were alive after having been given marrow transplants.[73,74] Other disorders treated successfully with bone marrow transplantation include X-linked hyper-IgM,[75] reticular dysgenesis,[76] purine nucleoside phosphorylase deficiency,[77] cartilage hair hypoplasia, and X-linked lymphoproliferative syndrome.

Patients with complete DiGeorge syndrome have undergone both marrow and thymic transplantations. Six of 9 such patients were reported to have survived 2 to 24 years after having received unfractionated HLA-identical sibling marrow[78]; however, possible publication bias was suggested, proposing that a number of patients who might not have survived had not been taken into account.[79] Because the underlying defect in this condition is absence of the thymus, a more direct approach is to perform

thymus transplantation. To this end, 54 infants with complete DiGeorge syndrome have undergone thymic transplantation with cultured HLA-unmatched unrelated thymic tissue, with a survival rate of 69%.[80] An important immunologic difference is that the transplanted thymus allows the development of naive T cells even with a disparate HLA haplotype between donor and recipient. In contrast, patients with complete DiGeorge syndrome who receive bone marrow transplants survive with a reduced T-cell number and absent naive T-cell population.

Patients with primarily phagocytic disorders also have been shown to benefit from bone marrow transplantation. Recently, a report from Europe included data from 24 patients with chronic granulomatous disease (CGD) who had received bone marrow transplants, with 19 patients surviving.[81] At Texas Children's Hospital (Houston, Texas), 11 patients with CGD (9 with X-linked CGD and 2 with autosomal recessive CGD) have undergone transplantation, with 10 patients surviving and immunoreconstituted and a median follow-up of 25 months (unpublished data). Four of these received HLA-matched related transplants, and 6 received HLA-matched unrelated grafts. One patient who received a mismatched related (HLA 5/6 matched) transplant did not survive. Other leukocyte disorders that have been successfully treated with bone marrow transplantation include pigmentary dilution (Griscelli) syndrome, Chediak-Higashi syndrome, familial hemophagocytic histiocytosis, severe congenital neutropenia, and leukocyte adhesion deficiency.[61,82]

Efficacy of bone marrow transplantation in malignancy

Bone marrow transplantation is the therapy of choice for leukemia, lymphoma, and myelodysplastic proliferative disorders.[83] The success of marrow transplantation in curing malignancy depends on a number of factors, the most important of which are the type of malignant disease, the stage of the disease, and the age of the recipient. Most patients with acute myelogenous leukemia achieve remission after chemotherapy; however, approximately 65% of patients will relapse within 2 years.[84] During the first complete remission, consolidation chemotherapy or bone marrow transplantation are possible alternatives. In patients with intermediate-risk disease, the projected disease-free survivals at 5 years are 52% for allogeneic transplantation and 45% for autologous transplantation.[85] For patients with chronic myelogenous leukemia, allogenic bone marrow transplantation is considered primarily for pediatric patients, with a success rate of more than 80%, and for those adults who have had unsuccessful medical treatment with tyrosine kinase inhibitors.[83,86] Three-year overall survival is variable among different series, reaching up to 80%. The best survival rates with the lowest probability of relapse occurred in patients younger than 20 years who had acute nonlymphocytic leukemia and underwent transplantation in first remission and in patients with chronic myelogenous leukemia who underwent transplantation in the chronic phase.[87]

The rationale for allogeneic bone marrow transplantation in patients with leukemia is the hope that the leukemic cells can be reduced or eliminated by means of irradiation or chemotherapy and that the grafted allogeneic normal T cells can then reject any remaining leukemic cells.[88] Supporting a need for T cells in the graft is the fact that T cell–depleted bone marrow transplants have been associated with a higher degree of leukemia recurrence.[89]

Efficacy of bone marrow transplantation in hemoglobinopathies, osteopetrosis, metabolic storage diseases, and severe autoimmunity

Bone marrow transplantation has been highly effective for the treatment of homozygous β-thalassemia, with survivals reaching 70% to 80% for marrow transplants from HLA-identical siblings.[90] Likewise, HLA-identical bone marrow transplantation has also been successful for patients with sickle cell disease, with 59 patients known to have been treated, 55 of whom were surviving, with 50 free of sickle cell disease.[91] The European Bone Marrow Transplantation Group reported on 69 patients with autosomal recessive osteopetrosis who received HLA-identical or haploidentical bone marrow transplants between 1976 and 1994.[92,93] Recipients of genotypically HLA-identical marrow had an actuarial probability for 5-year survival of up to 60%, with osteoclast function of 79% of the survivors. Mucopolysaccharidosis type I (Hurler disease) and adrenoleukodystrophy, but not other lysosomal storage diseases, have been successfully treated with bone marrow transplantation when performed before significant organ damage occurs, as an alternative to enzyme replacement.[94] Autologous and allogeneic bone marrow transplantation protocols have been used with relative success in patients with severe autoimmunity. In a large collaborative study of more than 500 patients with autoimmune conditions, survival was 80%, with sustained improvement in 70% of the survivors.[95]

Nonmyeloablative bone marrow transplantation

For patients with pre-existing organ damage, there is significant morbidity and mortality from traditional conditioning regimens with busulfan and cyclophosphamide or irradiation. Because of this, there has been increasing interest in developing conditioning regimens that are less toxic.[96]

This has been accomplished by using either total lymphoid irradiation or a combination of nucleoside analogs and anti–lymphocyte antibody preparations. Although these regimens are significantly less cytotoxic than high-dose alkylating agents and total-body irradiation, they are profoundly immunosuppressive. Opportunistic infections, such as the reactivation of cytomegalovirus, remain clinical obstacles when nonmyeloablative stem cell transplantations are performed with these agents, especially in elderly and previously immunosuppressed patients. GVHD prophylaxis with cyclosporine and methotrexate, with added mycophenolate mofetil in some cases, has been necessary because GVHD is common after nonmyeloablative transplantation.

Gene therapy for primary immunodeficiencies

Gene therapy trials in the last decade have shown "proof of concept" that genetic disorders can be modified and even cured. Significant progress was made in patients with X-linked SCID, ADA-deficient SCID, and X-linked CGD. The reports by Cavazzana-Calvo et al[97] and Hacein-Bey-Abina et al[98,99] of successful gene therapy in infants with X-linked SCID represented a major step forward because repeated efforts to achieve gene correction of ADA-deficient SCID had failed during the decade before 2000. Subsequently, Gaspar et al[100] reported a similar gene therapy protocol for X-linked SCID conducted in London, confirming the efficacy of this novel approach. The group at the Hôpital Necker in Paris treated 11 patients with X-linked SCID with

gene-corrected autologous bone marrow cells. Nine infants had normal T- and B-cell functions after the treatments. Two did not improve and were given allogeneic bone marrow transplants. The 9 patients who did acquire normal immune function did not require intravenous immunoglobulin infusions and were at home without any medication. Four of the 10 patients treated in London have poor B-cell reconstitution and are dependent on immunoglobulin supplementation. Natural killer cell reconstitution in this molecular type of SCID is also poor, which is similar to that seen in patients who receive bone marrow transplantation.

However, serious adverse events with this therapy occurred in 4 patients treated at the Hôpital Necker and 1 patient treated in London.[99] Shortly before varicella developed, the first patient was discovered to have a high white blood cell count as a result of an expanded clonal population of circulating γδ-positive T cells. The white blood cell count became much higher and became a leukemic-like process that was treated with chemotherapy. The T-cell clone was shown to carry the inserted retroviral gene vector within an intron in a gene on chromosome 11 called *LMO2*. *LMO2* is an oncogene that is aberrantly expressed in acute lymphoblastic leukemia of childhood.[101] Similarly, the other 3 patients in that protocol and 1 of the 10 patients treated in London had T-cell proliferation with upregulation of the expression of not only *LMO2* but also of other oncogenes. Fortunately, 4 of these patients responded to conventional chemotherapy regimens and are presently in remission, with a relatively normal quality of life. Insertional oncogenesis has long been known to be a potential complication of retroviral vector gene transfer because retrovirus integration might occur within oncogenes in the genome. This complication has been thought to be unlikely with such vectors because the vectors cannot reproduce themselves and cannot repeatedly insert into the cell's chromosomes to increase the likelihood of malignant change. Before these cases, malignant changes had not been seen in any human subjects given retroviral vectors for gene transfer. Considering the success of bone marrow transplantation for recipients of HLA-matched related donor grafts and for those who are treated in early infancy, new gene therapy trials for X-linked SCID are now being developed with the objective of reducing their oncogenesis potential, such as with the use of lentivirus-based gene vectors.[102]

Gene therapy trials for ADA deficiency were initiated in the early 1990s, with targeting of peripheral lymphocytes and later CD34-enriched bone marrow cells. The success of these trials was modest, resulting in detection of a small proportion of gene-modified cells in peripheral blood but no evidence of immunologic benefits.[103] The required concomitant use of polyethylene glycol–modified bovine ADA is considered to have been a contributing cause to the failures in the US trials. Recently, 2 European research groups reported gene therapy trials for ADA deficiency using low-dose busulfan pretherapy without polyethylene glycol–modified bovine ADA or (in those patients who were receiving it) withdrawing the enzyme for a few weeks before infusion of the gene-modified cells.[104,105] Eleven of the 15 patients treated with this approach (10 in Italy and 5 in London) showed good immunoreconstitution. Of note, there have not been cases of leukemia or lymphoma in the cases of ADA-deficient SCID that have been corrected by gene therapy, although insertions of gene vectors near oncogenes similar to the X-linked SCID trials have been observed.

A small number of patients with X-linked CGD have been treated with gene therapy approaches.[106] In the United States initial efforts in 1997 by Malech and collaborators resulted in the detection of genetically corrected cells, although in minimal proportion (<1% of granulocytes). A more recent European trial adding a myeloablative regimen before infusion of the gene-corrected cells showed a larger proportion of gene-modified cells, although with only transient expression of the gene. The treatment provided initial clinical benefit, including resolution of severe and chronic fungal and bacterial infections. Patients in one of the trials demonstrated cell expansion as a result of insertional mutagenesis and required bone marrow transplantation, which was curative in one of 2 patients.[107] Efforts aimed to improve the expression of the gene and to reduce oncogenesis are underway.

CONCLUSIONS

Advances in transplantation immunology have allowed the exponential growth of organ and tissue transplantation in medicine over the last 3 decades. Newer immunosuppressive agents have allowed the control of solid-organ and tissue rejection and GVHD, even when HLA incompatibility is present. For the treatment of hematologic disorders, including primary immunodeficiencies, hematopoietic stem cell transplantation is not only feasible but is also the treatment of choice in many cases. Future developments in the field of transplantation immunology will hopefully include novel immunosuppressors with less toxicity and more specificity to control graft rejection while sparing overall immunity and thereby enabling better infection control. Gene therapy has shown promise in curing severe primary immunodeficiencies; however, problems with this approach urgently need to be addressed, the most important of which is insertional mutagenesis seen with the gene vectors used to date.

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Embryonic and adult stem cell therapy

Anne C. Brignier, MD, and Alan M. Gewirtz, MD *Philadelphia, Pa*

There are many types of stem cells. All share the characteristics of being able to self-renew and to give rise to differentiated progeny. Over the last decades, great excitement has been generated by the prospect of being able to exploit these properties for the repair, improvement, and/or replacement of damaged organs. However, many hurdles, both scientific and ethical, remain in the path of using human embryonic stem cells for tissue-engineering purposes. In this report we review current strategies for isolating, enriching, and, most recently, inducing the development of human pluripotent stem cells. In so doing, we discuss the scientific and ethical issues associated with this endeavor. Finally, progress in the use of stem cells as therapies for type 1 diabetes mellitus, congestive heart failure, and various neurologic and immunohematologic disorders, and as vehicles for the delivery of gene therapy, is briefly discussed. (J Allergy Clin Immunol 2010;125:S336-44.)

Key words: *Stem cells, human embryonic stem cells, induced pluripotent stem cells, regenerative medicine, gene therapy, cell therapy*

Abbreviations used

AHSCT:	Autologous hematopoietic stem cell transplantation
G-CSF:	Granulocyte-colony stimulating factor
GVHD:	Graft-versus-host disease
GVL:	Graft-versus-leukemia
hESC:	Human embryonic stem cell
hESC-CM:	Human embryonic stem cell–derived cardiomyocyte
HSC:	Hematopoietic stem cell
HSCT:	Hematopoietic stem cell transplantation
iPSC:	Induced pluripotent stem cell
LVEF:	Left ventricular ejection fraction
MSC:	Mesenchymal stem cell
NK:	Natural killer
NSC:	Neural stem cell
SCID:	Severe combined immunodeficiency
T1DM:	Type 1 diabetes mellitus
UCB:	Umbilical cord blood

Stem cells are not homogeneous but exist instead as part of a developmental continuum. The most primitive of the cells is the totipotent stem cell. This cell has the potential to develop into a complete embryo (ie, to form any type of cell, including extraembryonic tissues [embryonic membranes, umbilical cord, and placenta]). This unique property is evanescent. It appears with fertilization of the egg and disappears by the time the embryo reaches the 4- to 8-cell stage. With subsequent divisions, embryonic stem cells lose the ability to generate an entire organism. However, they are capable of differentiating into cells present in all 3 embryonic germ layers, namely ectoderm, mesoderm, and endoderm, and on this basis are called pluripotent. With subsequent divisions, cells become more and more restricted in their ability to differentiate into multiple lineages. They are then called multipotent; that is, they are capable of forming a limited number of cell types. This is the property of adult stem cells, also referred to as somatic stem cells or nonembryonic stem cells, which are able to self-renew during the lifetime of the organism and to generate differentiated daughter cells. In the adult, tissues are in a perpetual state of flux under homeostatic conditions. Even in the absence of injury, they are continuously producing new cells to replace those that have worn out. For this reason, adult stem cells can be found in a metabolically quiescent state in most specialized tissues of the body, including the brain, bone marrow, liver, skin, and gastrointestinal tract. These cells are scarce, however, and with the relative exception of hematopoietic stem cells (HSCs), they are difficult to isolate. Typically, preparations of these cells are often contaminated with more differentiated progenitor cells, which decreases the long-term efficiency of the product because progenitor cells are fixed with respect to cell fate and do not self-renew.

One could argue that 3 major technologic achievements have driven the field of stem cell therapeutics. The first occurred in 1961, when the pioneering studies of Till and McCulloch,[1] using a revolutionary *in vivo* bioassay, unequivocally demonstrated the existence of HSCs. The second major enabling technologic leap occurred in 1998, when Thomson et al[2] reported the isolation of human embryonic stem cells (hESCs) from blastocysts and the creation of hESC lines for study. The most recent was reported by Yamanaka's group[3] in 2006, which induced the formation of pluripotent stem cells from murine fibroblasts.

Each of these advances has furthered the ability of researchers to use stem cells for basic research on cell-lineage fate and development, as well as for drug testing, modeling, and treating disease. It is in the latter area, in particular, that exciting progress has been made over the last few years.

SOURCES OF STEM CELLS

Having defined the different types of stem cells, we will now describe currently available sources of stem cells.

hESCs

hESCs are characterized by self-renewal, immortality, and pluripotency. Ongoing attempts to use hESCs in the laboratory finally came to fruition in 1998 with the creation of several human

From the Division of Hematology/Oncology, Department of Medicine, and the Abramson Family Cancer Research Institute, University of Pennsylvania School of Medicine.

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Address for reprints: Anne C. Brignier, MD, Division of Hematology/Oncology, Department of Medicine, Rm 727, BRB II/III, University of Pennsylvania School of Medicine, 421 Curie Blvd, Philadelphia, PA 19104. E-mail: brignier@free.fr.

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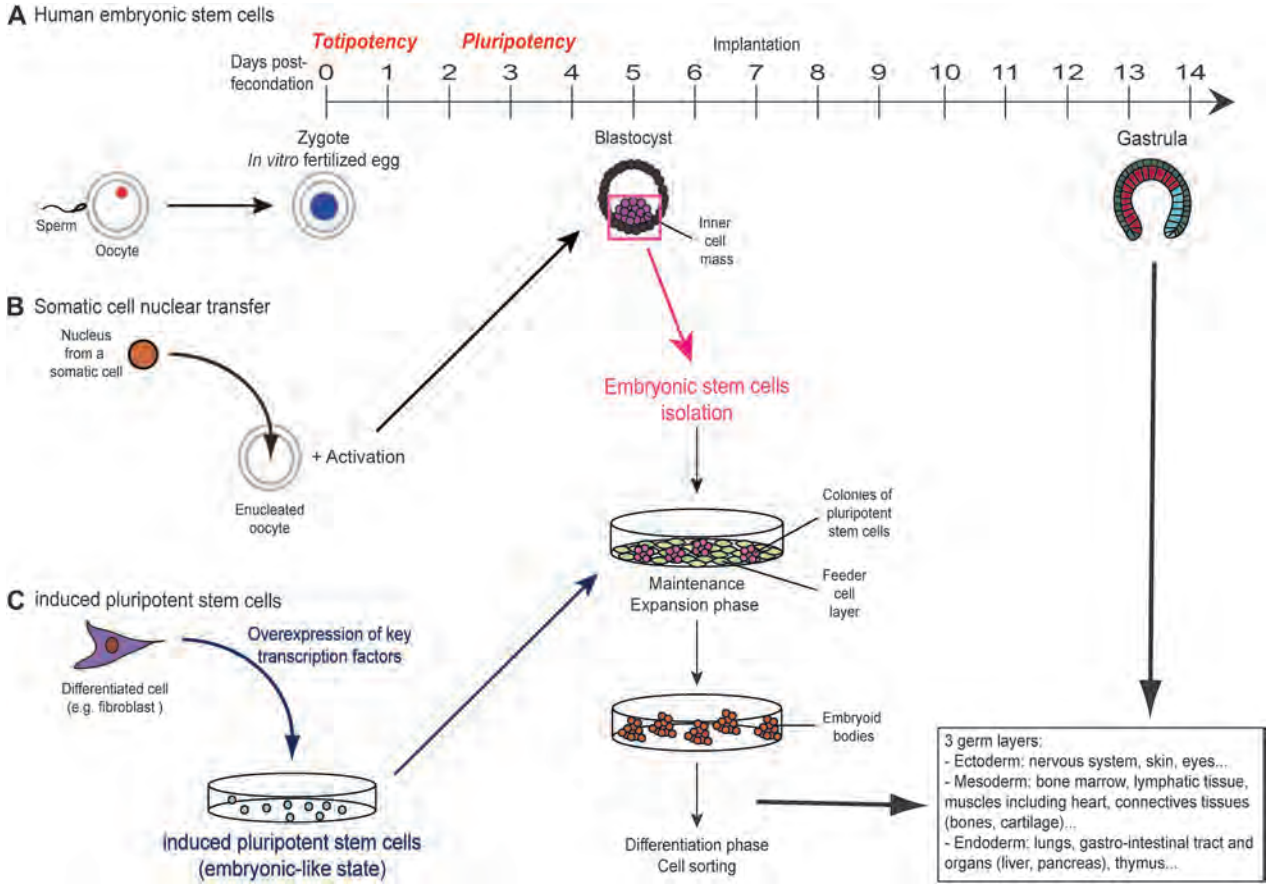

