Supplement to
THE JOURNAL OF
Allergy AND Clinical Immunology

VOLUME 125 • NUMBER 2

AMERICAN ACADEMY OF ALLERGY ASTHMA & IMMUNOLOGY

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

Supplement to
THE JOURNAL OF
Allergy AND Clinical Immunology

VOLUME 125 • NUMBER 2

AMERICAN ACADEMY OF ALLERGY ASTHMA & IMMUNOLOGY

OFFICIAL JOURNAL OF THE AMERICAN ACADEMY OF ALLERGY, ASTHMA & IMMUNOLOGY

2010 Primer on Allergic and Immunologic Diseases

CONTENTS

CONTINUED

CONTENTS

CONTINUED

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.

REFERENCES

1. Turvey SE, Broide DH. Innate immunity. J Allergy Clin Immunol 2010;125: S24-32.
2. Bonilla FA, Oettgen HC. Adaptive immunity. J Allergy Clin Immunol 2010;125: S33-40.
3. Schroeder HW Jr, Cavacini L. Structure and function of immunoglobulins. J Allergy Clin Immunol 2010;125:S41-52.
4. Castro C, Gourley M. Diagnostic testing and interpretation of tests for autoimmunity. J Allergy Clin Immunol 2010;125:S238-47.
5. Frank M. Complement disorders and hereditary angioedema. J Allergy Clin Immunol 2010;125:S262-71.
6. Simons FER. Anaphylaxis. J Allergy Clin Immunol 2010;125:S161-81.
7. Lee SJ, Chinen J, Kavanaugh A. Immunomodulator therapy: monoclonal antibodies, fusion proteins, cytokines, and immunoglobulins. J Allergy Clin Immunol 2010;125:S314-23.

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.

Disclosure of potential conflict of interest: W. T. Shearer and D. Y. M. Leung have declared that they have no conflicts of interest.

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.

J Allergy Clin Immunol 2010;125:S1-2.
0091-6749/$36.00

doi:10.1016/j.jaci.2009.12.019

8. Notarangelo LD. Primary immunodeficiencies. J Allergy Clin Immunol 2010;125: S182-94.
9. Holloway JW, Yang IA, Holgate ST. Genetics of allergic disease. J Allergy Clin Immunol 2010;125:S81-94.
10. Commins SP, Borish L, Steinke JW. Immunologic messenger molecules: cytokines, interferons, chemokines. J Allergy Clin Immunol 2010;125:S53-72.
11. Fonacier LS, Dreskin SC, Leung DYM. Allergic skin diseases. J Allergy Clin Immunol 2010;125:S138-49.
12. Atkins D, Furuta GT. Mucosal immunology, eosinophilic esophagitis, and other intestinal inflammatory diseases. J Allergy Clin Immunol 2010;125: S255-61.
13. Chinen J, Shearer WT. Secondary immunodefiencies, including HIV infection. J Allergy Clin Immunol 2010;125:S195-203.
14. Peden D, Reed CE. Environmental and occupational allergies. J Allergy Clin Immunol 2010;125:S150-60.
15. Khan DA, Solensky R. Drug allergy. J Allergy Clin Immunol 2010;125:S126-37.
16. Stone KD, Prussin C, Metcalfe DD. IgE, mast cells, basophils, and eosinophils. J Allergy Clin Immunol 2010;125:S73-80.
17. Lemanske RF Jr, Busse WW. Asthma: clinical expression and molecular mechanisms. J Allergy Clin Immunol 2010;125:S95-102.
18. Sicherer SH, Sampson HA. Food allergy. J Allergy Clin Immunol 2010;125: S116-25.
19. Dykewicz MS, Hamilos DL. Rhinitis and sinusitis. J Allergy Clin Immunol 2010; 125:S103-15.
20. Joseph A, Brasington R, Kahl L. Prabha Ranganathan P, Cheng TP, Atkinson J. Immunologic rheumatic disorders. J Allergy Clin Immunol 2010;125:S204-15.
21. Langford CA. Vasculitis. J Allergy Clin Immunol 2010;125:S216-25.
22. Michels AW, Eisenbarth GS. Immunologic endocrine disorders. J Allergy Clin Immunol 2010;125:S226-37.
23. Greenberger PA, Grammer LC. Pulmonary disorders including vocal cord dysfunction. J Allergy Clin Immunol 2010;125:S248-54.
24. Whiteside TL. Immune responses to malignancies. J Allergy Clin Immunol 2010; 125:S272-83.
25. Frew AJ. Allergen immunotherapy. J Allergy Clin Immunol 2010;125:S306-13.
26. Chinen J, Buckley RH. Transplantation immunology: solid organ and bone marrow. J Allergy Clin Immunol 2010;125:S324-35.
27. Brignier AC, Gewirtz AM. Embryonic and adult stem cell therapy. J Allergy Clin Immunol 2010;125:S336-44.
28. Chaplin DD. Overview of the immune response. J Allergy Clin Immunol 2010;125: S3-23.
29. Hamilton RG. Clinical laboratory assessment of immediate-type hypersensitivity. J Allergy Clin Immunol 2010;125:S284-96.
30. Oliveira JB, Fleisher TA. Laboratory evaluation of primary immunodeficiencies. J Allergy Clin Immunol 2010;125:S297-305.