FIG 1. Isolation, generation, and culture of pluripotent stem cells. **A**, After isolation, typically from the inner cell mass of the blastocyst made by means of *in vitro* fertilization, hESCs are expanded in culture. They are classically grown on feeder cell layers, the purpose of which is to expand the cells while maintaining their undifferentiated state (maintenance/expansion phase). Initially those feeder layers were of xenogeneic origin (irradiated murine embryonic fibroblasts), but human feeder layers are being developed and will likely be used with increasing frequency in the future. When removed from feeder layers and transferred to suspension cultures, hESCs begin to form 3-dimensional multicellular aggregates of differentiated and undifferentiated cells termed embryoid bodies. Plated cultures of embryoid bodies spontaneously display a variety of cellular types from the 3 germ lineages at various differentiation stages. Theoretically, cells can be sorted according to differentiation markers, can be differentiated into any desired cells by adding specific growth factors (differentiation phase), or both. On a more practical level, it is difficult to induce hESC differentiation into a specific lineage, and highly definite culture protocols have to be developed for each desired cell type. **B**, Somatic cell nuclear transfer consists of injecting the nucleus from a somatic cell into an enucleated oocyte, followed by activation stimuli. The resulting embryo can be used to generate an hESC line (therapeutic cloning). **C**, iPSCs are generated from differentiated cells that have been reprogrammed to acquire a pluripotent state through overexpression of the key transcription factors *Oct4*, *Sox2*, and either *c-Myc* and *Klf4* or *Nanog* and *Lin28*. Overexpression can be achieved with viral vectors or proteins with or without histone-modifying chemicals. Once they are undifferentiated, they can be grown in culture like hESCs.

hESC lines,[2,4] although the cloning efficiency of such lines is still very low. Typically, hESCs are derived from the inner cell mass of 5-day-old blastocysts. The blastocysts in turn are made from unused embryos generated by means of *in vitro* fertilization for infertility problems. (It is important to note that the unused embryos can only be used for research purposes with the written informed consent of the parents.) Cells derived from earlier developmental stages can also be used.[5] At the other extreme, pluripotent cells have been isolated from the primordial germ cells of the gonadal ridge of the 5- to 9-week-old embryo. These are cells that normally become either oocytes or spermatozoa. Ironically, spontaneous differentiation in long-term *in vitro* culture of these so-called embryonic germ cells impeded their availability for research.[6] Accordingly, other strategies were and are still being developed with different methods and cell sources. For example, single-cell biopsy of the embryo[5] using a procedure not dissimilar to that used in preimplantation genetic diagnosis and that critically avoids the destruction of the embryo has been used with success, as have parthenogenesis of an unfertilized oocyte[7] and spermatogonial cells from adult human testis.[8] The latter developments are particularly exciting because they would allow the production of histocompatible cells that could be used in the donor. Technical methods for culture of hESCs are depicted in Fig 1, *A*.

Hundreds of hESC lines have been generated thus far. The first human stem cell line bank opened in 2004 in the United Kingdom (http://www.ukstemcellbank.org.uk/). The National Institutes of

Health registry (http://stemcells.nih.gov/research/registry/) has also archived a number of hESC lines and established criteria for demonstration of the pluripotency of these lines. Specifically, cells should be able to give rise to any cell lineage of the body and thus to form a teratoma (a tumor containing tissues from the 3 primary germ layers) *in vivo* after injection in an immune-compromised animal and should be capable of unlimited self-renewal.

Nuclear reprogramming and induced pluripotency

Nuclear reprogramming is a procedure that causes changes in gene expression that allow a cell of one type to develop into a cell of another type.[9] Recent strategies for generating stem cells are focused on nuclear reprogramming of differentiated cells to force them to become pluripotent. An example is somatic cell nuclear transfer (Fig 1, *B*). This consists of injection of the nucleus of a somatic cell into an enucleated oocyte.[10] The resulting pluripotent cells are genetically matched with the cell donor (this technique is thereby often called "therapeutic cloning"), except for the mitochondrial DNA, which comes from the egg. Another method is accomplished by means of cell fusion with an hESC, which can produce cells with some stem cell characteristics.[11]

A very recent and very exciting advance in reprogramming has been the generation of induced pluripotent stem cells (iPSCs). First reported in 2006 using murine fibroblasts,[3] iPSCs can be made from multiple murine and human somatic cell types,[12,13] and it is now possible to create patient-specific iPSCs.[14] iPSCs can be generated from differentiated cells by using retroviral-mediated expression of core transcription factors known to be required for maintenance of pluripotency and proliferation of embryonic stem cells.[3] These genes are *Oct4*, *Sox2*, and either *c-Myc* and *Klf4* or *Nanog* and *Lin28* (Fig 1, *C*). iPSCs exhibit similar features to embryonic stem cells, including cell morphology, cell-surface markers, growth properties, telomerase activity, expression, and epigenetic marks (ie, methylation or acetylation of histones, which result in changes in gene expression) of pluripotent cell–specific genes[12,13] but not global gene expression signatures.[15] They can give rise to cells derived from all 3 germ layers *in vitro* and *in vivo*, and murine iPSCs injected into murine blastocysts have been shown to contribute to embryonic development.[3]

Using pluripotent stem cells in the clinic: Scientific/medical issues

A number of scientific/medical issues need to be addressed before stem cells can be considered safe for clinical applications. The first hurdle is the tumorigenic potential of pluripotent cells (hESCs and iPSCs). Because pluripotency is evidenced by the ability to form teratomas when transplanted in immunodeficient mice, the concern exists that these cells could form malignant tumors in their new host. One strategy for dealing with this problem is to select pure populations of more committed cells for transfer. Demonstrating genetic and epigenetic stability will therefore be important before these cells are used clinically. In fact, karyotypic abnormalities have been described in several hESC lines, although changes might be at least partially dependent on culture techniques.[16]

In additional to biologic issues directly affecting the stem cell product, it is imperative that controlled, standardized practices and procedures be followed to maintain the integrity, uniformity, and reliability of the human stem cell preparations. Because stem cells are both maintained and expanded *in vitro* before transplantation, culture conditions compatible with human administration must be used. Feeder cells and sera of animal origin have to be reduced and ideally avoided to reduce the potential risk of contamination by xenogeneic protein and pathogens. Finally, transplantation of hESCs into patients is also limited by potential HLA incompatibility. Consequently, life-long immunosuppressive therapy, which can lead to infections and organ-based toxic side effects, such as nephropathy, might be required to prevent graft rejection. In this regard iPSCs hold great promise because they are histocompatible with the patient and because their use avoids one of the major ethical concerns (see below) associated with hESCs.

Although iPSCs solve the tissue-barrier problem, they too have technical drawbacks that are presently limiting their use. First is the issue of the risk of insertional mutagenesis caused by viral integration into the genome. This is of particular concern because patients who have received gene-modified lymphoid cells have had aggressive leukemias as a result of this phenomenon (see below).[17] The possibility that iPSCs might be generated with nonintegrating expression plasmids or adenoviral vectors is being explored in the murine system and appears possible.[18,19]

Another risk is reactivation of a viral oncogene, such as *c-Myc*, used to engineer the cells. Here there are data to suggest that the use of histone-modifying chemicals, such as the histone deacetylase inhibitor valproic acid, improves reprogramming efficiency and avoids the need to use *c-Myc* and that *Oct4* and *Sox2* alone are then sufficient in the generation of human iPSCs.[20] Recently, iPSCs were successfully obtained from murine fibroblasts cultured without any genetic material at all by using only valproic acid and recombinant proteins for the necessary transcription factors.[21] However, even as technical hurdles are overcome, generation of iPSCs still suffers from low efficiency and high cost, although no doubt these problems will be solved in time as well. In particular, the reprogramming efficiency is typically less than 1% but could depend on the differentiation stage of the cells. Indeed, the efficiency of iPSC generation has been recently increased to 28% with the use of hematopoietic stem and progenitor cells.[22]

Adult stem cells

The best-known example of the adult stem cell is the HSC, which is located in the bone marrow niche. HSCs and progenitors can be readily harvested from bone marrow and umbilical cord blood (UCB). They can even be collected from peripheral blood after mobilization from the marrow with granulocyte colony-stimulating factor (G-CSF) with or without CXCR4 antagonist.[23] HSCs are characterized by the expression of cell-surface markers, which allows for their isolation. In human subjects the HSC surface phenotype is typically lineage-specific antigen negative (lin^-), $CD34^+CD38^-CD133^+$c-Kit/$CD117^+CD59^+$Thy1/$CD90^+CXCR4^+$. Apart from differentiating into all myeloid and lymphoid lineages, HSCs have been shown to be able to differentiate *in vitro* into cells of nonhematopoietic lineages. However, such plasticity was probably an experimental artifact that is currently explained by the presence of heterogeneous populations of non-HSCs in hematopoietic organs or by the phenomenon of cell fusion.

Mesenchymal stem cells (MSCs) are another type of adult multipotent cells that are capable of differentiating into various mesodermal cell lineages, including myocytes, osteoblasts,

chondroblasts, fibroblasts, adipocytes, and other stromal elements. MSCs are present in almost all organs, but for therapeutic purposes, they are most conveniently isolated from bone marrow and UCB. MSCs can be organ specific. Consequently, populations isolated from various sources, although morphologically similar, might be functionally different. For example, MSCs isolated from the umbilical cord do not have the same abilities to give rise to osteoblasts, chondrocytes, and cardiomyocytes as bone marrow–derived MSCs.[24] MSCs can be readily expanded *ex vivo* and manipulated, if needed, to acquire specific properties. The International Society for Cellular Therapy recommended changing their name to "multipotent mesenchymal stromal cells" because the majority of MSCs lack complete "stemness" property and proposed minimal criteria for standardization of preparations.[25] Human MSCs must be plastic adherent; express CD105, CD73 and CD90; lack hematopoietic markers (CD45, CD34, CD14 or CD11b, CD79α or CD19 m and HLA-DR); and be able to differentiate into osteoblasts, adipocytes, and chondroblasts *in vitro*. MSCs display trophic, anti-inflammatory, and immunomodulatory capacities, both through secretion of soluble factors (indoleamine 2,3-dioxygenase, IL-6, TGF-β1, hepatocyte growth factor, inducible nitric oxide synthase, and prostaglandin) and direct cell-to-cell interaction with immune cells. *In vitro* MSCs suppress effector and cytotoxic T-cell, B-cell, natural killer (NK) cell, and dendritic cell activity and can induce regulatory T cells.[26] However, how MSCs assist in repairing a damaged organ is still unclear. Mounting evidence suggests that direct substitution of injured cells by *in situ* differentiated MSCs is unlikely (although still argued). Establishment of a favorable environment or niche for reconstruction of the tissue by intrinsic stem cells *per se* seems more likely. Regardless, because of their low immunogenicity and claimed beneficial effects on organ regeneration (whatever the mechanism), MSCs are being examined in an increasing number of regenerative medicine applications, as well as in inflammatory and immunologic diseases.

Finally, amniotic fluid, UCB, and the placenta are other sources of nonembryonic stem cells. However, it is not clear yet whether they are pluripotent or multipotent and how clinically useful they will be.

POTENTIAL CLINICAL USES OF STEM CELLS

Stem cells are postulated to have a tremendous number of applications, but tissue engineering seems to generate the greatest excitement. Stem cells can be used in regenerative medicine, immunotherapy, and gene therapy. Animal models and clinical studies have shown that transplantation of stem cells from diverse origins can successfully treat many acute and chronic diseases, such as immunohematologic disorders, type 1 diabetes mellitus (T1DM), Parkinson disease, neuronal destruction, and congestive heart failure.

Hematology-immunology

During the last 50 years, allogeneic hemopoietic stem cell transplantation (HSCT) has progressively become a common procedure for the treatment of a variety of inherited or acquired immunohematologic diseases, including thalassemias, sickle cell disease, Fanconi anemia, inborn errors of metabolism, severe aplastic anemia, severe combined immunodeficiency (SCID), and other primary immune deficiencies. HSCT is also widely used for the treatment of hematologic malignancies, such as acute myeloid and lymphoid leukemias, chronic myeloid leukemia and other myeloproliferative syndromes, myelodysplastic disorders, lymphoma, myeloma, and even solid tumors, such as renal cell cancer, breast cancer, ovarian carcinoma, and neuroblastoma.[27]

The aim of allogeneic HSCT in malignancies is not only a substitution of the malignant bone marrow but also a form of adoptive immunotherapy. In the context of HLA compatibility, donor allogeneic T lymphocytes detect differences in minor histocompatibility antigens in both the host and the tumor and can destroy the residual malignant cells, thereby contributing to the cure of the patient. This is the graft-versus-tumor or graft-versus-leukemia (GVL) effect. Particularly in the case of disease relapse after transplantation, donor lymphocyte infusions might induce or enhance a GVL effect and reinduce the patient into remission.

Major complications of HSCT include organ toxicity from the conditioning regimen used and graft-versus-host disease (GVHD), in which the donor's immune system destroys the recipient's normal tissues, particularly the skin, gastrointestinal tract, and liver. Other important complications of HSCT are graft failure, infertility, growth retardation in children, and secondary cancers thought to arise as a result of chronic immunosuppression and DNA-damaging preparative regimens.

In an effort to decrease these complications, several strategies have been developed. First among these are the reduced-intensity conditioning regimens, or so-called minitransplantations, which have made HSCT available to older and less fit patients.[28] Significant immunosuppression in these patients and GVHD as a result of the conditioning regimen remain serious problems and have suggested to many that the use of the term "mini" is misleading with respect to potential complications.

Second, the cell source has also been examined with respect to complications. Several studies convincingly show that $CD34^+$ cells harvested from peripheral blood engraft faster but are associated with a higher incidence of GVHD.[29]

Finding a histocompatible donor remains a problem for many patients in need of HSCT. Ideally, one would like to use an HLA-matched sibling as a donor. If such a donor is not available, then a matched unrelated donor is an acceptable alternative. This can be a major problem for minority groups underrepresented in registries of volunteer donors, information on which is centralized in the Bone Marrow Donors Worldwide database. In the absence of a compatible donor, HLA-haploidentical mismatched HSCT from a relative can be performed,[30] generally T depleted to avoid GVHD. In that context NK cells could facilitate engraftment and display an antileukemic activity without GVHD.[31]

Finally, UCB transplantation has the potential to significantly enlarge the number of potential HSCT recipients. UCBs are rapidly and easily available from cord blood banks and can be used when only partially HLA matched because they are much less likely to induce acute and chronic GVHD.[32] Remarkably, the GVL effect seems to be preserved, likely as a result of NK cells present in the cord blood preparation.[33] Issues that remain to be solved are delayed engraftment, prolonged T lymphopenia, and defective thymopoiesis.[34] In addition, it is not currently possible to perform donor lymphocyte infusions in the case of relapse, but an ongoing clinical trial is testing *ex vivo* expansion of UCB T cells. Because the number of HSCs per unit of UCB is small, thereby limiting their ability to be used for adult patients, strategies using combined units or *ex vivo* expanded cells are being developed.[35,36]

Autologous hematopoietic stem cell transplantation (AHSCT) is commonly performed in certain settings as well. Its main role is

to lessen the period of aplasia (rescue therapy) after high-dose chemotherapy and thereby lessen the risk of infection and bleeding. When used for the treatment of hematologic malignancies and solid tumors, HSCs to be used for AHSCT are commonly collected after few cycles of chemotherapy to lessen contamination of the graft by tumor cells. Moreover, autoimmune diseases, such as multiple sclerosis, rheumatoid arthritis, and systemic lupus erythematosus, might benefit from AHSCT. It is thought that immunoablative therapy resets the immune system by eliminating autoreactive T and B lymphocytes, lessening memory T cells, increasing thymus-derived naive T cells, generating a diverse but distinct T-cell receptor repertoire,[37] and promoting regulatory T cells.[38] Several recent studies have shown, as might be expected, that nonmyeloablative and low-intensity myeloablative regimens have fewer treatment-related complications and mortality than high-intensity myeloablative regimens[39] and that best results might be obtained during the inflammatory phase of autoimmune disease. Allogeneic HSCT has also been used for the treatment of autoimmune disease on the theory of both immune reset and correction of the genetic predisposition, but the risks of GVHD and infection are substantial, and therefore this form of therapy should be reserved only for treatment of very serious and refractory disease.[39]

Finally, the immunomodulatory capability of MSCs is being tested in the treatment of patients with systemic lupus erythematosus, multiple sclerosis, Crohn disease, amyotrophic lateral sclerosis, and T1DM. In solid organ transplantation and HSCT, results from preclinical animal studies, although controversial, suggest that donor-derived MSCs could have a tolerogenic effect and might therefore be used for prevention and/or treatment of graft rejection and GVHD.[40-42]

T1DM

T1DM is an autoimmune disease resulting from the destruction of pancreatic insulin-producing β cells in the islets of Langerhans. Insulin replacement therapy, even when rigorously controlled, is often not efficient enough to prevent long-term complications of the disease. Transplantation of a whole pancreas or isolated mature islets can restore proper glucose regulation, but the former is a morbid high-complication procedure, and the latter appears to be only a transient solution.[43] For these reasons, other sources of β cells suitable for transplantation are being sought. Pancreatic stem cells have been identified in ductal epithelium of injured pancreas on the basis of expression of the transcription factor neurogenin 3.[44] Efforts are now being directed toward methods to expand these cells *ex vivo* or to stimulate their proliferation *in vivo*.

Generation of insulin-secreting cells from hESCs and iPSCs holds great promise for the cure of T1DM. hESC-derived pancreatic endoderm can be differentiated *in vivo* into glucose-responsive insulin-secreting cells in immunodeficient mice with streptozotocin-induced diabetes (a β cell–selective destruction).[45] Unfortunately, teratoma formation was found in approximately 15% of the recipient mice and is a safety concern. The immunologic incompatibility of hESCs could be resolved by using bioengineered porous capsules, which are designed to protect the graft from immune cells but remain permissive to the passage of small molecules. In regard to iPSCs, a particularly exciting study from Zhou et al[46] reported the reprogramming of differentiated murine pancreatic exocrine cells into β-like cells *in vivo*. These investigators found that transient expression of 3 key developmental transcription factors, neurogenin 3, MafA, and Pancreatic duodenal homeobox-1 (Pdx1), by adenoviral vectors injected directly into the pancreas was sufficient to reprogram exocrine cells into insulin-secreting cells responding to hyperglycemia.

The immunomodulatory effects of MSCs are also being explored in the setting of T1DM. At the time of diagnosis, β-cell destruction is often not yet complete. In theory, amelioration of the immune attack might allow the survival of the residual islet cells. In diabetic immunodeficient mice human MSCs decreased hyperglycemia and increased endogen insulin levels and β-cell numbers.[47] Clinical studies testing allogeneic MSCs in patients with recently diagnosed diabetes are ongoing.

Finally, nonmyeloablative AHSCT was performed in 23 patients with early-onset T1DM.[48] Twenty enjoyed a variable insulin-free period, and 12 of these patients remained insulin free after a mean follow-up of 31 months. Interestingly, benefit was demonstrated in those patients with transient responses as well. In this small group (8 patients) daily insulin doses were significantly diminished, and C-peptide levels (reflective of endogenous insulin synthesis) were increased.

Diseases of the nervous system

It was thought for a long time that nerve cells do not divide in the adult mammalian brain, but this has now been shown to be incorrect. Neurogenesis not only occurs during prenatal and postnatal development but also in adults. Neurogenic niches have been identified in the subventricular zone of the lateral ventricles and in the subgranular zone of the dentate gyrus of the hippocampus. In brain-injury models neural stem cells (NSCs) proliferate in those neurogenic regions and are even able to migrate toward the site of damage.[49] NSCs are multipotent and capable of self-renewing. *In vitro* they cluster in "neurospheres," which are able to differentiate into the 3 major neuroectodermal lineages (neurons, astrocytes, and oligodendrocytes).

Neural stem/progenitor cells can be isolated from embryonic, fetal, or adult brain tissue by sorting cells on the basis of nestin expression primarily, as well as other markers, and can then be placed into culture for expansion. Clearly, for autologous stem cell therapy, more accessible NSCs are required. Curiously, dental pulp and peridontium have been shown to be sources of NSC, as well as olfactory mucosa, which is readily harvested by means of nasal biopsy.[50] Investigations for NSC-based therapy are ongoing for various neurologic diseases. The neural repair probably results from a replacement of defective cells but also from neuroprotective, trophic, and immunomodulatory effects. To date, the ideal NSC source, schedule, and route of transplantation have not been established and are likely to be disease specific. The use of NSCs to treat Parkinson disease might be instructive in this regard.

Parkinson disease is an incurable, progressive, neurodegenerative disease that affects dopaminergic neurons. Levodopa, which is converted to dopamine in the brain, is the mainstay of treatment, but most patients acquire tachyphylaxis to its effects over time. In contrast to patients with diabetes, in whom transplantation of islet cells is a therapeutic option, implantation of fully differentiated dopamine-releasing neurons into the brain is not presently feasible because such cells do not survive. Transplantation of embryonic/fetal nigral dopaminergic neurons was tested in 2 double-blind, placebo-controlled trials, but results

were not as encouraging as those from previous open-trial reports.[51,52] However, modest clinical improvement was noted in some patients, and striatal fluorodopa uptake was significantly enhanced. Unfortunately, several patients subsequently had dyskinesias. Postmortem examination of the brains of some patients provided evidence that transplanted dopamine neurons can differentiate and survive for many years without immunosuppression. However, it appeared that at least some of the grafted tissue was involved by disease over a period lasting from 9 to 16 years.[53] Widespread application of this therapy will likely be limited as long as access to fetal donor tissue is required. Moreover, the safety of those cells is not completely assessed because they have not been tested in a large number of patients and because development of cerebral mass lesions of donor origin was reported in a patient less than 6 years after fetal transplantation for Huntington disease.[54] Accordingly, finding alternative sources of NSCs for therapeutic purposes is the object of intense investigation.

In one case autologous NSCs were harvested by means of cerebral biopsy, expanded, and differentiated into dopaminergic neurons *ex vivo* and then injected back to the patient's putamen 9 months later.[55] Clinical evaluation and fluorodopa uptake were improved after transplantation, but all benefits had disappeared by 5 years. In animal studies other stem cell types partially alleviated Parkinson symptoms, including MSCs,[56] olfactory NSCs,[57] hESC-derived neurons,[58] and iPSCs.[59] Human clinical trials with cells of these types can therefore be anticipated.

Unlike Parkinson disease, which theoretically requires replacement of only 1 cell type, therapies for other neurologic diseases, such as stroke and spinal cord injury, in which large numbers of cells of many types (neurons, glia, and endothelial cells) are destroyed, face much larger hurdles. In recent years, human NSCs from diverse origins, including hESCs, HSCs, and MSCs, have been tested in preclinical models of ischemic stroke. These experiments have enabled the development of treatment strategies and have demonstrated the critical importance of transplantation timing for clinical success. Only a few clinical studies have been performed for the treatment of stroke. Stereotactic injection of neuronal cells derived from embryonal carcinoma cell line (NT2/D1) did not display significant benefits compared with control results, but some patients experienced improvement.[60] In other studies an investigation into the utility of fetal porcine cells was stopped because of adverse events (temporary worsening of deficits and seizures) in 2 of 5 patients,[61] whereas intravenous infusion of MSCs was shown to be safe without significant benefit. HSCs are currently being evaluated in phase I/II protocols. The injection of specific growth factors to stimulate proliferation of intrinsic neuroprogenitors in the brain is a novel approach to this problem.[62]

Spinal cord injury often results in permanent motor deficiency, sensory deficiency, or both, thereby rendering treatment particularly challenging. In a small number of paraplegic and quadriplegic patients, olfactory NSCs have been injected into intralesional and perilesional areas.[50] Feasibility and safety were acceptable, but unfortunately, clinical improvement remained slight after 3 years of follow-up. Logically, early-phase treatment might yield the best results. In fact, a phase I/II clinical trial tested infusion of HSCs into the spinal cord associated with G-CSF injections: the Association Impairment Scale grade improved in 30.4% of patients treated quite early (<8 weeks) after the initial lesion; however, no enhancement was noted when the treatment was performed later.[63]

Cardiac repair

Congestive heart failure afflicts millions of persons around the world, with 400,000 new cases being reported each year in the United States alone. The most common cause is coronary artery disease. After myocardial necrosis has occurred, the cell loss is irreversible, and although many medical and surgical treatments are available for the subsequent congestive heart failure, the long-term prognosis of these patients remains guarded, with a 5-year mortality of 50%. Transplanting stem cells would have clear advantages to transplanting a heart because it would obviate the constraint of a donor and, in case of autologous cells, for the requisite immunosuppression.

hESC-derived cardiomyocytes (hESC-CMs) have been successfully generated. Intramyocardial injection of hESC-CMs a few days after infarction in immunodeficient rodents seemed to enhance left ventricular ejection fraction (LVEF) compared with that seen in a control group when evaluated at 4 weeks.[64] Unfortunately, this enhancement was not sustained after 12 weeks of follow-up. Another study suggested that a coinfusion of hESC-CMs and MSCs in mice was of benefit because, according to the authors, a "synergistic trophic effect that enhanced repair of injured host tissue" was brought about.[65] Importantly, no teratoma was found in animals receiving hESC-CMs.[64,65]

Despite a controversial plasticity *in vitro*, a considerable amount of data from actual preclinical studies suggest that it is unlikely that transdifferentiation of HSCs and MSCs into functional cardiomyocytes happens to any significant degree *in vivo*. In very specific culture conditions, MSCs might be driven toward differentiating into cardiomyocyte-like cells at a very low frequency (approximately 0.07%) that would not be enough for cardiac repair.[24] It is now generally agreed that the transplanted cells exert their beneficial role through paracrine effects and by creating a favorable trophic environment for intrinsic cell recovery, enhancing angiogenesis, and limiting ventricular remodeling.[66] A study comparing the efficacy of transplanted bone marrow mononuclear cells, MSCs, skeletal myoblasts, and fibroblasts in mice with experimental myocardial infarcts was carried out and showed that HSCs had the most beneficial effect on left ventricular function in that model.[67] Recently, infusion of endogenous cardiac stem cells isolated from endomyocardial biopsy specimens and expanded *ex vivo* appeared to enhance myocardial viability and LVEF in a murine infarction model.[68]

Intracoronary infusions of HSCs[69] or MSCs[70] have been performed a few days after percutaneous coronary intervention for acute myocardial infarction. Despite contradictory results of clinical trials, meta-analyses reported moderate but significant benefits of such therapy compared with the condition of control patients, with improvement in LVEF, infarct size, and end-systolic volume.[71] In patients with chronic ischemic disease, intracoronary and intramyocardial injections of HSCs are associated with modest enhancements as well.[71] Other cell types are also being tested, such as skeletal myoblasts (although lack of electrical synchronization with cardiomyocytes could potentially be arrhythmogenic) or endothelial progenitor cells.

Stem cells and gene therapy

The goal of gene therapy is to cure diseases caused by malfunctioning genes. It does so by substituting the function of a normal gene for the one that causes disease. Until now, the most commonly used procedure in human gene therapy clinical trials is

the insertion of a normal copy of the target gene in a nonspecific location into the host genomic DNA. The therapeutic transgene is packaged into a delivery vehicle, which is typically a replication-deficient virus. Nonintegrating virus (adenovirus or adeno-associated virus) can be used in nondividing cells, such as neurons and cardiomyocytes. In dividing cells, such as stem cells, vectors that integrate into host DNA, such as γ-retrovirus or lentivirus, are required to have a transmission to daughter cells.

Stem cells are of great benefit to cell-based gene therapy because they are self-renewing and thus might reduce or eliminate the necessity for repeated administrations of the therapeutic cells. Single-gene inherited diseases are particularly good candidates for gene therapy. In theory the host's own stem cells can be repaired through genetic engineering and then used in an autologous transplantation. This avoids all the risks of transplanted allogeneic cells, including the risks associated with long-term immunosuppression, as well as GVHD, in patients receiving HSCT. The first clinical trials with engineered HSCs involved patients with genetic immunodeficiency diseases, such as adenosine deaminase–deficient SCID.[72] Trials have also been carried out in patients with X-linked SCID (γ-common [γ-c] cytokine receptor deficient or SCID-X1)[17] and chronic granulomatous disease.[73] The clinical results have been quite promising but have been marred by the development of leukemia, which has been shown to be caused by insertional mutagenesis in a number of these patients (see below). Gene therapy for hemoglobinopathies, such as β-thalassemia and sickle cell disease, are ongoing. Easily accessible mucosal and skin stem cells are also being used, for example in treatment of diseases such as junctional epidermolysis bullosa.[74]

These early studies revealed problems that need to be addressed, such as difficulties controlling protein levels without endogenous gene regulatory regions, maintenance of gene expression through long periods, low protein production, and insertional mutagenesis of the retroviral transgene vector. Indeed, the major side effect was thus far the occurrence of T cell–acute lymphoblastic leukemia in 5 of 19 patients successfully treated for SCID-X1 in 2 distinct French and British trials.[17,75] In all cases the retroviral vector was found in the leukemic clone, integrated near a proto-oncogene, and particularly before the LIM domain–only 2 in 4 cases and was associated with acquired somatic mutations. γ-Retroviral vectors were subsequently shown to integrate preferentially in the 5′ ends of genes[76] near transcription start sites in a nonrandom manner near genes that provide selective advantage to the clone. Interestingly, when the same retroviral vector, the murine leukemia virus, was used to deliver other transgenes, no case of leukemia was observed, suggesting that γ-c receptor overexpression might be involved in the oncogenesis.

New techniques are being developed to enhance efficiency and to avoid the risk of insertional oncogenesis. First, safer delivery systems are being developed. For example, HIV-derived lentivirus is able to transduce nondividing cells, is easier to use, and can induce less mutagenesis than γ-retrovirus, as assessed in murine models.[77] Other modifications under development include the use of inducible and tissue-specific promoters, a weaker viral promoter/enhancer, and self-inactivating retroviral vectors and introduction of suicide genes. At last, the improvement of direct gene correction with homologous recombination (a normal copy of the gene is switched with the defective allele) is promising in murine models.[78] That last technology could be particularly significant in the treatment of dominant genetic diseases.

The potential utility of hESCs and iPSCs was discussed earlier, but the use of such cells is under active investigation for human gene therapy.

Finally, new attempts are focusing on cell-based delivery vehicles for tumor-specific therapy. MSCs appear to be good agents for this purpose because, in addition to their properties described above, they have been shown to migrate toward tumors. For example, MSCs engineered to express the TNF-related apoptosis-inducing ligand induced apoptosis in tumor cells, reduced tumor growth *in vivo*, and prolonged survival in murine models of human glioma.[79]

ETHICAL ISSUES

The use of hESCs in medical research has drawn much attention from many sectors of the public. Religious, historical, cultural, medical, and other points of view have contributed to a very vigorous and wide-ranging discourse over the use of these materials.[80] Some consider research with hESCs to be inherently immoral because these individual's believe that life begins with fertilization of the ovum, and the destruction of an embryo with the potential to develop into a viable human being is thought tantamount to infanticide. For this reason, the American federal government severely restricted access and use of hESCs in 2001. These restrictions have now been largely overturned by the Obama administration. In contrast, proponents of this line of research insist that the potential benefits to humankind from this research mitigate such concerns. They also argue that hESCs are made from unwanted fertilized ovum that would likely be destroyed in any event.

Stem cells created by means of nuclear transfer share the same ethical concerns. Furthermore, because these cells have the potential to generate a complete embryo, they also raise the even more highly charged possibility of cloning human beings, so-called reproductive cloning. Many organizations and countries have already banned reproductive cloning of human beings. Because this procedure can be used to generate stem cells for therapeutic purposes, in countries where this type of cloning is legal, such as Australia and the United Kingdom, the created embryos must be destroyed within 14 days. Federal laws in the United States are not clear on the legality of therapeutic cloning, but the Obama administration has pledged establishment of strict guidelines to ensure that cloning research will not be used for human reproduction.

Because of the shortage of human oocytes, generation of human-animal chimeras was legalized in 2008 in the United Kingdom for research purposes only. A human nucleus is transferred into an animal's oocyte, creating a hybrid embryo that must be destroyed within 2 weeks and cannot be implanted. Clearly, creation of such tissues raises even more complex issues.

Finally, the issue of financial compensation for embryo and gamete donors is also controversial, with guidelines for this problem being proposed by the International Society of Stem Cell Research (http://www.isscr.org/guidelines/index.htm). All parties involved in the debate want very much to avoid the development of an underground black market in spare embryos.

CONCLUSIONS AND PERSPECTIVES

The promise of stem cell therapeutics powers the field of regenerative medicine and has generated a huge amount of

excitement, anticipation, and hope. Accordingly, research with hESCs is increasing exponentially worldwide, particularly in the United States, where important limitations on research with such cells were overturned in 2009. Furthermore, the US Food and Drug Administration recently approved the world's first phase I clinical trial using hESC-based therapy in patients with spinal cord injury.

Nonetheless, a number of substantive scientific and ethical issues remain to be resolved before hESCs can enter the therapeutic mainstream. In the meantime, recent breakthroughs in generating iPSCs would obviate the need to solve the most vexing of these problems. In fact, it seems reasonable to hope that in the next few years many of the enabling issues relevant to iPSCs will be solved, allowing the field of regenerative medicine to deliver on its vast potential promise.

Although it is difficult to predict the ultimate utility of stem cell–based therapy at this time, it is not difficult to conclude that this is an extremely important area of scientific research. Surrounded by controversy and a good many ethical concerns, thoughtful legislative action could both foster the field and ensure continued progress. This would clearly be more desirable than having the whole endeavor driven underground and potentially into the hands of less ethical and less regulated scientists. Open discussions between political bodies and the various interest groups in the scientific, medical, and religious communities need to take place to address the concerns of each and to provide an ultimate solution that is clearly in the interest of humanity.

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Study questions

Overview of the immune response

Learning objectives: "Overview of the immune response"

1. To understand the fundamental ways in which the innate and the adaptive arms of the immune system work together to help the host recognize, inactivate, and clear pathogenic microbes, neoplastic cells, toxins, and other exogenous threats.
2. To understand the mechanisms used by the innate and the adaptive arms of the immune response to distinguish self from non-self so that the immune effector mechanisms can be focused on appropriate targets and avoid damage to the host's normal tissues.

Question 1. For $CD8^+$ T lymphocytes to recognize virally infected cells, the infected cells must —

A. express functional HLA-DM molecules.
B. express functional transporter associated with antigen presentation (TAP) 1 and 2 proteins.
C. have trafficked through a germinal center.
D. extinguish expression of its class I HLA molecules.

Question 2. The HLA invariant chain —

A. controls loading of viral peptide fragments into class I HLA molecules.
B. is delivered to HLA molecules in endosomes.
C. prevents loading of antigenic peptides into class II HLA molecules until it is proteolytically degraded.
D. differs in primary amino acid sequence among different subjects in the population.

Question 3. For T_H cells, a functional T-cell receptor requires all of the following except

A. the CD3 complex.
B. coexpression of CD4.
C. rearranged α and β chains.
D. β_2-microglobulin.

Question 4. Which of the following statements is true?

A. The extracellular domains of Toll-like receptors (TLRs) are homologous to the corresponding domains of the IL-1 receptor.
B. All TLRs are cell-surface proteins.
C. TLRs are found on macrophages, dendritic cells, neutrophils, and endothelial cells.
D. TLR4 is activated by CpG DNA.

Study questions

Innate immunity

Learning objectives: "Innate immunity"

1. To appreciate the contribution of the innate immune system to host defense.
2. To understand the cellular and humoral elements involved in innate immune responses.
3. To be aware of the molecular strategies used by the innate immune system to sense infection or tissue damage.
4. To recognize how innate immune defects contribute to human disease and how the innate immune system can be modulated to prevent or treat illness.

Question 1. Which of the following statements regarding activation of Toll-like receptor (TLR) 9 in allergic responses is true?

A. Activation of TLR9 expressed by eosinophils inhibits generation of prostaglandin D_2.
B. Activation of TLR9 expressed by $CD4^+$ cells inhibits generation of T_H2 cytokines.
C. Activation of TLR9 expressed by dendritic cells inhibits T_H2 cell generation of cytokines.
D. Activation of TLR9 expressed by endothelial cells inhibits recruitment of T_H2 cells to sites of allergic inflammation.

Question 2. Which of the following human diseases is primarily caused by a defect in the innate immune system?

A. IL-1 receptor–associated kinase 4 (IRAK4) deficiency
B. severe combined immunodeficiency (SCID)
C. X-linked agammaglobulinemia (XLA)
D. DiGeorge syndrome

Question 3. One recognition strategy used by the innate immune system is to detect conserved microbial components. Which of the following is an example of a well-characterized innate immune system receptor-ligand pair?

A. TLR4 and peptidoglycan
B. T-cell receptor and H1N1 influenza A peptide
C. caspase 1 and the muramyl dipeptide component of peptidoglycan
D. TLR5 and flagellin

Question 4. Aluminum-containing vaccine adjuvants (alum) appear to mediate their beneficial immunostimulatory effects through which of the following molecules of the innate immune system?