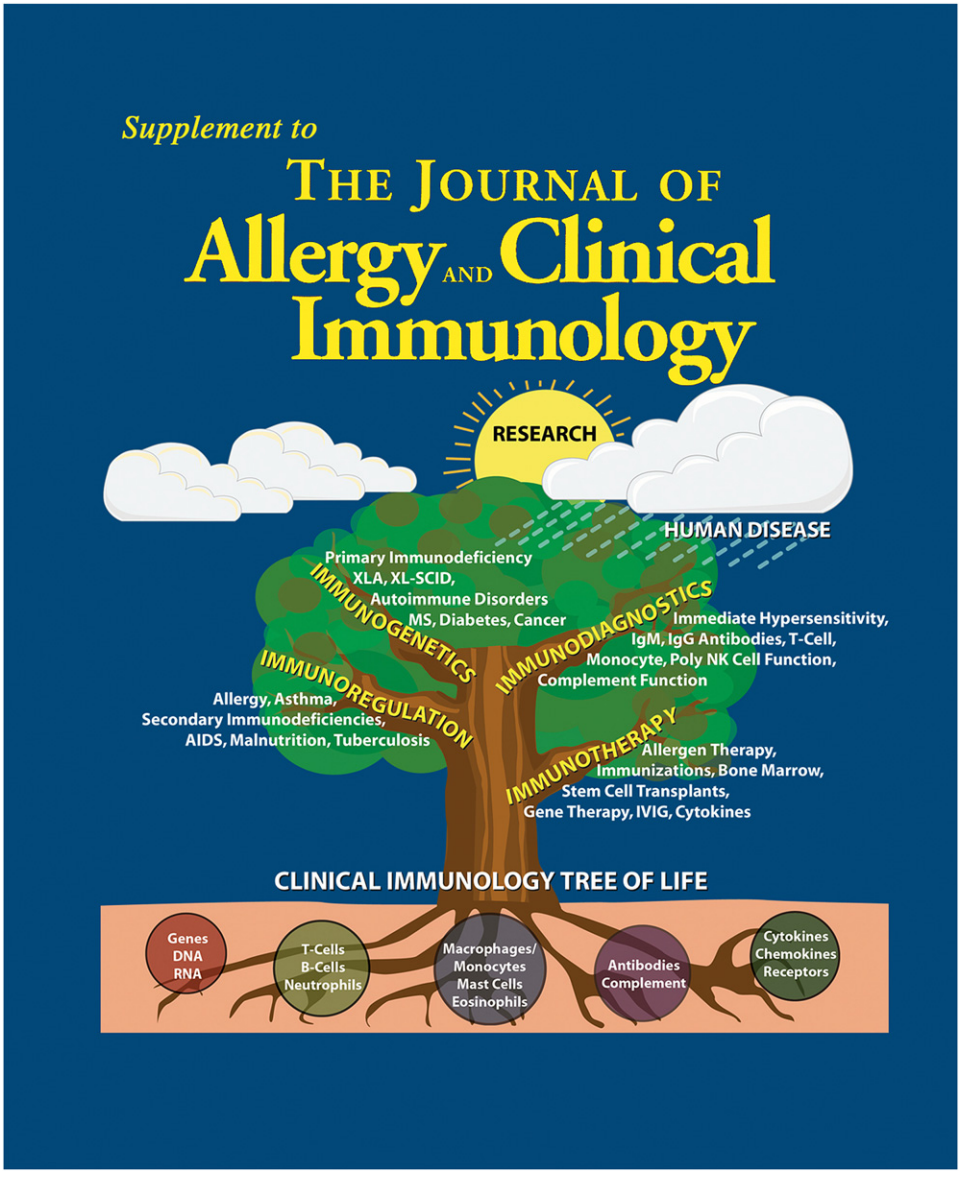

FIG 1. The clinical immunology tree of life. Used with permission from Primer 2003, 5th edition.

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.

Disclosure of potential conflict of interest: D. D. Chaplin is a grantee and reviewer of and has received research support from the National Institutes of Health.

Received for publication October 25, 2009; revised December 18, 2009; accepted for publication December 21, 2009.

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.

0091-6749/$36.00

doi:10.1016/j.jaci.2009.12.980

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

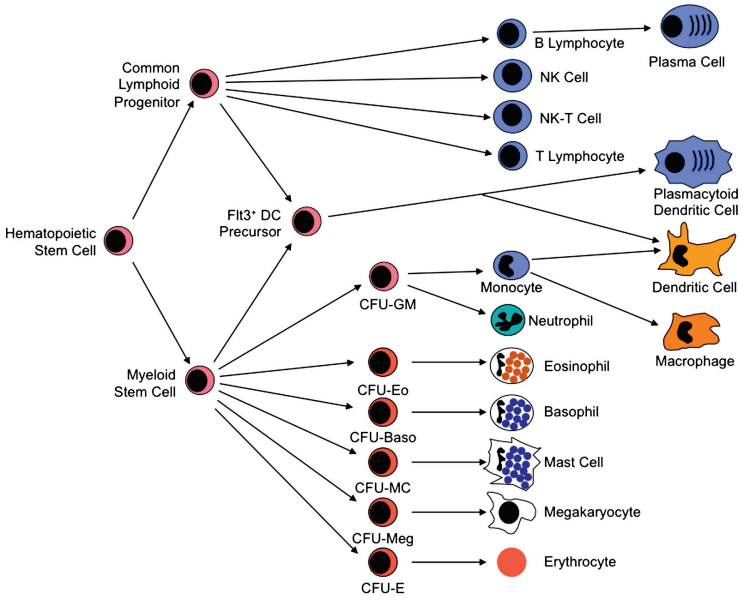

FIG 1. Hematopoietic stem cell–derived cell lineages. Pluripotent hematopoietic stem cells differentiate in bone marrow into common lymphoid progenitor cells or myeloid stem cells. Lymphoid stem cells give rise to B-cell, T-cell, and NK cell lineages. Myeloid stem cells give rise to a second level of 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