A. TLR1/6 heterodimers
B. nucleotide oligomerization domain–like receptor family, pyrin domain–containing 3 (NLRP3, also called *NALP3* or *cryopyrin*)
C. myeloid differentiation primary response gene 88 (MyD88)
D. killer cell immunoglobulin-like receptor (KIR)

Study questions

Adaptive immunity

Learning objectives: "Adaptive immunity"

1. To understand the process of T-cell development, including the mechanisms of somatic genetic rearrangements that generate T-cell receptor diversity.
2. To recognize the different functional subsets of effector T cells.
3. To know the subunits that comprise the immunoglobulin pre-B cell receptor, which is critical for B-cell development in the bone marrow.
4. To know the critical processes of antigen-dependent B-cell development, which takes place in germinal centers.

Question 1. Which of the following statements concerning T cells is true?

A. On full maturation, T cells exiting the thymus express both CD4 and CD8.
B. CD8 serves as a coreceptor by binding to nonpolymorphic domains on MHC class II molecules.
C. T-cell activation leads both to release of intracellular calcium stores and to influx of extracellular calcium.
D. Newborn screening for severe combined immunodeficiency is performed by counting T cells on a blood spot.

Question 2. Which of the following statements concerning effector T cells is true?

A. $CD25^+$ regulatory T cells express the transcription factor retinoic acid receptor related orphan receptor γt (RORγt).
B. T_H17 cells arise from T_H0 precursors under the influence of IL-4 and IFN-γ.
C. Killing of virally infected target cells by cytolytic T lymphocytes is mediated by complement.
D. Natural killer T cells express αβ T-cell receptors and CD56.

Question 3. The pre-B cell receptor expressed on developing B cells in the bone marrow consists of —

A. IgM heavy chain, κ or λ light chain, Ig-α and Ig-β.
B. IgM heavy chain, surrogate light chain, CD20.
C. IgD heavy chain, surrogate light chain, Ig-α and Ig-β.
D. IgM heavy chain, surrogate light chain, Ig-α and Ig-β.

Question 4. Which of the following takes place predominantly in germinal centers?

A. immunoglobulin gene rearrangement
B. expression of IgD on the B-cell surface
C. immunoglobulin class-switching and somatic hypermutation
D. large-scale antibody secretion

Study questions

Structure and function of immunoglobulins

Learning objectives: "Structure and function of immunoglobulins"

1. To understand the molecular basis of immunoglobulin gene rearrangement.
2. To gain insight into the structural features of immunoglobulin that allow an individual antibody to distinguish between antigens.
3. To understand the contribution of immunoglobulin heavy chain structure to effector functions, such as complement activation and antibody-dependent cellular cytotoxicity.
4. To recognize that classes of immunoglobulin heavy chains differentially contribute to innate and adaptive immune responses.

Question 1. To generate their antigen receptors, developing B cells must undergo a complex process of DNA gene rearrangements that begins with precise cutting of the DNA strands and ends with the imprecise, in-frame joining of the ends of the nonhomologous sequences that encode the various portions of the future variable domain. Which of the following proteins is most critical for immunoglobulin rearrangement?

A. activation-induced cytidine deaminase
B. κ light chain
C. recombination-activating gene (RAG) 1 and 2
D. surrogate light chain (λ14.1 [λ5] and V_{preB})
E. terminal deoxynucleotidyl transferase (TdT)

Question 2. Activation of complement is one mechanism by which antibodies can kill cells. However, not all antibodies can bind complement, and some bind it better than others. Of the following isotypes, which one activates complement best?

A. IgA
B. IgD
C. IgE
D. IgG3
E. IgG4

Question 3. Which of the following functions cannot be performed by IgA?

A. binding Fcϵ receptors on mast cells
B. blocking pathogen adhesion
C. facilitation of antibody-dependent cellular cytotoxicity
D. mucosal transport
E. neutralizing toxins

Question 4. As a glycoprotein, there are potential N- and O-linked sites on the protein backbone of an immunoglobulin. Which of the following statements regarding immunoglobulin glycosylation is true?

A. All immunoglobulins are glycosylated the same.
B. Aglycosylated immunoglobulins function the same as glycosylated immunoglobulins.
C. Aberrantly glycosylated immunoglobulins play a role in some disease manifestations.
D. Fucose is the only sugar moiety on an immunoglobulin.

Study questions

Immunologic messenger molecules: Cytokines, interferons, and chemokines

Learning objectives: "Immunologic messenger molecules: Cytokines, interferons, and chemokines"

1. To recognize how different cytokines modulate cellular immune function.
2. To describe how the different T-cell populations develop and the role that cytokines play in modulating this response.
3. To understand how chemokines are grouped into separate families based on structure and function to modulate cell recruitment under inflammatory and homeostatic conditions.

Question 1. IL-6 and the IL-6 family of cytokines trigger signal transducer and activator of transcription 3 phosphorylation through which of the following receptors?

A. glycoprotein 130
B. shared γ chain
C. shared β chain (CD131)
D. nuclear factor IL-6
E. oncostatin M receptor α chain

Question 2. Which of the following is the master regulator for T_H17-like lymphocytes?

A. T-bet transcription factor
B. GATA-3
C. retinoic acid receptor–related orphan receptor γt
D. signal transducer and activator of transcription 3
E. Forkhead box protein 3

Question 3. Which of the following cytokines does not use the shared γ chain as part of its receptor?

A. IL-4
B. thymic stromal lymphopoietin
C. IL-7
D. IL-15
E. IL-21

Question 4. Which of the following chemokines is not involved in T_H1-like recruitment?

A. CCL3 (macrophage inflammatory protein 1α)
B. CCL4 (macrophage inflammatory protein 1β)
C. CCL5 (RANTES)
D. CCL11 (eotaxin)
E. CCL17 (thymus and activation-regulated chemokine).

Study questions

IgE, mast cells, basophils, and eosinophils

Learning objectives: "IgE, mast cells, basophils, and eosinophils"

1. To understand the biology of IgE, mast cells, basophils, and eosinophils.
2. To understand the role of IgE, mast cells, basophils, and eosinophils in disease.

Question 1. Which of the following regarding the high-affinity IgE receptor FcεRI is true?

A. The γ chain amplifies signaling through the receptor.
B. The β chain is absent on basophils.
C. The α chain binds to the C2 domain of the Fc region of IgE.
D. The β chain associates with Lyn kinase.

Question 2. All of the following are produced by basophils after activation except —

A. GM-CSF.
B. granzyme B.
C. IL-4.
D. prostaglandin D_2.

Question 3. Which of the following is associated with eosinopenia?

A. Addison disease
B. sepsis
C. Kimura disease
D. Omenn syndrome

Question 4. Which of the following statements is true regarding tryptase?

A. Anaphylaxis to food allergens is always associated with an increase in total serum tryptase levels.
B. Baseline serum tryptase is composed of predominantly the mature β-tryptase.
C. Tryptase is stabilized in secretory granules by heparin.
D. Protryptase is the predominant form of tryptase stored in the secretory granules of mast cells.

Study questions

Genetics of allergic disease

Learning objectives: "Genetics of allergic disease"

1. To comprehend the principles of study design for genetic and genomic approaches to studying allergic disease.
2. To identify single nucleotide polymorphisms (SNPs) that have been identified as potential markers for allergic disease in the latest genome-wide association studies.
3. To apply knowledge of genetic studies to the pharmacogenetics of allergic disease.
4. To analyze mechanisms of genetic susceptibility to allergic disease and their associated candidate genes.

Question 1. Which of the following approaches to studying the genetics of allergic disease would be most appropriate to identifying the role of variation in a candidate gene in susceptibility to allergic disease?

A. positional cloning/linkage studies examining transmission of genetic markers with clinical phenotype in families
B. examination of "tagging" SNPs that capture the common variation in a defined region of the genome in a case-control cohort
C. using a genome-wide association study approach to assess variation across the whole genome to find polymorphisms associated with allergic disease
D. examining the effect of an amino acid variant on protein function in *in vitro* studies

Question 2. SNPs in or near which of the following genes have been found to be associated with asthma or allergic phenotypes in genome-wide association studies?

A. *ORMDL*
B. *CHRNA3* (nicotinic acetylcholine receptor subunit)
C. *IL13* (IL-13)
D. *SH2B3* (SH2B adaptor protein 3)

Question 3. Which of the following genes have SNPs that have been associated with pharmacogenetic responses in asthma treatment?

A. *CYP1A1* (cytochrome P450 1A1)
B. *ADRB2* (β_2-adrenergic receptor)
C. *IL5* (IL-5)
D. *CD14*

Question 4. Which of the following pairs of mechanisms and genes correctly matches a proposed disease susceptibility mechanism for allergic disease with a relevant candidate gene?

A. modulation of the effect of environmental risk factors for allergic disease–*IL13*
B. loss of epithelial barrier function–*FLG* (filaggrin)
C. regulation of atopic inflammation–*ORMDL3*
D. tissue response genes–*PHF11*

Study questions

Asthma: Clinical expression and molecular mechanisms

Learning objectives: "Asthma: Clinical expression and molecular mechanisms"

1. To understand the importance of viral respiratory tract infections in asthma inception and exacerbations.
2. To recognize the potential contribution of various comorbidities to asthma control.
3. To understand the contribution of allergic sensitization to asthma expression and management.

Question 1. When asthma severity and control are being evaluated, which of the following factors is part of the assessment of the risk domain?

A. pulmonary function
B. symptoms
C. exacerbations
D. rescue albuterol use

Question 2. Which of the following viruses is the most frequent respiratory tract infection associated with asthma exacerbations?

A. metapneumovirus
B. rhinovirus
C. respiratory syncytial virus
D. parainfluenza

Question 3. Which of the following pain medications can be safely given to an asthmatic subject sensitive to aspirin?

A. ibuprofen
B. naproxen
C. acetaminophen
D. indomethacin

Question 4. Which of the following medications has been associated with an increased risk for severe asthma exacerbations when used as the only treatment?

A. inhaled corticosteroids
B. theophylline
C. leukotriene receptor antagonists
D. long-acting β-agonists

Study questions

Rhinitis and sinusitis

Learning objectives: "Rhinitis and sinusitis"

1. To understand the mechanism of dust mite allergen sensitization in the nasal mucosa.
2. To understand the association between nonallergic rhinitis and eosinophilia.
3. To understand the pathologic processes involved in chronic rhinosinusitis (CRS) with or without nasal polyps.
4. To learn the clinical significance of hyperdensities on sinus computed tomographic (CT) scanning in a patient with CRS.

Question 1. Which of the following processes involved during natural allergen sensitization through the nasal mucosa in patients with allergic rhinitis is most specific for dust mite antigen?

A. elaboration of thymic stromal lymphopoietin by nasal epithelial cells
B. local and systemic production of allergen-specific IgE
C. enhancement through induction of Toll-like receptor 4 (TLR4) signaling
D. interaction of dust mite antigen with interepithelial and subepithelial dendritic cells

Question 2. Which of the following subtypes of nonallergic rhinitis is most likely to be associated with eosinophilia?

A. irritant-induced rhinitis
B. cold-induced rhinitis
C. vasomotor rhinitis
D. rhinitis associated with aspirin sensitivity (aspirin-exacerbated respiratory disease)

Question 3. Which of the following pathologic processes implicated in the pathogenesis of CRS is most specific for CRS with nasal polyposis?

A. T_H2-type immune hyperresponsiveness (production of IL-5 and IL-13) directed toward colonizing fungi in sinus secretions
B. glandular hyperplasia
C. formation of bacterial biofilm on sinus mucosal tissue
D. local production of IgE directed against staphylococcal enterotoxins (ie, superantigens) from *Staphylococcus aureus*

Question 4. In patients with CRS, the sinus CT scan might reveal hyperdensities within an opacified sinus cavity. Which of the following statements best describes the significance of hyperdensities?

A. They are pathognomonic of allergic fungal rhinosinusitis.
B. They are suggestive of the presence of necrotizing infection (abscess formation).
C. They are often associated with mucocele formation.
D. They are suggestive of the presence of thick inspissated secretions containing large numbers of degranulated eosinophils (allergic mucin) and possibly colonizing fungi.

Dr. Mark Dykewicz, as a member of the Board of Directors of the American Board of Allergy and Immunology, did not participate in the development or review of these questions.

Study questions

Food allergy

Learning objectives: "Food allergy"

1. To understand the epidemiologic aspects of food hypersensitivity disorders.
2. To understand the pathogenesis of food allergy.
3. To understand the clinical manifestations of food allergy.
4. To understand current and future diagnosis and management.

Question 1. Which of the following most accurately describes an epidemiologic feature of food allergy?

A. Allergy to fish/shellfish is more prevalent among children than among adults.
B. On the basis of studies from a referral center in the United States, allergy to milk and egg might be more persistent than noted in past decades, with fewer than 20% resolving these allergies by age 4 years.
C. Food allergy has approximately doubled in children over the past decade.
D. Peanut allergy resolves by school age for 35% of children given diagnoses at less than 2 years of age.

Question 2. A 27-year-old atopic man experienced mild oral pruritus when eating raw apples but tolerates apple juice and baked apple. Which of the following is most likely to be true?

A. He has an increased IgE level that binds lipid transfer protein in apple.
B. He has positive skin test results to commercial extract of apple.
C. He has an increased IgE level to Bet v 1.
D. The Maillard reaction during heating apple results in a change in conformation that abrogates IgE binding for this subject.

Question 3. Which of the following clinical descriptions is most likely to represent a food allergy?

A. A 3-year-old experiences acute, transient, nonpruritic erythema over the left cheek minutes after she ingests, on separate occasions, lemonade, spicy potato chips, and sour candy.
B. A breast-fed 5-month-old infant experiences severe vomiting, lethargy, and an increased white blood cell count with bandemia 2 hours after she is fed rice cereal. Skin test results to rice are negative.
C. An 18-year-old experiences cramps and diarrhea after ingesting a large milk shake.
D. A 47-year-old experiences facial flushing and a tingling sensation in the mouth after ingesting tuna in a restaurant. He previously tolerated all fish.

Question 4. An infant experienced urticaria and angioedema when introduced to egg, and the egg-specific IgE concentration was 4.7 kIU/L. At age 2 years, she accidentally ingested a bite of egg and experienced wheezing and generalized urticaria and around that time had an egg IgE level of 1.7 kIU/L. At age 3 years, she accidentally ingested a small amount of egg and experienced generalized urticaria. At age 3½ years, she presents for evaluation, and the serum egg IgE level was less than 0.35 kIU/L. Which of the following would be the most reasonable next step toward diagnosis?

A. Perform an open oral food challenge to egg.
B. Perform a double-blind, placebo-controlled oral food challenge to egg.
C. Perform a skin prick test to egg.
D. Allow the child to add egg to the diet.

Study questions

Drug allergy

Learning objectives: "Drug allergy"

1. To recognize the limitations of diagnostic testing in most patients with drug allergy.
2. To gain an understanding of the negative predictive value of penicillin skin testing.
3. To gain an understanding of duration, indications, and contraindications of procedures to induce drug tolerance.
4. To be able to differentiate the various drug-induced allergic reactions to aspirin and nonsteroidal anti-inflammatory drugs.

Question 1. In evaluation of a patient with drug allergies, which of the following is generally the best tool to guide management?

A. skin testing
B. *in vitro* tests
C. detailed history
D. Gell and Coombs classification

Question 2. Which of the following is true regarding penicillin allergy?

A. History is adequate for diagnosis.
B. Skin testing has high negative predictive value.
C. Cross-reactivity with cephalosporins is high.
D. Resensitization is common.

Question 3. Procedures to induce drug tolerance —

A. involve only IgE-mediated allergy.
B. cause permanent drug tolerance.
C. can take days to weeks to complete.
D. are indicated for Stevens-Johnson syndrome reactions.

Question 4. A 30-year-old man has a history of shortness of breath, urticaria, and lightheadedness 30 minutes after ingesting ibuprofen. He most likely —

A. has asthma.
B. has nasal polyposis.
C. will react to celecoxib.
D. will tolerate aspirin.

Study questions

Allergic skin diseases

Learning objectives: "Allergic skin diseases"

1. To identify common clinical patterns and sensitizing allergens in patients with contact dermatitis.
2. To understand the newest concepts regarding the immunology and treatment of chronic urticaria.

Question 1. Which of the following statements concerning autoantibodies in patients with chronic urticaria is true?

A. The autologous serum skin test is the gold standard.
B. Commercially available tests for autoantibody activity are well established.
C. The presence, titer, or both of autoantibodies to the high-affinity receptor for IgE, FcεRIα, predict clinical outcome.
D. Approximately 40% of patients with chronic urticaria have evidence of autoantibodies with the ability to activate mast cells.

Question 2. For patients with chronic urticaria unresponsive to high doses of antihistamines, the immunomodulatory drug with the best efficacy data is —

A. hydroxychloroquine.
B. cyclosporin A.
C. sulfasalazine.
D. mycophenolate.

Question 3. Which of the following statements would be true for a patient with contact dermatitis?

A. Irritant contact dermatitis commonly presents as a generalized dermatitis with vesicles extending beyond the area of contact and involving the whole hand, including the webs of fingers and the dorsal and ventral surfaces of the hands.
B. Allergic contact dermatitis often has vesicles that favor the dorsum of the hands and, less commonly, involve the palms.
C. Atopic dermatitis is not an important factor in susceptibility to persistent postoccupational dermatitis.
D. A patient with allergic contact dermatitis can use "unscented" products and botanical extracts because these products are typically free of classic fragrance ingredients.

Question 4. Which of the following is true for patch testing?

A. Patch test results are affected by oral corticosteroids, cancer chemotherapy, immunosuppressive drugs, and antihistamines but not by topical corticosteroids.
B. Allergens not found on commercially available screening series in the United States are generally irrelevant reactions, and personal products have no use as supplements in patch testing.
C. Metals (gold, potassium dichromate, nickel, and cobalt), topical antibiotics (neomycin and bacitracin), topical corticosteroids, and paraphenylenediamine (PPD) might produce positive results after 7 days.
D. Hairdressers allergic to glycerol thioglycolate in permanent wave solution and PPD in hair dye might be able to cut hair after it has been rinsed out.
E. Lanolin in medicaments is less sensitizing than lanolin in cosmetics and is a weak sensitizer in normal skin but a stronger sensitizer in damaged skin.

Study questions

Environmental and occupational allergies

Learning objectives: "Environmental and occupational allergies"

1. To know what allergens can occur in the air both indoors and outdoors and how to identify which ones are important to an individual patient.
2. To understand the methods of reducing exposure to these allergens.
3. To understand the effects of indoor and outdoor air pollution.
4. To recognize and diagnose occupational asthma.

Question 1. Concentrations of which of the following allergens are most closely related to indoor humidity?

A. *Dermatophagoides farinae*
B. *Blatella germanica*
C. *Alternaria alternata*
D. *Felis domesticus*

Question 2. Which of the following methods of controlling exposure to cat allergen is most useful?

A. keep the cat out of the bedroom
B. dispose of the cat
C. wash the cat once a week
D. use high-efficiency particle filtration

Question 3. Which of the following air pollutants increases production of IgE antibodies?

A. sulfur dioxide
B. nitric oxide
C. diesel exhaust particles
D. ozone.

Question 4. Which of the following statements about occupational asthma is true?