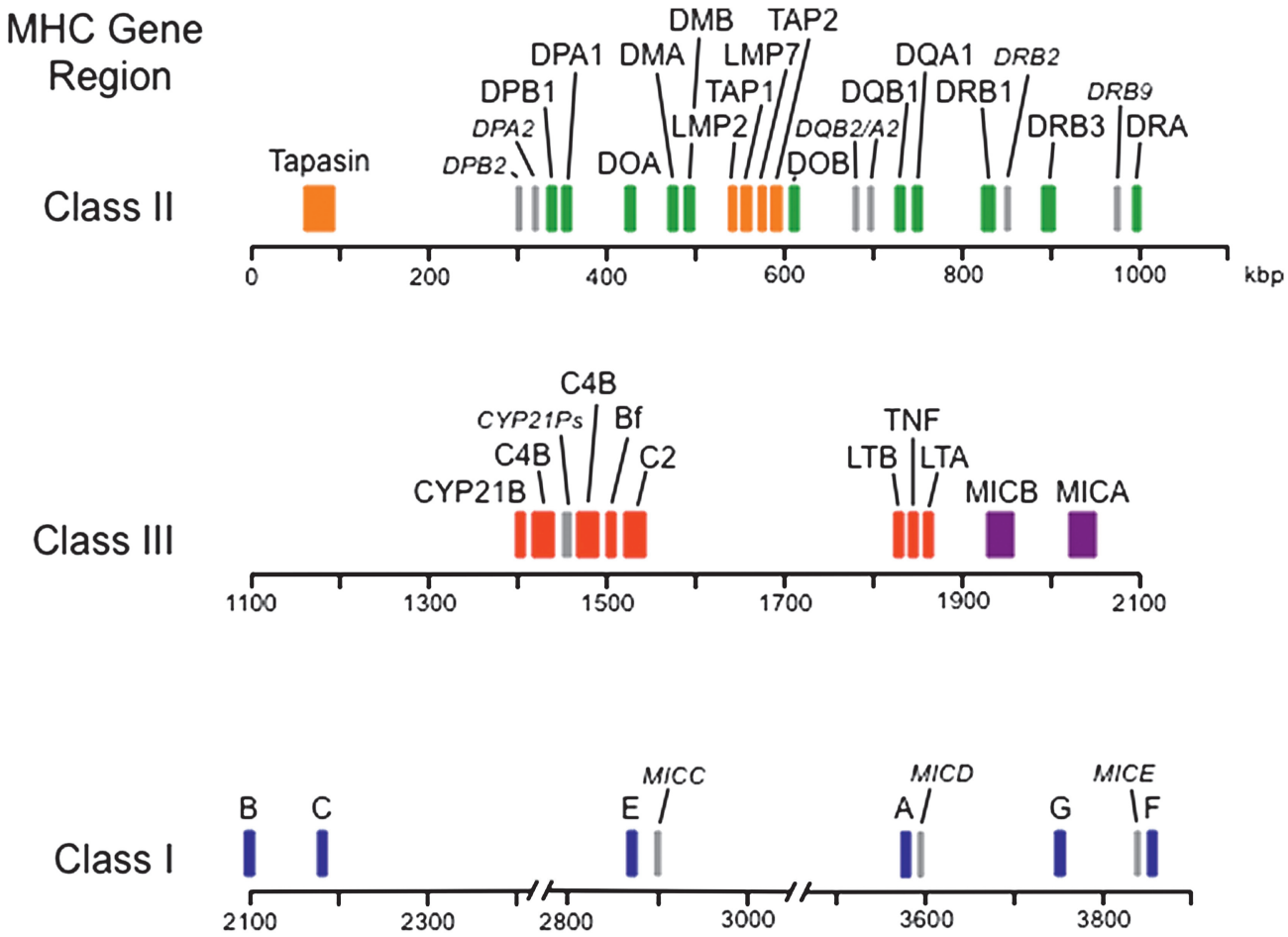

FIG 2. Molecular map of the human MHC. The human MHC, designated HLA, is encoded on the short arm of chromosome 6. The locations of the major HLA and related genes are shown above a scale showing approximate genetic distances in kilobase pairs of DNA *(kbp)*. The genes encoding the class I HLA heavy 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

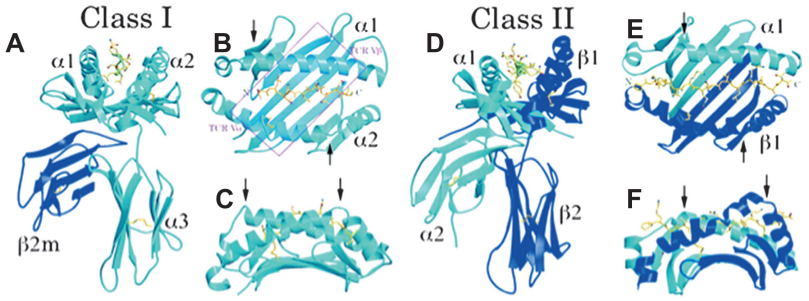

FIG 3. Structure of HLA molecules. Molecular models derived from crystal structures of class I **(A-C)** and class II **(D-F)** HLA molecules. Fig 3, *A*, the class I α_1, α_2, and α_3 domains are shown (light blue) in noncovalent association with the β_2-microglobulin molecule (dark blue). *Coils* represent 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