A. Symptoms occur only on days when the patient is at work.
B. Bronchial provocation tests are required to confirm the diagnosis.
C. Approximately 5% of asthma beginning in adulthood is due to occupational exposure.
D. All the exposures that cause occupational asthma elicit an IgE response.

Study questions

Anaphylaxis

Learning objectives: "Anaphylaxis"

1. To describe the triggers, mechanisms, and patient-specific risk factors in anaphylaxis.
2. To state the principles of risk assessment in anaphylaxis.
3. To discuss long-term risk reduction in anaphylaxis: preventive measures and emergency preparedness.

Question 1. The lifelong prevalence of anaphylaxis from all triggers in the general population is estimated at —

A. 0.001%.
B. 0.01%.
C. 0.1%.
D. 0.05% to 2%.

Question 2. You diagnose idiopathic anaphylaxis in a 50 year-old woman who has had 2 episodes during the past year. What should you do next?

A. Refer her for a bone marrow biopsy.
B. Measure her serum total tryptase level.
C. Advise her to avoid peanut, tree nuts, shellfish, and fish.
D. Prescribe prednisone, 60 mg daily, for a week and then taper the dose.

Question 3. For which of the following is a 3- to 5-year course of subcutaneous immunotherapy recommended based on randomized, double-blind, placebo-controlled trials?

A. food-induced anaphylaxis
B. medication-induced anaphylaxis
C. stinging insect venom–induced anaphylaxis
D. natural rubber latex–induced anaphylaxis

Question 4. Epinephrine is the drug of first choice in anaphylaxis because —

A. its α_1-adrenergic vasoconstrictor effects decrease mucosal edema and increase peripheral vascular resistance.
B. its α_2-adrenergic receptor effects decrease release of insulin and norepinephrine.
C. its β_1-adrenergic effects increase the rate and force of cardiac contractions.
D. its β_2-adrenergic effects increase bronchodilation and decrease mediator release.

Study questions

Primary immunodeficiencies

Learning objectives: "Primary immunodeficiencies"

1. To recognize the key diagnostic features of congenital defects of lymphocyte development and neutrophil function.
2. To learn the mainstay of treatment for patients with antibody deficiency.

Question 1. Which of the following statements concerning severe combined immunodeficiency (SCID) is true?

A. SCID is characterized by severe deficiency of T cells.
B. SCID is characterized by severe deficiency of T and B cells.
C. SCID is characterized by severe deficiency of T, B, and natural killer cells.
D. SCID is characterized by severe deficiency of both lymphocytes and neutrophils.

Question 2. Which of the following statements concerning X-linked agammaglobulinemia (XLA) is true?

A. XLA is characterized by lack of immunoglobulins in spite of a normal number of circulating B cells.
B. XLA is characterized by a virtual lack of circulating B cells.
C. In patients with XLA, the profound deficiency of immunoglobulins reflects defects of T_H lymphocytes.
D. The mainstay of treatment of patients with XLA is antibiotic prophylaxis.

Question 3. Which of the following presentations is common in patients with chronic granulomatous disease?

A. autoimmune manifestations resembling systemic lupus erythematosus
B. interstitial pneumonia caused by *Pneumocystis jiroveci*
C. purulent lymphadenitis
D. recurrent otitis media

Question 4. Which of the following statements concerning treatment with immunoglobulins is true?

A. Initial treatment for patients with agammaglobulinemia should be with intravenous immunoglobulin, 100 mg/kg every 3 weeks.
B. Subcutaneous immunoglobulins should be used at the dose of 100 mg/kg/wk in children less than 14 years of age. Beyond that age, the dose for adults is 4 g/wk.
C. The usual dose for subcutaneous immunoglobulins is 100 mg/kg/wk.
D. Patients with IgA deficiency should receive preparations enriched in IgA.

Study questions

Secondary immunodeficiencies, including HIV infection

Learning objectives: "Secondary immunodeficiencies, including HIV infection"

1. To define the concept of secondary immunodeficiency as a clinical condition in which the immune response is adversely affected by extrinsic factors.
2. To realize that the frequency of patients with secondary immunodeficiencies far exceeds the frequency of those with primary (genetic) immunodeficiencies.
3. To appreciate the many diverse factors and conditions that produce secondary immunodeficiency.
4. To understand that HIV infection is one of the best understood yet most challenging examples of a secondary immunodeficiency.

Question 1. An 18-year-old woman presents with a history of recurrent respiratory tract infections in the past 3 months. She has been previously healthy. Which of the following is the most likely cause of immunodeficiency in this patient?

A. severe combined immunodeficiency
B. HIV infection
C. X-linked agammaglobulinemia
D. hyper-IgM syndrome

Question 2. Which of the following is a characteristic of a secondary immunodeficiency?

A. The clinical presentation is variable.
B. A defect in T-cell function can always be identified.
C. Management should prioritize immunoglobulin supplementation in all cases.
D. Phagocyte chemotaxis is normal.

Question 3. Calcineurin inhibitors primarily inhibit—

A. oxidative burst.
B. complement activation.
C. T-cell activation.
D. calcium membrane receptor.

Question 4. In patients with HIV infection, which of the following is true?

A. AIDS, the advanced stage of HIV infection, develops when B cells are depleted.
B. The presence of the chemokine receptor CCR5 in cell membrane blocks HIV infection.
C. The presence of the chemokine receptor CCR5 in cell membrane is necessary for HIV infection.
D. An adenovirus-based anti-HIV vaccine has been demonstrated to reduce HIV infection in a large placebo-controlled trial.

Study questions

Immunologic rheumatic disorders

Learning objectives: "Immunologic rheumatic disorders"

1. To recognize the diagnostic utility and the prognostic significance of the rheumatoid factor and anti–cyclic citrullinated peptide (anti-CCP) antibody tests in patients with rheumatoid arthritis (RA).
2. To understand the basic mechanisms behind the biologic disease-modifying medications currently available to treat RA.
3. To identify the antibody tests that are useful in making the diagnosis of systemic lupus erythematosus (SLE) while recognizing that the diagnosis is based on the whole clinical picture and not simply the laboratory findings.

Question 1. Which of the following statements is true regarding anti-CCP antibodies in patients with RA?

A. The appearance of anti-CCP antibodies in the bloodstream coincides with the onset of clinical RA.
B. Anti-CCP antibodies are highly specific for RA.
C. Patients with RA who have anti-CCP antibodies tend to have milder disease than those who do not have the antibodies.
D. Rheumatoid factor and anti-CCP antibodies are about equally specific for RA.

Question 2. Comparing the traditional disease-modifying antirheumatic drugs (DMARDs) used to treat RA with the new biologic DMARDS, which of the following statements is true?

A. The biologic DMARDs target specific factors in the immune system, such as proinflammatory cytokines.
B. Current American College of Rheumatology recommendations include initiation of biologic DMARDs within 3 months of diagnosis of RA.
C. Traditional DMARDs, such as methotrexate, increase cardiovascular risk in patients with RA.
D. Combining biologic DMARDs with traditional DMARDs does not increase efficacy in patients with RA.

Question 3. Which of the following diseases is more common in men than in women?

A. RA
B. seronegative spondyloarthropathies
C. SLE
D. Sjögren syndrome

Question 4. Which of the following statements is true regarding antibodies in SLE?

A. A patient with arthralgias and a low titer of antinuclear antibody (ANA) is likely to have SLE.
B. Patients with SLE frequently have negative ANA test results.
C. The presence of anti-Ro (SSA) rules out SLE in favor of Sjögren syndrome.
D. Anti-Smith antibodies are highly specific for SLE.

Study questions

Vasculitis

Learning objectives: "Vasculitis"

1. To describe the diagnostic yield from biopsy specimens of different organs in patients with Wegener granulomatosis.
2. To identify the sites of organ involvement in patients with Churg-Strauss syndrome.
3. To recognize the antigen associations of antineutrophil cytoplasmic antibodies (ANCAs).
4. To distinguish the prominent clinical features of giant cell arteritis (GCA).

Question 1. Which of the following biopsies of a clinically involved site has the highest likelihood of yielding a diagnosis of Wegener granulomatosis?

A. sinus
B. kidney
C. lung
D. gastrointestinal mucosa

Question 2. Which of the following is the most common organ system affected by vasculitis in patients with Churg-Strauss syndrome?

A. peripheral nerve
B. heart
C. kidney
D. gastrointestinal tract

Question 3. Which of the following antigens do ANCAs most commonly target in patients with Wegener granulomatosis?

A. myeloperoxidase
B. proteinase 3
C. human neutrophil elastase
D. bactericidal permeability–increasing protein

Question 4. Which of the following statements is true regarding GCA?

A. Visual loss occurs in 50% to 60% of patients.
B. Isolated polymyalgia rheumatica requires treatment with 40 to 60 mg/d prednisone.
C. An increased sedimentation rate occurs in less than 50% of patients with GCA.
D. Large-vessel involvement of the aorta or its primary branches occurs in 27% of cases.

Study questions

Immunologic endocrine disorders

Learning objectives: "Immunologic endocrine disorders"

1. To understand general HLA and autoantibody testing for type 1 diabetes and the associated celiac disease.
2. To understand the genetics of 2 major monogenic forms of autoimmune type 1 diabetes.
3. To become familiar with major autoantibodies measured in patients with polyendocrine syndromes.
4. To recognize the similarities and differences between the major autoimmune polyendocrine syndromes.

Question 1. Which of the following statements concerning type 1 diabetes is true?

A. The highest-risk genotype for type 1 diabetes is DR4/4.
B. Islet autoantibodies typically appear years before the development of diabetes.
C. Insulin autoantibodies are most common in adults rather than children with diabetes.
D. Celiac disease and anti-transglutaminase autoantibodies are not increased in patients with type 1 diabetes.

Question 2. Immune dysfunction, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome and autoimmune polyendocrine syndrome type 1 (APS-1) are —

A. monogenic disorders influencing the development of regulatory T cells or expression of peripheral antigens in the thymus.
B. inherited in an autosomal recessive manner.
C. polygenic disorders.
D. common.

Question 3. Which of the following statements concerning autoantibodies is true?

A. Anti–21-hydroxylase autoantibodies are diagnostic of Addison disease.
B. Anti-insulin autoantibodies develop in most individuals treated with subcutaneous insulin, including patients with type 2 diabetes.
C. Transglutaminase autoantibodies are the best marker of celiac disease.
D. All of the above

Question 4. APS-1 differs from autoimmune polyendocrine syndrome type 2 (APS-2) in that —

A. patients with APS-1 have a mutation of *AIRE*.
B. Addison disease and type 1 diabetes occur in both syndromes.
C. mucocutaneous candidiasis is present only in patients with APS-1.
D. All of the above

Study questions

Diagnostic testing and interpretation of tests for autoimmunity

Learning objectives: "Diagnostic testing and interpretation of tests for autoimmunity"

1. To understand the usefulness of autoantibodies and immunologic studies.
2. To understand the limitations of these studies.
3. To understand the major clinical presentations of autoimmune diseases.

Question 1. A 36-year-old woman is seen in the emergency department for new-onset shortness of breath with wheezing. After bronchodilation therapy, the patient no longer wheezes. Other than chronic sinusitis, she has been well. Examination shows her to be comfortable, afebrile, and normotensive, with a respiratory rate of 14 breaths/min. No wheezes are auscultated. Tender subcutaneous nodules are discovered on her right anterior leg. Screening laboratory tests reveal a mild anemia and slightly increased white blood cell count with increased eosinophil numbers. The Westergren erythrocyte sedimentation rate (ESR) is 88 mm, and the high-sensitivity C-reactive protein level is 46 mg/dL. Chest radiography shows patchy opacities without lobar or segmental distribution. What laboratory test would be highly suggestive that this is Churg-Strauss syndrome?

A. antinuclear antibody (ANA)
B. anti–proteinase 3
C. anti-myeloperoxidase (anti-MPO)
D. anti–extractible nuclear antigen (anti-ENA)
E. anti–double-stranded DNA (anti-dsDNA)

Question 2. What is the most sensitive serologic test for systemic lupus erythematosus (SLE)?

A. anti-dsDNA
B. ANA
C. anti-Smith
D. anti-ENA
E. ESR

Question 3. What is the most specific serologic test for SLE?

A. anti-dsDNA
B. ANA
C. anti-Smith
D. anti-ENA
E. ESR

Question 4. In immune complex deposition diseases, such as vasculitis, which is associated with rheumatoid arthritis, serum C3 levels will most commonly —

A. increase.
B. decrease.
C. remain unchanged.
D. decrease and then increase.

Study questions

Pulmonary disorders, including vocal cord dysfunction

Learning objectives: "Pulmonary disorders, including vocal cord dysfunction"

1. To explore the classification of pulmonary disorders with various immunologic processes.
2. To consider the causes of pulmonary eosinophilia syndromes or conditions.
3. To differentiate granulomatous T_H1 and T_H2 inflammatory conditions.
4. To appreciate the variable aspects of diagnosis of vocal cord dysfunction.

Question 1. In patients with pulmonary tuberculosis, the number of $CD4^+CD25^+$ regulatory T cells is —

A. decreased.
B. increased.
C. very low or absent.
D. very high.

Question 2. Antibodies in classic cases of Churg-Strauss syndrome are directed against —

A. proteinase-3.
B. myeloperoxidase.
C. single-stranded DNA.
D. Churg-Strauss syndrome protein.

Question 3. The expected finding in bronchoalveolar lavage differential count in a patient with acute hypersensitivity pneumonitis is —

A. macrophages of 95%.
B. lymphocytes of 60%.
C. eosinophils of 40%.
D. polymorphonuclear leukocytes of 60%.

Question 4. A characteristic feature of the reactive airways dysfunction syndrome (RADS) is that —

A. the period for sensitization is usually 1 to 5 years before onset.
B. respiratory symptoms resolve by 3 months after exposure.
C. bronchial hyperresponsiveness is present.
D. bronchial biopsy demonstrating eosinophilia is consistent with the diagnosis.

Study questions

Mucosal immunology, eosinophilic esophagitis, and other intestinal inflammatory diseases

Learning objectives: "Mucosal immunology, eosinophilic esophagitis, and other intestinal inflammatory diseases"

1. To identify anatomic features of the gastrointestinal mucosal immune system.
2. To recognize clinicopathologic features of common gastrointestinal diseases that are linked by perturbations in the mucosal immune system.

Question 1. Defects in which of the following cell types lead to a syndrome termed immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome?

A. B cells
B. mast cells
C. regulatory T cells
D. eosinophils

Question 2. Which of the following are the correct diagnostic features of eosinophilic esophagitis in a child or adult?

A. esophageal inflammation consisting of more than 15 eosinophils per high-power field
B. dysphagia and food impaction that persists despite acid blockade
C. symptoms consistent with esophageal dysfunction and more than 15 eosinophils per high-power field in which other causes have been ruled out
D. feeding dysfunction and esophageal inflammation consisting of more than 15 eosinophils per high-power field

Question 3. Which of the following diseases can be treated primarily with dietary exclusion?

A. Crohn disease
B. ulcerative colitis
C. celiac disease
D. gastroesophageal reflux disease

Question 4. Which of the following is a feature of defensins?

A. biochemical characterized as charge neutral molecules
B. They are primarily produced by Paneth cells.
C. They participate as an element of the acquired immune system.
D. Three different types have been identified.

Study questions

Complement disorders and hereditary angioedema

Learning objectives: "Complement disorders and hereditary angioedema"

1. To understand the role of complement in host defense.
2. To be able to identify and understand the difference between the various complement pathways.
3. To understand hereditary angioedema, its relation to complement and kinins, and the new therapeutic approaches.
4. To understand the consequences of genetic deficiency of complement proteins and regulatory proteins.

Question 1. The complement pathway initiated by the binding of a plasma protein to sugars on the surface of a microbe is —

A. the lectin pathway.
B. the alternative pathway.
C. the classical pathway.
D. the kinin pathway.

Question 2. Therapy for hereditary angioedema is directed at which of the following outcomes?

A. control of C1 esterase inhibitor levels to greater than 75% activity
B. decrease in the number of angioedema episodes to maximize quality of life with minimal adverse effects
C. control of C4 levels to normality
D. control of all episodes of angioedema

Question 3. The pathway of complement activation activated by antibody usually is —

A. the lectin pathway.
B. the alternative pathway.
C. the classical pathway.
D. the lectin pathway.

Question 4. What is the genetic defect that is frequently associated with high-grade pathogen infection, such as with pneumococci or staphylococci?

A. C8 deficiency
B. factor B deficiency
C. C3 deficiency
D. mannose-binding lectin deficiency

Study questions

Immune responses to malignancies

Learning objectives: "Immune responses to malignancies"

1. To understand the role of the immune system in control of tumor progression.
2. To recognize the effect of the tumor microenvironment on functions of immune cells.
3. To understand the molecular and cellular mechanisms used by the tumor for its escape from the host immune system.

Question 1. What type of evidence do we have that a patient with cancer makes an immune response specifically directed at his or her own tumor?

A. Tumors induce apoptosis of $CD8^+$ T cells.
B. Tumor cells release tumor-associated antigens (TAs) into the circulation.
C. Antibodies to MHC molecules are detectable in patients' sera.
D. A measurable or increased (relative to that seen in healthy control subjects) frequency in the blood of $CD8^+$ cytotoxic T lymphocytes that stain with TA-specific tetramers is present.

Question 2. Dendritic cells (DCs) are professional antigen-presenting cells that play a key role in the induction of adaptive immune responses to TAs. DCs in patients with cancer do not efficiently cross-present TAs to T cells because —

A. they do not home to the tumor or tumor-draining lymph nodes.
B. they are enriched in class I and class II MHC molecules relative to DCs in healthy control subjects.
C. they are immature because of the presence of soluble tumor-derived factors.
D. they produce excessive levels of IL-12.

Question 3. Regulatory T cells are in part responsible for down-regulation of antitumor activity in patients with cancer. These T cells —

A. mediate suppression of other immune cells through cell-to-cell contact.
B. secrete IFN-γ and IL-2.
C. are decreased in number in the peripheral blood of patients with cancer.
D. do not kill other T or B cells.

Question 4. Inflammatory infiltrates into human solid tumors have been carefully examined because of their potential prognostic significance. These infiltrates have the following characteristic.

A. They resemble acute inflammatory infiltrates into healing wounds.
B. They are enriched in natural killer cells.
C. The ratio of $CD8^+/CD4^+$ T cells is always high because of the excess of $CD8^+$ T cells.
D. They are a major source of proinflammatory cytokines that support tumor growth.

Study questions

Clinical laboratory assessment of immediate-type hypersensitivity

Learning objectives: "Clinical laboratory assessment of immediate-type hypersensitivity"

1. To understand the principal properties of human IgE antibodies.
2. To describe the components of the diagnostic algorithm that are used in the evaluation of a patient with a suspected allergic disease.
3. To define laboratory methods for studying IgE antibody cross-reactivity with structurally similar allergens (eg, Hymenoptera venoms).
4. To define the humoral immune response parameters that determine the most effective translation of an IgE antibody response into mediator release from mast cells and basophils.
5. To understand when detection of IgG antibody responses can be diagnostically useful in the evaluation of lung-related hypersensitivity states.

Question 1. IgE is the immunoglobulin that has been called the "gatekeeper" of the allergic response. Once bound to the surface of basophils or mast cells, it serves to mediate vasoactive mediator release after cross-linking by allergenic molecules. Which of the following is a property of human IgE antibodies?

A. Its molecular weight is approximately 150,000 d.
B. It freely passes the placenta to contribute to neonatal total serum IgE levels that allow identification of a neonate's atopic predisposition.
C. Its concentration in serum is highly age-dependent.
D. It constitutes 2% of the total serum immunoglobulin.

Question 2. The diagnostic algorithm for allergic disease begins with a carefully collected clinical history. If the history indicates a highly probable association between the patient's reported upper airway allergic symptoms and a probable aeroallergen exposure, what is the next recommended step in the diagnostic process based on the practice parameters?

A. Immediately quantify mast cell α-tryptase within 30 minutes to 4 hours after symptom initiation.
B. Perform serology or skin test measurements (depending on the suspected allergen specificity) to verify that the patient is sensitized (IgE antibody positive).
C. Perform a direct allergen challenge of the indicated target organ.
D. Perform an allergen-specific IgG measurement to verify exposure.

Question 3. IgE antibody can be present in the absence of any evident clinical symptoms. Which one of the following humoral immune response–related conditions enhances the effectiveness of IgE antibody responses to induce mediator release from mast cells and basophils?

A. lower allergen-specific IgE antibody concentration in circulation
B. less mature specificity directed at the allergen's or allergens' specific epitopes
C. lower proportion of specific IgE to total IgE in a patient's serum
D. higher affinity of the IgE antibody for specific allergen

Question 4. In which one of the following situations are specific IgG antibody responses considered diagnostically useful in the workup of a patient?

A. evaluation of food allergy symptoms for the planning of food-elimination diets
B. assessment of patients with rhinitis associated with seasonal aeroallergen exposure
C. testing of a child with spina bifida who experienced anaphylaxis after the insertion of a *Hevea brasiliensis* latex catheter
D. evaluation of a patient suspected of hypersensitivity pneumonitis as a result of exposure to organic dusts

Study questions

Laboratory evaluation of primary immunodeficiencies

Learning objectives: "Laboratory evaluation of primary immunodeficiencies"

1. To recognize the clinical symptoms of the most common primary immunodeficiencies.
2. To describe the appropriate laboratory approach for investigating general categories of primary immunodeficiencies.

Question 1. Choose the alternative that best describes the most appropriate initial laboratory test to evaluate a patient with recurrent bacterial sinopulmonary infections and chronic diarrhea caused by *Giardia lamblia*.

A. lymphocyte immunophenotyping
B. mitogen-induced lymphocyte proliferation
C. oxidative burst by dihydrorhodamine 123 (DHR)
D. serum immunoglobulin levels

Question 2. Recurrent infections by a narrow range of pathogens, such as *Streptococcus pneumoniae* and *Staphylococcus aureus*, together with a poor fever response to infection is most consistent with a defect in signaling by which of the following?

A. IFN-γ receptor
B. IL-12 receptor
C. Toll-like receptors
D. GM-CSF receptor

Question 3. The results of which of the following laboratory tests would most likely be abnormal in a 2 month-old infant with failure to thrive, persistent diarrhea, and oral thrush?

A. DHR
B. CH50
C. lymphocyte count
D. serum immunoglobulin measurement

Question 4. Which of the following assays correlates most closely with CD45RA expression on CD4 T cells?

A. T-cell receptor excision circles
B. T-cell receptor diversity
C. T cell–mediated cytotoxicity
D. T-cell response to mitogens

Study questions

Allergen immunotherapy

Learning objectives: "Allergen immunotherapy"

1. To have a clear understanding of the mechanisms believed to be responsible for the beneficial effects of allergen immunotherapy.
2. To be able to explain the principles of patient selection and safe administration of immunotherapy.
3. To be aware of the scope for improving immunotherapy in the future.

Question 1. Which of these conditions is not an indication for specific immunotherapy (SIT)?

A. bee venom–induced anaphylaxis
B. aspirin-induced asthma
C. allergic rhinitis
D. cat dander–induced asthma

Question 2. Sublingual immunotherapy —

A. uses similar doses of allergen to conventional (injected) SIT.
B. has a large evidence base for use in children.
C. works by induction of local (mucosal) tolerance.
D. has been show to induce antigen-specific regulatory T cells.

Question 3. Venom immunotherapy (VIT) —

A. abolishes the risk of anaphylaxis to subsequent stings.
B. provides protection against large local reactions.
C. offers protection once the maintenance dose is reached.
D. needs to be continued indefinitely in most patients.

Question 4. Recombinant allergens —

A. are more effective than conventional extracts in clinical trials in patients with allergic rhinitis.
B. are inherently less allergenic than natural allergens.
C. could allow the development of patient-tailored therapy.
D. work better if coupled to CpG oligodeoxynucleotides.

Study questions

Immunomodulator therapy: Monoclonal antibodies, fusion proteins, cytokines, and immunoglobulins

Learning objectives: "Immunomodulator therapy: Monoclonal antibodies, fusion proteins, cytokines, and immunoglobulins"

1. To review potential therapeutic roles of mAbs and fusion proteins in the treatment of autoimmune conditions.
2. To review the mechanism of action for biologic agents commonly used in the treatment of inflammatory arthritis.
3. To recognize potential safety concerns related to mAbs and fusion proteins used in the treatment of autoimmune diseases.

Question 1. TNF inhibitors have been shown to be effective in the treatment of several autoimmune diseases. Which of following statements is true regarding the efficacy of TNF inhibitors?

A. All approved TNF inhibitors are effective in the treatment of rheumatoid arthritis (RA).
B. Etanercept is effective in the treatment of inflammatory bowel disease (eg, Crohn disease).
C. TNF inhibitors are effective in the treatment of congestive heart failure.
D. TNF inhibitors are effective in the treatment of demyelinating diseases (eg, multiple sclerosis).

Question 2. Biologic agents have significantly improved the clinical outcomes of many autoimmune diseases. However, they are also associated with potentially serious adverse events. Which of the following statements on safety considerations of biologic agents is true?

A. The risk of infection does not increase when TNF inhibitors are combined with other biologic agents.
B. TNF inhibitors have been associated with increased risk of tuberculosis.
C. No cases of progressive multifocal leukoencephalopathy have been reported among patients treated with rituximab.
D. Rituximab is safe to use in patients with hepatitis B.

Question 3. Autoreactive T cells, especially $CD4^+$ T_H1 T cells, serve a key role in orchestrating the immune-driven inflammatory responses in autoimmune diseases. Which of the following statements on T-cell activation is true?

A. The binding of specific antigen-associated MHC class II molecules to the T-cell receptor is sufficient to activate $CD4^+$ T cells.
B. The binding of CD28 to CD80/CD86 results in T-cell inhibition and anergy.
C. Cytotoxic T lymphocyte–associated antigen 4 (CTLA-4) binds to CD80/CD86 with higher affinity than CD28 and inhibits T-cell costimulation.
D. The binding of lymphocyte function–associated antigen 3 to $CD2^+$ T cells activates memory T cells.

Question 4. Rituximab therapy has been approved for the treatment of non-Hodgkin lymphoma and RA. Which of the following statements on the potential mechanism of action is true?

A. Rituximab binds to CD20, which is present in both mature B cells and plasma cells.
B. Rituximab can be used alone or in combination with other disease-modifying antirheumatic drugs in the treatment of RA.
C. Seropositivity for rheumatoid factor does not appear to affect the efficacy of rituximab among patients with RA.
D. B-cell depletion after a course of rituximab rarely lasts longer than 3 months.

Study questions

Transplantation immunology: Solid organ and bone marrow

Learning objectives: "Transplantation immunology: Solid organ and bone marrow"

1. To recognize the central role of donor-recipient HLA matching in transplant outcomes.
2. To know the most common diseases that benefit from hematopoietic stem cell transplantation (HSCT).

Question 1. Disparity of the HLA proteins between a transplant donor and the recipient results in —

A. immune tolerance.
B. immune activation.
C. transplant engraftment.
D. no immune effect.

Question 2. In solid-organ transplantation a characteristic of hyperacute rejection is —

A. that it usually occurs within 48 hours of transplantation.
B. that it involves the new development of anti-HLA antibodies.
C. that it is mostly mediated by T cells.
D. that treatment based on steroids is usually successful.

Question 3. Low risk of graft-versus-host disease (GVHD) in HSCT can be predicted when the graft is —

A. a cord blood unit with only 1 of 6 HLA antigens matched with the recipient.
B. bone marrow from an HLA-haploidentical related donor that has not been T-cell depleted.
C. non–T cell–depleted bone marrow from an unrelated donor with a match of 4 of 6 HLA antigens.
D. non–T cell–depleted bone marrow from an HLA-matched related donor.

Question 4. HSCT is indicated in which one of the following primary immunodeficiencies?

A. X-linked agammaglobulinemia
B. C2 complement deficiency
C. severe combined immunodeficiency
D. partial DiGeorge syndrome

Study questions

Embryonic and adult stem cell therapy

Learning objectives: "Embryonic and adult stem cell therapy"

1. To develop a basic understanding of the processes involved in stem cell development.
2. To understand the complexity of and recent advances in stem cell programming.
3. To appreciate the therapeutic possibilities and problems associated with the transplantation of manipulated stem cells.
4. To appreciate the ethical and political debate surrounding the use of human stem cells.

Question 1. Allogeneic hematopoietic stem cell transplantation is often used for the treatment of acute leukemia. The most desirable source of donor cells is from —

A. a parent.
B. an HLA-matched sibling.
C. HLA-matched umbilical cord blood.
D. an HLA-matched unrelated donor.

Question 2. When and why would one use donor lymphocyte infusion during the course of allogeneic transplantation for malignant hematopoietic disorders?

A. 1 week before to facilitate engraftment
B. 1 month after to consolidate engraftment
C. At the time of relapse to obtain remission
D. Never, because it has too many side effects

Question 3. Haploidentical hematopoietic stem cell transplantation is made possible by what manipulation of the donor cells?

A. T-cell depletion
B. B-cell depletion
C. natural killer cell depletion
D. That procedure is currently not possible in human subjects.

Question 4. Human embryonic stem cells can be derived from which of the following sources?

A. aborted fetus
B. hematopoietic stem cells
C. living embryos *in utero*
D. unused embryos made by means of *in vitro* fertilization for infertility problems

Author index

Subject index

Answers to Study questions

Chapter 1: Overview of the immune response

1. Answer: B

Explanation: In virus-infected cells, some viral proteins are degraded in the cellular proteasome, the major cellular protein degrading organelle. TAP-1 and TAP-2 are proteins that participate in the transport of peptide fragments from the proteasome into the endoplasmic reticulum where they are loaded into newly synthesized class I MHC molecules. Without TAP-1 or TAP-2, viral peptides are not loaded into class I molecules, and recognition by $CD8^+$ T cells does not occur. HLA-DM is required for peptide loading into class II MHC molecules, permitting recognition by $CD4^+$ T cells. B-lymphocytes must traffic through the germinal center in order to undergo somatic mutation and differentiation to high affinity antibody producing cells. Trafficking through the germinal center is not required for $CD8^+$ T cells development or differentiation. Some viruses down-regulate (extinguish) expression of the class I proteins of the cell they have infected. This is a strategy for avoiding the CD8 host immune response. Sustained class I molecule expression is necessary for recognition by $CD8^+$ T cells.

2. Answer: C

Explanation: Loading of peptide fragment from exogenous antigens into class II HLA molecules occurs when the acidified endosome fuses with the class II loading compartment. This fusion results in the proteolytic degradation of the invariant chain that then allows peptides to associate with the class II molecules. The invariant chain is not associated with peptide loading into class I HLA molecules. The invariant chain associates with the newly synthesized class II HLA molecules in the endoplasmic reticulum. The invariant chain is designated as 'invariant' because is shows very low sequence variability in different individuals in the population.

3. Answer: D

Explanation: β_2-Microglobulin associates with class I MHC molecules and several other molecules with class I–like structures. The MHC molecules that are the targets of T_H cells ($CD4^+$ T cells) are class II MHC molecules. These do not contain β_2-microglobulin. The CD3 complex, rearranged α and β chains of the T-cell receptor, and the CD4 molecule are all important components for recognition of peptide antigens associated with class II MHC molecules.

4. Answer: C

Explanation: TLRs are found on many somatic cells, particularly ones that are involved in early contact with microbes that invade the body through skin and mucosal tissues. The *intracellular* domains of TLRs are homologous to the corresponding domains of the IL-1 receptor. Most TLRs are cell-surface proteins, but TLR3 and TLR9 are intracellular proteins that interact with their ligands during the intracellular part of their lifecycle. TLR4 is activated by LPS. TLR9, in contrast, is activated by bacterial CpG DNA.

Chapter 2: Innate immunity

1. Answer: C

Explanation: Because TLRs are highly expressed on dendritic cells but not on T cells, the goal of TLR-based therapies in patients with allergy and asthma is to activate dendritic cells to produce a cytokine milieu (eg, IL-12 and interferons) that favors inhibition of the T_H2 immune response.

2. Answer: A

Explanation: IRAK4 deficiency is a novel primary immunodeficiency specifically affecting TLR function, which is a component of the innate immune system. IRAK4 is involved in downstream signaling from most TLRs.

3. Answer: D

Explanation: Flagellin is the ligand for TLR5.

4. Answer: B

Explanation: The NLRP3 (NALP3) inflammasome is involved in mediating the adjuvant effects of alum. This adjuvancy can occur directly through the triggering of the NALP3 inflammasome by alum crystals or indirectly through release of the endogenous danger signal uric acid, which subsequently activates NLRP3.

Chapter 3: Adaptive immunity

1. Answer: C

Explanation: T-cell receptor activation leads to release of intracellular calcium stores, as well as influx of extracellular calcium. Mature T cells express either CD4 or CD8 but not both. CD8 serves as a coreceptor for MHC class I and not MHC class II molecules. The newborn screening for severe combined immunodeficiency is performed by means of PCR quantitation of T-cell receptor excision circles on blood spots.

2. Answer: D

Explanation: Natural killer T cells express markers of both T ($\alpha\beta$) and natural killer (CD56) cells. $CD25^+$ regulatory T cells express forkhead box protein 3. RORγt is present in T_H17 cells, which arise under the influence of IL-6 and TGF-β. Target cell killing by cytolytic T lymphocytes is complement independent and involves perforin, granzymes, and Fas-mediated apoptotic mechanisms.

3. Answer: D

Explanation: The complete pre-B cell receptor is made up of the IgM heavy chain, the surrogate light chain, and the Ig-α and Ig-β signal-transducing molecules. The κ and λ light chains and IgD heavy chains are contained in mature B-cell immunoglobulin receptors. CD20 is a B-cell marker that is not a component of the immunoglobulin receptor.

4. Answer: C

Explanation: Immunoglobulin class-switching and somatic hypermutation are the critical processes of antigen-dependent B-cell development that take place in germinal centers. Immunoglobulin gene rearrangement occurs mainly during B-cell development in the bone marrow, IgD is expressed on the surfaces of mature IgM-expressing B cells in the spleen and in the circulation, and large-scale antibody production by plasma cells occurs mainly in the spleen, bone marrow, and mucosal sites.

Chapter 4: Structure and function of immunoglobulins

1. Answer: C

Explanation: Activation-induced cytidine deaminase deaminates cytosine to produce uracil, which in turn can be removed from DNA through the action of uracil DNA glycosylase to permit either double-stranded DNA breaks that permit class-switch recombination or substitution of nucleotide sequence to advance somatic hypermutation. κ Light chains can be replaced by λ light chains; hence although the repertoire is restricted in their absence, rearrangement can still occur in the heavy chain and λ locus to permit immunoglobulin formation. RAG1 and RAG2 directly catalyze V(D)J recombination. In their absence VDJ recombination does not occur. The result is a complete deficiency of B and T cells. Surrogate light chain plays a key role in checking the function of new heavy chains after they have rearranged. In the absence of surrogate light chain, B-cell development is blocked at the pre–B-cell stage, creating agammaglobulinemia. However, this occurs after heavy chain rearrangement. TdT adds nucleotides at random to rearranging gene segments, but it is not necessary for the rearrangement process itself. Indeed, fetal mice lack TdT expression entirely.

2. Answer: D

Explanation: Of the isotypes, IgM is the most potent at activating complement, followed by IgG. Within IgG subclasses, IgG2 and IgG4 are very ineffective at activating complement, with considerable activity by IgG1 and IgG3.

3. Answer: A

Explanation: Effector cells differ in their expression of Fcε receptors, and IgA receptors are not present on mast cells.

4. Answer: C

Explanation: Proper glycosylation of immunoglobulin is very important to immunoglobulin function, metabolism, and half-life. Aberrant glycosylation can result in altered clearance and/or be recognized as foreign by the immune system and lead to autoimmune manifestations.

Chapter 5: Immunologic messenger molecules: Cytokines, interferons, and chemokines

1. Answer: A

Explanation: IL-6 signals through a ligand-binding IL-6 receptor α chain (CD126) and the signal-transducing component glycoprotein 130 (CD130). CD130 is the common signal transducer for several cytokines in the IL-6 family and is ubiquitously expressed.

2. Answer: C

Explanation: As a unique regulator of T_H17 development, retinoic acid receptor–related orphan receptor γt through stimulation with IL-6 acting in the additional presence of TGF-β is responsible for differentiation of T_H17 cells.

3. Answer: B

Explanation: Unlike many cytokines that use the shared γ chain, TSLP receptor is a heterodimer composed of a unique TSLP-specific receptor and the IL-7 receptor α chain (CD127).

4. Answer: D

Explanation: Expression of CCL17, which can be induced by IL-4 and IL-13, promotes T_H2 cell development.

Chapter 6: IgE, mast cells, basophils, and eosinophils

1. Answer: D

Explanation: The tetrameric form of the FcεRI receptor ($\alpha\beta\gamma2$) is present on mast cells and basophils, whereas the trimeric form ($\alpha\gamma2$), lacking the β chain, is present on antigen-presenting cells. The β chain stabilizes the receptor and amplifies signaling. The α subunit of FcεRI binds the c3 domain of the Fc region of IgE, the same domain recognized by omalizumab.

2. Answer: D

Explanation: Unlike mast cells, prostaglandin D_2 is not produced in significant quantities by basophils. Production of GM-CSF, IL-4, and granzyme B by basophils has been reported.

3. Answer: B

Explanation: Peripheral eosinophilia is present in hypoadrenalism (Addison disease); some primary immunodeficiency diseases, including Omenn syndrome; and Kimura disease. Eosinopenia is common in the setting of acute bacterial or viral infections, such as sepsis.

4. Answer: C

Explanation: Anaphylaxis to parenteral agents is usually associated with increased serum tryptase levels, whereas anaphylaxis to oral agents frequently is not associated with increased serum tryptase levels. Baseline serum tryptase levels are composed primarily of protryptases, whereas mature β-tryptase is the form stored in secretory granules and secreted after mast cell activation. Tryptase is stabilized in the secretory granules by heparin.

Chapter 7: Genetics of allergic disease

1. Answer: B

Explanation: The most efficient approach to studying whether genetic variation affecting the expression level of a candidate gene or function of the encoded protein alters susceptibility to allergic disease would be to use a panel of genetic variations across the gene selected on the basis of linkage disequilibrium patterns to tag all common variations in the gene region to genotype a case-control cohort. Genome-wide association study approaches, or genome-wide positional cloning in families, are hypothesis-independent approaches in which the entire genome is assessed for gene regions/genes that are associated/linked to the phenotype being assessed. Hence these are best suited to finding novel genes whose encoded proteins by definition must play important roles in disease pathophysiology, or genetic variation affecting their expression, function, or both would not be associated with disease.

2. Answer: A

Explanation: *CHRNA3* is a candidate gene identified for lung cancer, chronic obstructive pulmonary disease, and smoking behavior. Genetic variation in the promoter and coding region of the gene encoding IL-13 has been shown to be associated with atopy and asthma phenotypes in a number of case-control candidate gene studies. *SH2B3* was identified as a gene associated with blood eosinophil levels in a genome-wide association approach but was not associated with asthma; rather, it was strongly associated with risk of myocardial infarction. The genetic region around the *ORMDL3* gene was the first locus identified for asthma susceptibility by using a genome-wide association approach.

3. Answer: B

Explanation: *ADRB2* polymorphisms have been associated with bronchodilator responses in asthma. Polymorphisms in the genes encoding IL-5 and CD14 have been associated with asthma and atopy phenotypes in case-control studies. The cytochrome P450 gene *CYP1A1* might potentially modify responses to therapeutics metabolized by this isoenzyme but has not been identified as playing an important role in modulating responses to asthma therapy.

4. Answer: B

Explanation: *IL13* can regulate both atopic inflammation through its effect on B-cell IgE production and tissue responses through effects on structural cells, such as promoting mucus hypersecretion by airway epithelial cells and collagen production by airway fibroblasts. *FLG* polymorphisms do modulate epidermal barrier function and are the strongest genetic risk factor for atopic dermatitis, although they are not expressed in the lung, and association with asthma can occur through increased allergen sensitization as a result of a poor epidermal barrier. *ORMDL3*, although of unknown function, is expressed in epithelial cells and might be important in epithelial barrier function. *PHF11* is a candidate gene encoding a transcription factor that is likely to be involved with the atopic immune response.

Chapter 8: Asthma: Clinical expression and molecular mechanisms

1. Answer: C

Explanation: Assessment of the risk domain involves an evaluation of the following over time: rates of exacerbations, loss of lung function, and side effects from medications. In contrast, an assessment of pulmonary function, symptoms, and albuterol use are factors that one evaluates in assessing current impairment.

2. Answer: B
Explanation: In both children and adults, the virus most frequently found to be associated with asthma exacerbations is rhinovirus.

3. Answer: C
Explanation: The only pain medication not in the class of COX pathway inhibitors is acetaminophen.

4. Answer: D
Explanation: The use of long-acting β-agonists as monotherapy has been demonstrated in a number of studies to increase risk for loss of control, exacerbations, and perhaps mortality from asthma.

Chapter 9: Rhinitis and sinusitis

1. Answer: C
Explanation: The dust mite antigen has proteolytic activity that cleaves tight junctions in the airway epithelium. Activated epithelial cells produce thymic stromal lymphopoietin, a protein that interacts with interepithelial and subepithelial dendritic cells to skew T-cell development toward T_H2 allergic sensitization. The house dust mite allergen Der p 2 has a unique property, namely that it mimics MD-2, the LPS-binding component of the TLR4 signaling complex, and facilitates TLR4 signaling and airway T_H2-type inflammation. In the nose allergens are processed by antigen-presenting cells (dendritic cells expressing CD1a and CD11c and macrophages) in the nasal epithelial mucosa, with subsequent presentation of allergenic peptides by MHC class II molecules to T-cell receptors on resting $CD4^+$ T lymphocytes in regional lymph nodes.

2. Answer: D
Explanation: Nonallergic rhinitis often occurs without eosinophilia. The terms *nonallergic rhinitis without eosinophilia* and *idiopathic rhinitis* are used interchangeably. Irritant-induced rhinitis, cold-induced rhinitis, and vasomotor rhinitis are all considered subsets of this condition. *Vasomotor rhinitis* is sometimes used synonymously with *nonallergic rhinitis without eosinophilia*, but it sometimes can more specifically connote nasal symptoms that occur in response to environmental conditions, such as changes in temperature or relative humidity, odors (eg, perfume or cleaning materials), passive tobacco smoke, alcohol, sexual arousal, and emotional factors. Nonallergic rhinitis with aspirin sensitivity is usually associated with marked tissue eosinophilia (ie, nonallergic rhinitis with eosinophilia).

3. Answer: D
Explanation: T_H2-type immune hyperresponsiveness in sinus tissue is an important feature of CRS without distinction for the presence of nasal polyps. Patients with CRS typically have fungi, such as *Alternaria* species, in the mucus secretions and *in vitro* hyperresponsiveness to *Alternaria* species, with production of IL-5 and IL-13. Local production of IgE against staphylococcal enterotoxins (superantigens) has been found in homogenates of nasal polyps and is regarded as specific for CRS with nasal polyps. Production of bacterial biofilm on sinus mucosal tissue has been demonstrated in several studies without distinction for the presence of nasal polyps. Glandular hyperplasia is a feature of CRS without nasal polyps.

4. Answer: D
Explanation: Opacified sinus cavities might contain inspissated mucus that produces an inhomogeneous hyperdense pattern on sinus CT scanning. Hyperdensities suggest the presence of allergic mucin. They are a classic feature of allergic fungal rhinosinusitis (in which case the allergic mucin also contains fungal hyphae), but they can be seen in both patients with CRS without nasal polyps and patients with CRS with nasal polyps.

Chapter 10: Food allergy

1. Answer: B
Explanation: Studies of a referral population in the United States indicated that only 11% resolved egg and 19% resolved milk allergy by age 4 years; however, about 80% resolved these allergies by age 16 years. Allergy to fish/shellfish is reported more often in adults compared with children. Although several studies showed an increase, approximately doubling, in peanut allergy among children in the past 10 to 15 years, there are no data to indicate a general doubling of food allergy. Peanut allergy resolves for about 20% of young children by school age.

2. Answer: C
Explanation: The symptom complex of having mild oral pruritis to raw apple but tolerating cooked apple is consistent with a diagnosis of oral allergy syndrome/pollen-food syndrome, in which initial sensitization to pollen results in reactions to homologous proteins in a raw food. Here there was likely sensitization to birch pollen protein in this "atopic" man; the birch pollen protein Bet v 1 is homologous to Mal d 1 in apple. Lipid transfer protein is more stable to heat and less likely to result in mild symptoms. Although he might have a positive skin test result to commercial apple extract, the birch-related protein is less stable, and testing with fresh raw juice of an apple is more likely to show a positive result in this scenario. Although heating apple reduces the Mal d 1 protein level and

generally results in a form of the food that does not trigger symptoms in persons with birch pollen–related allergy, this is not a Maillard reaction. High heat resulting in a Maillard reaction, a chemical reaction between an amino acid and a reducing sugar, has been proposed to increase the allergenicity of some foods (roasted peanut) by increasing the stability of allergens.

3. Answer: B

Explanation: Food allergy requires an adverse immune response. This description fits rice-induced enterocolitis syndrome. This is a non–IgE-mediated food allergy, and results of skin testing are expected to be negative. Choice A describes auriculotemporal syndrome, which is a neurologic response to the spicy or tart triggers for the child described. Choice C describes lactose intolerance, which is dose dependent. Choice D most likely describes an episode of scombroid fish poisoning.

4. Answer: C

Explanation: Increasingly larger food-specific skin test results and increasingly higher food-specific serum IgE levels are associated with higher risks of clinical allergy. However, false-negative test results are possible, and the history is important in assessing the prior probability of allergy. This child had repeated allergic responses to egg, including a significant reaction 6 months before the most recent serum testing that was "undetectable" by using this assay. Therefore performing a food challenge next might be a poor choice given a relatively recent reaction. Seeing a decrease in serum IgE levels to egg is an encouraging indication that the egg allergy might be resolving. However, some egg-reactive children (approximately 20%) might have negative test results on the serum test and still react clinically on challenge. In this setting a skin test might be helpful as additional information before deciding on an oral food challenge.

Chapter 11: Drug allergy

1. Answer: c

Explanation: A detailed history is essential to the management of patients with drug allergy. Skin testing and *in vitro* testing might be helpful in a limited number of drug-induced allergic reactions. Some, but not all, drug-induced allergic reactions can be classified by using the Gell and Coombs system.

2. Answer: B

Explanation: Skin testing has a very high negative predictive value in patients with penicillin allergy, and resensitization to penicillin is rare, especially with oral courses of penicillin. Although the history is suggestive, it is usually not adequate for confirming or negating a history of penicillin allergy. Cross-reactivity between cephalosporins and penicillin is low.

3. Answer: C

Explanation: Induction of drug tolerance procedures can involve IgE-mediated and non–IgE-mediated processes and cause temporary tolerance to the drug. These procedures can take days or weeks to complete for non–IgE-mediated reactions and are generally contraindicated in patients with life-threatening cutaneous drug reactions.

4. Answer: D

Explanation: This patient's history is consistent with anaphylaxis. Anaphylactic reactions to nonsteroidal anti-inflammatory drugs are drug-specific reactions, and therefore he should tolerate aspirin.

Chapter 12: Allergic skin diseases

1. Answer: D

Explanation: It is clear that sera from patients with chronic urticaria have a biologic activity that can activate donor mast cells, and most of this activity is found in the IgG fraction. However, there is no gold standard for measuring these antibodies, and the value of this finding for predicting prognosis or decisions regarding management is unclear.

2. Answer: B

Explanation: Although there are many case reports and case series suggesting efficacy of a variety of immunomodulatory drugs, only cyclosporin A has been studied in double-blind placebo-controlled trials.

3. Answer: B

Explanation: Answer A is false. Irritant contact dermatitis commonly presents as a localized dermatitis without vesicles more common in the palms and ventral surfaces of the hands and rarely extending beyond the area of contact. Answer B is true. Answer C is false. Atopic dermatitis is an important factor in susceptibility to persistent postoccupational dermatitis. Answer D is false. "Unscented" might erroneously suggest absence of fragrance when, in fact, a masking fragrance is present. "Fragrance-free" products are typically free of classic fragrance ingredients and are generally acceptable for the patient with allergic contact dermatitis.

4. Answer: C

Explanation: Answer A is false. Patch test results are affected by oral corticosteroids, cancer chemotherapy, and immunosuppressive drugs but not by antihistamines. Answer B is false. Allergens not found on commercially available screening series in the United States

frequently produce relevant reactions, and personal products are a useful supplement, especially in facial or periorbital dermatitis. Answer C is true. Answer D is false. Glycerol thioglycolate is the active ingredient in permanent wave solution. Unlike PPD, the thioglycolates can remain allergenic in the hair long after it has been rinsed out. Answer E is false. Medicaments containing lanolin are more sensitizing than lanolin-containing cosmetics. It is a weak sensitizer in normal skin but a stronger sensitizer in damaged skin. Thus patients with chronic dermatitis, especially stasis dermatitis, are at higher risk of lanolin sensitivity.

Chapter 13: Environmental and occupational allergies

1. Answer: A

Explanation: This is important not only because control of indoor humidity is important in reducing mite exposure but also because the presence of house dust mites is less in arid climates or at higher altitudes.

2. Answer: B

Explanation: Because cat allergens are widespread throughout the home, it is important to end the generation of the contamination. Even so, it takes several weeks to eradicate the allergen from the home.

3. Answer: C

Explanation: The prevalence of allergy and asthma is higher in subjects who live near highly traveled roads.

4. Answer: C

Explanation: Onset of symptoms can be delayed for several hours after exposure. Bronchial provocation tests are appropriate only in research centers. Many low-molecular-weight agents do not elicit an IgE response.

Chapter 14: Anaphylaxis

1. Answer: D

Explanation: Accurate community-based population estimates are difficult to obtain because of underdiagnosis, underreporting, and miscoding; however, lifelong prevalence of anaphylaxis from all triggers in the general population is estimated at 0.05% to 2%.

2. Answer: B

Explanation: In patients with newly diagnosed idiopathic anaphylaxis, the serum total tryptase level should be measured. This test reflects the increased burden of mast cells in all forms of mastocytosis and is therefore an important screening test for mastocytosis. If the total tryptase level is greater than 11.4 ng/mL, the new upper limit of normal, meticulous examination for cutaneous mastocytosis is indicated, and if the level is greater than 20 ng/mL, a bone marrow biopsy is indicated, even if cutaneous manifestations are absent.

3. Answer: C

Explanation: A 3- to 5-year course of subcutaneous injections of the relevant standardized insect venom or venoms reduces the risk of anaphylaxis from a subsequent sting, based on randomized, double-blind, placebo-controlled trials. In children, a 98% protection rate can be achieved.

4. Answer: A

Explanation: Epinephrine's multiple pharmacologic effects in many organ systems are useful in anaphylaxis; however, its α_1-adrenergic vasoconstrictor effects in the small arterioles and precapillary sphincters are unique among medications used in the prehospital treatment of anaphylaxis. By decreasing mucosal edema, it prevents and relieves upper airway obstruction. It also prevents and relieves hypotension and shock. When used in first-aid treatment, prompt injection is important. The epinephrine doses currently available in autoinjectors for outpatient use are too low for use in cardiopulmonary resuscitation.

Chapter 15: Primary immunodeficiencies

1. Answer: A

Explanation: SCID includes a heterogeneous group of disorders characterized by severe defects in T-cell development. Some (but not all) forms of SCID also have defects in B-cell development, natural killer cell development, or both, whereas impaired myeloid differentiation is restricted to a few rare forms of SCID. Regardless of the presence or absence of B cells, patients with SCID have a severe defect in antibody production, reflecting a lack of T lymphocytes.

2. Answer: B

Explanation: XLA and all other forms of congenital agammaglobulinemia are caused by genetic defects that affect signaling through the pre–B-cell receptor in the bone marrow. Therefore patients with congenital agammaglobulinemia typically lack circulating mature B cells.

3. Answer: C

Explanation: Neutrophils are important in the defense against bacteria and fungi. Patients with neutrophil defects often present with severe infections, among which purulent lymphadenitis is common.

4. Answer: C

Explanation: It is important that patients with antibody deficiency receive appropriate replacement treatment. This is usually achieved with 400 mg/kg/mo intravenous immunoglobulins or with weekly injections of subcutaneous immunoglobulins at a dose of 100 mg/kg/wk. This regimen applies to patients of any age.

Chapter 16: Secondary immunodeficiencies, including HIV infection

1. Answer: B

Explanation: From the 4 options, option B is the most likely answer. HIV infection can be considered as a cause of immunodeficiency at any age. Options A, C, and D are primary immunodeficiencies that present clinically in infancy or early childhood.

2. Answer: A

Explanation: Secondary immunodeficiencies have a variable clinical presentation. T-cell, B-cell, or innate immunity components, including phagocyte function, might or might not be affected. Management should include immunoglobulin supplementation only if humoral responses are not restored despite optimal control of the primary disease.

3. Answer: C

Explanation: Calcineurin inhibitors suppress IL-2–induced T-cell activation and proliferation, by binding immunophilin proteins in the cytoplasm. They do not affect the oxidative burst, complement activity, or calcium receptors.

4. Answer: C

Explanation: HIV infects its target cell by using the CD4 molecule in the cell membrane and the chemokine receptors CCR5 and CXCR4. Cells presenting with CCR5 deletions are not permissive for HIV infection. AIDS develops when there is severe depletion of T cells. Although an adenovirus-based anti-HIV vaccine has been shown to elicit specific immunologic responses, it did not demonstrate protection in a large trial of 3,000 subjects.

Chapter 17: Immunologic rheumatic disorders

1. Answer: B

Explanation: Anti-CCP antibodies can be present years before the onset of clinical disease. Patients with RA who have anti-CCP antibodies tend to have more aggressive erosive disease. Anti-CCP antibodies are more specific but less sensitive than rheumatoid factor for diagnosing RA.

2. Answer: A

Explanation: DMARDs should be initiated within 3 months of diagnosis, but traditional DMARDs are currently used in most cases before initiating biologic DMARDs. Nonsteroidal anti-inflammatory drugs have been shown to increase cardiovascular risk in patients with RA, but DMARDs have not. Adding a biologic DMARD to a traditional DMARD generally increases efficacy.

3. Answer: B

Explanation: Many immunologic based rheumatic diseases such as SLE, RA and SS are more common in women than men but the seronegative spondyloarthropathies are a prominent exception. The biologic basis for these findings is unknown.

4. Answer: D

Explanation: Patients with a low titer of ANA are less likely to have SLE than those with high-titer ANA, and the absence of typical clinical features of SLE makes the diagnosis even more unlikely. ANA-negative SLE is rare as long as the testing is done by means of indirect immunofluorescence. Anti-Ro (SSA) antibody is present in about 25% of patients with SLE, although it is seen in up to 75% of patients with Sjögren syndrome.

Chapter 18: Vasculitis

1. Answer: C

Explanation: The diagnosis of Wegener granulomatosis is usually made by means of biopsy, with nonrenal tissues demonstrating the presence of granulomatous inflammation and necrosis with necrotizing or granulomatous vasculitis. Surgically obtained biopsy specimens of abnormal pulmonary parenchyma demonstrate diagnostic changes in 91% of cases, which provides the highest diagnostic yield. Biopsy of the upper airways is less invasive but demonstrates diagnostic features only 21% of the time. The gastrointestinal tract is involved in less than 5% of patients with Wegener granulomatosis, with biopsy specimens of mucosa rarely revealing vasculitis.

2. Answer: A

Explanation: Vasculitis of small- to medium-sized vessels is a prominent feature of Churg-Strauss syndrome and is typically accompanied by prior or concurrent allergic rhinitis, asthma, and eosinophilia. The organ site most commonly affected by vasculitis is the peripheral nerve, which is involved in 70% to 80% of patients and manifests as a mononeuritis multiplex. Glomerulonephritis can lead to renal failure but only develops in 10% to 40% of patients. Gastrointestinal involvement occurs in 30% to 50% of patients and can be associated with mortality. The highest rate of mortality is seen with cardiac involvement, which occurs in 10% to 40% of patients.

3. Answer: B

Explanation: Two main antigen associations are seen in conjunction with ANCAs in patients with vasculitis. ANCAs directed against the neutrophil serine protease proteinase 3, which causes a cytoplasmic immunofluorescence pattern (cANCA) on ethanol-fixed neutrophils, are seen in 75% to 90% of patients with active generalized Wegener granulomatosis. ANCAs directed against the neutrophil enzyme myeloperoxidase that produce a perinuclear pattern (pANCA) are seen in 5% to 20% of patients with Wegener granulomatosis and are more common in microscopic polyangiitis. ANCAs directed against human neutrophil elastase can be seen in patients with cocaine-induced sinonasal destructive disease, which can be a mimic of Wegener granulomatosis. Bactericidal permeability–increasing protein is a target antigen for pANCA that has been described in patients with cystic fibrosis and ulcerative colitis.

4. Answer: D

Explanation: GCA is the most common form of systemic vasculitis that affects human subjects. GCA can be thought of as having 4 phenotypes that include cranial disease, PMR, systemic inflammatory disease, and large-vessel involvement. Large-vessel involvement of the aorta or its primary branches occurs in 27% of cases. The most dreaded complication of cranial disease is vision loss, which can occur in 14% of patients and is caused by optic nerve ischemia from arteritis involving vessels of the ocular circulation. A marker of systemic inflammation is an increased erythrocyte sedimentation rate, which occurs in more than 80% of patients. PMR can occur in conjunction with other features of GCA or in isolation. Although cranial or large-vessel GCA should be treated with 40 to 60 mg/d prednisone, isolated PMR can be treated with 10 to 20 mg/d prednisone.

Chapter 19: Immunologic endocrine disorders

1. Answer: B

Explanation: DR3/4 is the highest-risk genotype for type 1 diabetes. Insulin autoantibodies are remarkably inversely related to age of onset of type 1 diabetes, with levels being highest in the youngest children presenting with diabetes. Transglutaminase autoantibodies occur in approximately 10% of patients with type 1 diabetes, and half of these patients have high levels associated with a positive intestinal biopsy result for celiac disease.

2. Answer: A

Explanation: IPEX syndrome results from mutation of the forkhead box protein 3 gene *(FOXP3)*, which controls regulatory T cells and is X-linked recessive. APS-1 results from mutation of the autoimmune regulator gene *(AIRE)*, which controls peripheral antigen expression in the thymus and is almost always autosomal recessive (1 autosomal dominant family has been described). Both disorders are rare.

3. Answer: D

Explanation: We measure transglutaminase and 21-hydroxylase autoantibodies to screen for Addison disease and celiac disease. A major caveat with testing for insulin autoantibodies to aid in the diagnosis of type 1A diabetes (immune mediated) is that essentially everyone treated with subcutaneous insulin for more than 1 to 2 weeks had insulin antibodies that cannot be distinguished from the autoantibodies.

4. Answer: D

Explanation: APS-1 is a monogenic disorder, whereas APS-2 is a polygenic disorder, even though Addison disease occurs in both disorders. Mucocutaneous candidiasis is characteristic of APS-1, as is hypoparathyroidism, both of which rarely occur in patients with APS-2.

Chapter 20: Diagnostic testing and interpretation of tests for autoimmunity

1. Answer: C

Explanation: MPO is a serine protease that constitutes approximately 5% of the total protein content of a neutrophil. The autoantibodies directed against MPO are more often seen in patients with Churg-Strauss syndrome. The combination of the perinuclear antineutrophil cytoplasmic antibody pattern and MPO or MPO–antineutrophil cytoplasmic antibody is strongly associated with Churg-Strauss syndrome.

2. Answer: B

Explanation: ANA is seen in more than 90% of patients with SLE. However, it is not specific for SLE. ANA can also be seen in a variety of other autoimmune diseases, such as scleroderma, mixed connective tissue disease, polymyositis/dermatomyositis, and rheumatoid arthritis. Once an increased ANA level is documented, it cannot be used to measure disease activity.

3. Answer C

Explanation: Anti-Smith antibodies are highly specific for SLE (approximately 55% to 100%), but they are not very sensitive. These antibodies can remain positive when titers of anti-dsDNA antibodies are within a normal range and clinical activity of SLE has decreased. Therefore the anti-Smith titers can be useful diagnostically when anti-dsDNA antibodies are not detectable.

4. Answer: B

Explanation: In patients with rheumatoid arthritis, serum complement levels are generally normal or even increased during active disease because this is a reflection of the acute-phase response. However, in patients with rheumatoid vasculitis, hypocomplementemia is common. The combination of increased rheumatoid factor and decreased C3 levels favors rheumatoid vasculitis. There is a high prevalence of IgA immune complex deposits plus C3 deposits in the affected skin of patients with rheumatoid vasculitis.

Chapter 21: Pulmonary disorders, including vocal cord dysfunction

1. Answer: B

Explanation: In pulmonary TB lesions, there are reduced numbers of cytolytic T cells expressing low levels of perforin and granulysin. In addition, there are increased numbers of CD4+CD25+ Tregs, suggesting that an imbalance in the proportion of effector T cells to Treg cells may contribute to establishment of granulomas in TB infection.

2. Answer: B

Explanation: Approximately one third of CSS patients have antineutrophil cytoplasmic antibodies (ANCAs). Myeloperoxidase (MPO) is the antigen against which the antibodies are directed.

3. Answer: B

Explanation: In BAL from normal individuals, the usual cell percentages are 83-88% macrophages; 7-12% lymphs, 1-2% PMNs; rare basophils, eosinophils or ciliated cells. In patients with acute HP lymphocytes represent 40-60% of total cells, usually with a CD8+ predominance. Eosinophils can be significantly increased in diseases such as CSS, APBA and acute eosinophilic pneumonia.

4. Answer: C

Explanation: The clinical criteria for the diagnosis of RADS, as published by Brooks in 1985 include onset of symptoms occurred after a single specific exposure incident, onset of symptoms occurred within 24 hours after exposure and persisted for at least 3 months, methacholine challenge testing was positive, symptoms simulating asthma, and other types of pulmonary disease were ruled out.

Chapter 22: Mucosal immunology, eosinophilic esophagitis, and other intestinal inflammatory diseases

1. Answer: C

Explanation: The mucosal immune system consists of a variety of immune cells that orchestrate a complex series of tightly controlled responses that protect the host from luminal triggers. Mutations in the gene encoding the forkhead box protein 3 regulatory T cell–specific transcription factor lead to a syndrome in which patients have the gastrointestinal manifestations of diarrhea and intestinal inflammation. Defects in other cell types are not of immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome.

2. Answer: C

Explanation: Eosinophilic esophagitis is a clinicopathological disease characterized by upper intestinal symptoms and dense esophageal eosinophilia with normal gastric and duodenal mucosa. Other causes for these findings must be ruled out, especially gastroesophageal reflux disease, a more common condition, for which eosinophilic esophagitis is commonly mistaken. The acronym EE is commonly mistaken for erosive esophagitis, hence the adaptation of EoE for eosinophilic esophagitis in the gastrointestinal specialty.

3. Answer: C

Explanation: Celiac disease is treated with the complete exclusion of gluten from the diet. A body of experience and literature supports the use of dietary modifications/exclusions in the treatment of inflammatory bowel diseases. Current treatments include the use of corticosteroids, aminosalicylates, immunosuppressive agents, and biological agents. Patients with gastroesophageal reflux disease might benefit from limiting certain foods and weight loss, but antacid medications form the primary mode of treatment.

4. Answer: B

Explanation: Defensins are synthesized primarily by Paneth cells and function as one part of the innate immune system. Six different subtypes of these highly charged molecules have been thus far identified.

Chapter 23: Complement disorders and hereditary angioedema

1. Answer: A

Explanation: The mannose-binding lectin pathway is initiated by binding of mannose-binding lectin to a variety of sugars on the surface of microbes.

2. Answer: B

Explanation: Therapy is directed at controlling the disease, improving quality of life, and minimizing side effects to minimize side effects and cost. Often the patients will continue to have some attacks.

3. Answer: C

Explanation: The classical pathway is the usual pathway activated by antibody. IgM and IgG subclasses 1 and 3 are best at activating the classical pathway.

4. Answer: C

Explanation: C3 is a critical opsonin and is particularly important with high-grade pathogens.

Chapter 24: Immune responses to malignancies

1. Answer: D

Explanation: Although human solid tumors can induce apoptosis of $CD8^+$ T cells and release TAs into the circulation, the only evidence that a tumor-specific immune response is made to these TAs comes from the presence in the circulation or lymphoid tissues of effector T cells capable of binding tetramers, which are reagents containing the TA-derived peptide sitting in the groove of MHC molecules.

2. Answer: C

Explanation: In tumor-bearing hosts DCs are found at the tumor site and in tumor-draining lymph nodes. However, these DCs are immature, have low expression levels of MHC molecules, and produce immunosuppressive cytokines, such as IL-10. Tumor-derived factors, including vascular endothelial growth factor, GM-CSF, and IL-10, recruit myeloid-derived suppressor cells from the bone marrow, which migrate to lymph nodes or tumor sites and block DC maturation.

3. Answer: A

Explanation: Regulatory T cells accumulate in the peripheral blood and tumor tissues of patients with cancer and suppress functions of other T cells by secreting the immunosuppressive cytokines IL-10, TGF-β1, or both or by producing cytolysins, granzyme B, and perforin, which mediate death of effector T or B cells. This type of suppression requires cell-to-cell contact.

4. Answer: D

Explanation: Inflammatory infiltrates seen in human solid tumors are chronic in nature. They are characterized by the paucity of natural killer cells and usually contain variable proportions of $CD8^+$ and $CD4^+$ T cells. These infiltrating cells produce the proinflammatory cytokines IL-6, TNF-α, and IL-8, which can promote tumor growth.

Chapter 25: Clinical laboratory assessment of immediate-type hypersensitivity

1. Answer: C

Explanation: IgE's molecular weight is approximately 190,000 d, and it is known to not readily pass the placenta. Thus low levels of IgE are found in cord blood, with final total serum IgE concentrations representing approximately 0.004% of the total immunoglobulin in circulation. Because of the wide overlap in total serum IgE levels between atopic and nonatopic populations, IgE levels in serum are not considered a definitive discriminator for the presence of atopy. Total serum IgE levels are known to be highly age dependent, and thus evaluation of total serum IgE levels should be judged in relation to an age-adjusted mean from a clearly nonatopic population.

2. Answer: B

Explanation: Once a history indicates a high probability of allergic disease, allergen-specific IgE antibody in the skin or blood is measured to confirm sensitization and verify the specificity of the IgE antibody response. The precise method chosen as the primary confirmatory test (serology or puncture or intradermal skin testing) depends on the allergen specificity (eg, suspected food vs Hymenoptera venom sensitivity will require the use of different primary confirmatory tests). If there is a history of an anaphylactic event, a β-tryptase measurement can be useful as a subsequent measurement after IgE antibody testing. α-Tryptase is less useful as an

indicator of an immediate release of mast cell mediators. It is correct that it should be collected between 30 minutes and 4 hours after a systemic allergic reaction. Provocation tests are only done as a last resort because they tend to be risky and difficult to standardize. Allergen-specific IgG antibody measurements are not considered diagnostic for human allergic disease and thus are contraindicated in the diagnostic process.

3. Answer: D

Explanation: A higher allergen-specific IgE concentration, more mature IgE antibody specificity, higher specific IgE/total IgE molar ratio in serum, and higher IgE antibody affinity directed at the specific allergen all contribute to an enhanced translation of an IgE antibody's response into more effective basophil mediator release.

4. Answer: D

Explanation: IgG antibody is generally viewed as a marker of antigen exposure and is not diagnostic of an immediate-type hypersensitivity response. Specific IgG antibody responses are contraindicated in the assessment of food allergy because they are not diagnostic. They are also not useful in the evaluation of rhinitic conditions associated with aeroallergen exposure and the evaluation of latex allergy questions. Precipitating IgG antibody has been used as a diagnostic indicator for the evaluation of patients suspected of hypersensitivity pneumonitis after inhalation of organic dusts (molds: farmer's lung; fecal material dust from bird droppings: pigeon breeder's disease).

Chapter 26: Laboratory evaluation of primary immunodeficiencies

1. Answer: D

Explanation: The clinical symptoms of recurrent sinopulmonary infections and chronic diarrhea point to an antibody deficiency syndrome, which should be initially screened by means of measurement of serum immunoglobulin levels. Lymphocyte immunophenotyping and mitogen proliferation assays are particularly useful in the evaluation of cellular immunodeficiencies, although B-cell immunophenotyping has utility as a secondary test in evaluating humoral immunodeficiencies. DHR is used to evaluate phagocyte defects in oxidative burst, which are primarily seen in chronic granulomatous disorder.

2. Answer: C

Explanation: Toll-like receptor pathway defects, such as IL-1 receptor–associated kinase 4 and MYD88 defects, are associated almost exclusively with pyogenic bacterial infections and poor inflammatory responses. IFN-γ and IL-12 defects result in infections by mycobacterial species, whereas GM-CSF defects are associated with pulmonary alveolar proteinosis.

3. Answer: C

Explanation: The clinical symptoms in this patient are suggestive of a cellular immune defect, most likely severe combined immunodeficiency, and a lymphocyte count would likely demonstrate significant lymphopenia. DHR is directed at evaluating oxidative burst, and results are abnormal in patients with chronic granulomatous disease; the clinical picture is not consistent with this diagnosis. The CH50 assay is focused on classical component complement defects that typically would not present in infancy and usually involve bacterial infections. Immunoglobulin levels would primarily reflect maternal IgG, and defects in antibody production typically present later in infancy and show primarily bacterial infections of the sinopulmonary tract.

4. Answer: A

Explanation: T-cell receptor excision circles are present at high levels in naive $CD45RA^+$ T cells, which are not yet antigen experienced. T-cell receptor diversity is dependent on normal thymic function, and therefore it might be altered in settings of abnormal T-cell development but is not directly linked to CD45RA expression. T-cell functional capacity (cytotoxicity and mitogen proliferation) is also linked to normal T-cell development but cannot be specifically correlated with CD45RA expression.

Chapter 27: Allergen immunotherapy

1. Answer: B

Explanation: Patients with aspirin-exacerbated respiratory disease can be tolerized by repeated administration of aspirin, but this is not SIT. The other indications are appropriate.

2. Answer: D

Explanation: Sublingual immunotherapy uses high doses of allergen (up to 400 times higher than conventional SIT). There is relatively little evidence for its use in children. The exact mechanism is not known, but regulatory T cells have been demonstrated.

3. Answer: C

Explanation: VIT offers protection quite early on, during the build-up phase but certainly by the time the maintenance dose is achieved. Large local reactions are not an indication, and moreover, there is no hard evidence that they are relieved by VIT. Most patients can stop after 3 to 5 years, but a low risk of anaphylaxis remains, although the reactions are likely to be mild.

4. Answer: C

Explanation: Although it is not clear how this would be regarded by the regulatory authorities, using recombinant allergens will definitely allow us to dissect out patients' profiles of IgE response and then put together a treatment cocktail. However, in the medium term, it is more likely they will improve standardization of vaccines. They are as allergenic as natural allergens (unless genetically modified), and there is no evidence that they work better (or worse) if coupled to CpG.

Chapter 28: Immunomodulator therapy: Monoclonal antibodies, fusion proteins, cytokines, and immunoglobulins

1. Answer: A

Explanation: All TNF inhibitors have been shown to improve the signs and symptoms of RA. Although anti-TNF mAbs have been effective in the treatment of Crohn disease, the fusion protein etanercept has not been effective. Despite increased levels of TNF in patients with congestive heart failure and multiple sclerosis, TNF inhibitors have not improved and have sometimes worsened clinical outcomes.

2. Answer: B

Explanation: TNF inhibitors are generally well tolerated but have been associated with an increased risk of infections, including tuberculosis and opportunistic infections. The risk of infection is increased when combined with another biologic agent. Rituximab has been associated with rare but fatal cases of progressive multifocal leukoencephalopathy and reactivation of hepatitis B.

3. Answer: C

Explanation: Productive $CD4^+$ T-cell responses require 2 signals: binding of specific antigen-associated MHC class II molecules to the T-cell receptor complex and a second signal from costimulatory molecules (CD80 and CD86). CD28 and its natural inhibitor, CTLA-4 (CD152), are present on T cells and bind to CD80 and CD86 on antigen-presenting cells. CD28 ligation results in stimulation of T cells, whereas CTLA-4 serves an inhibitory role. CTLA-4, which binds CD80 and CD86 with substantially higher affinity than CD28, inhibits the stimulatory effects of CD28 by competitively binding to CD80 and CD86.

4. Answer: C

Explanation: Rituximab binds to CD20 on the surface of pre-B through activated mature B cells only and can deplete B cells up to 9 months or longer after a single course. Rituximab can be used alone or in combination with disease-modifying antirheumatic drugs and yields better clinical outcomes in patients with RA who are seropositive for rheumatoid factor.

Chapter 29: Transplantation immunology: Solid organ and bone marrow

1. Answer: B

Explanation: When the transplant donor HLA antigens are different from the recipient, the graft is recognized as "nonself" by the immune system, which gets activated and develops an immune response. This response eventually destroys the graft.

2. Answer: A

Explanation: Graft rejection can be classified according to the time it takes to develop. Hyperacute rejections usually occur within 48 hours of transplantation, and the injury is mediated by preformed alloantibodies and complement targeting the vascular endothelium. Treatment is generally unsuccessful.

3. Answer: D

Explanation: The lowest risk of GVHD in patients undergoing HSCT is when the donor and the recipient are HLA-matched siblings. Cord blood transplantation can be performed with an HLA mismatch of up to 4 of 6 antigens. HLA-haploidentical bone marrow transplant results in a high percentage of GVHD if T cells are not depleted from the graft. Although peripherally isolated $CD34^+$ cells have low T-cell concentrations, this small number would produce GVHD if there were no HLA compatibility.

4. Answer: C

Explanation: HSCT is the treatment of choice for patients with severe combined immunodeficiency, who otherwise would succumb early to severe and opportunistic infections. The balance of risk and benefits of HSCT is not favorable for patients with X-linked agammaglobulinemia. Partial DiGeorge syndrome and complement deficiencies might not be corrected by HSCT.

Chapter 30: Embryonic and adult stem cell therapy

1. Answer: B

Explanation: Parents are haploidentical with their children. One fourth of siblings can be HLA genoidentical to the patient. A matched unrelated donor would be HLA phenoidentical. The risk of graft-versus-host disease increases with differences in minor antigens, leading to increased morbidity/mortality in nonrelated recipients.

2. Answer: C

Explanation: Donor lymphocyte infusion is a therapy that might induce or enhance a graft-versus-leukemia effect and thus reinduce the patient into remission.

3. Answer: A

Explanation: Graft-versus-host disease is a consequence of alloreactive T cells.

4. Answer: D

Explanation: Human embryonic stem cells are currently derived from the blastocyst or sometimes earlier stages of unused embryos made by means of *in vitro* fertilization for infertility problems, with the written informed consent of the parents.