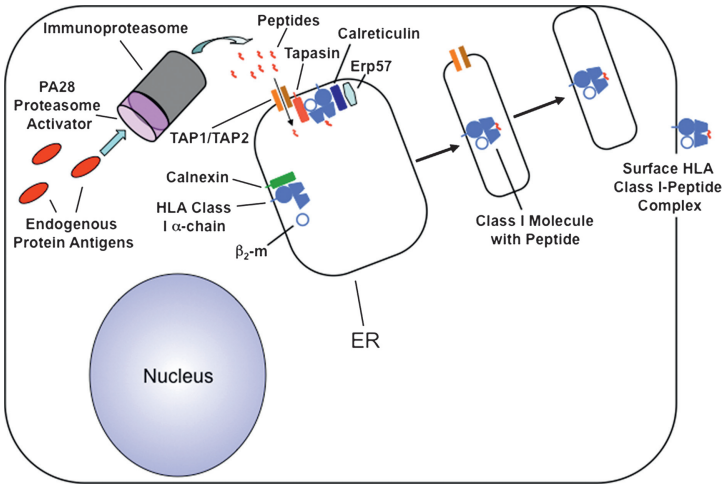

FIG 4. Cellular pathway for processing and presentation of endogenous antigens. Endogenous proteins are digested by the immunoproteasome to small peptide fragments. Production of the immunoproteasome is induced by IFN-γ, which leads to expression of LMP2 and LMP7 (which replace certain components of 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

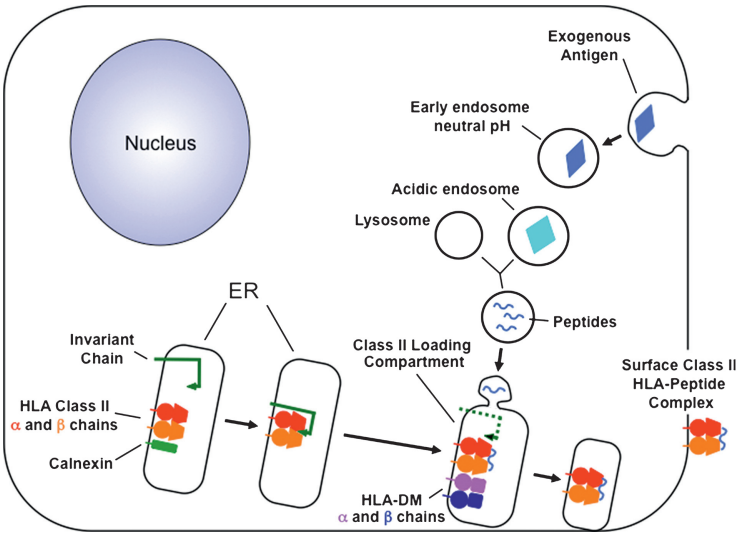

FIG 5. Cellular pathway for processing and presentation of exogenous antigens. In the ER newly synthesized class II proteins associate with the help of calnexin with an Ii protein that protects the antigen-binding groove of the class II molecule until it is transported to the class II^+ endosomal protein–loading 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

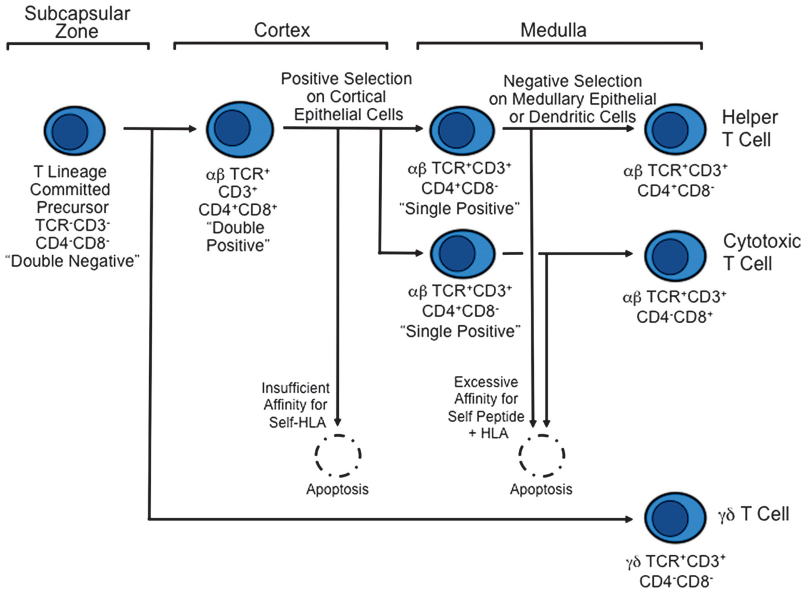

FIG 6. Differentiation and maturation of T cells in the thymus. Hematopoietic stem cells, which do not express CD3, CD4, or CD8 but are committed to T-cell differentiation, move from the bone marrow to the thymic subcapsular zone. There they begin rearrangement of the TCR genes. Once a productive TCR 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

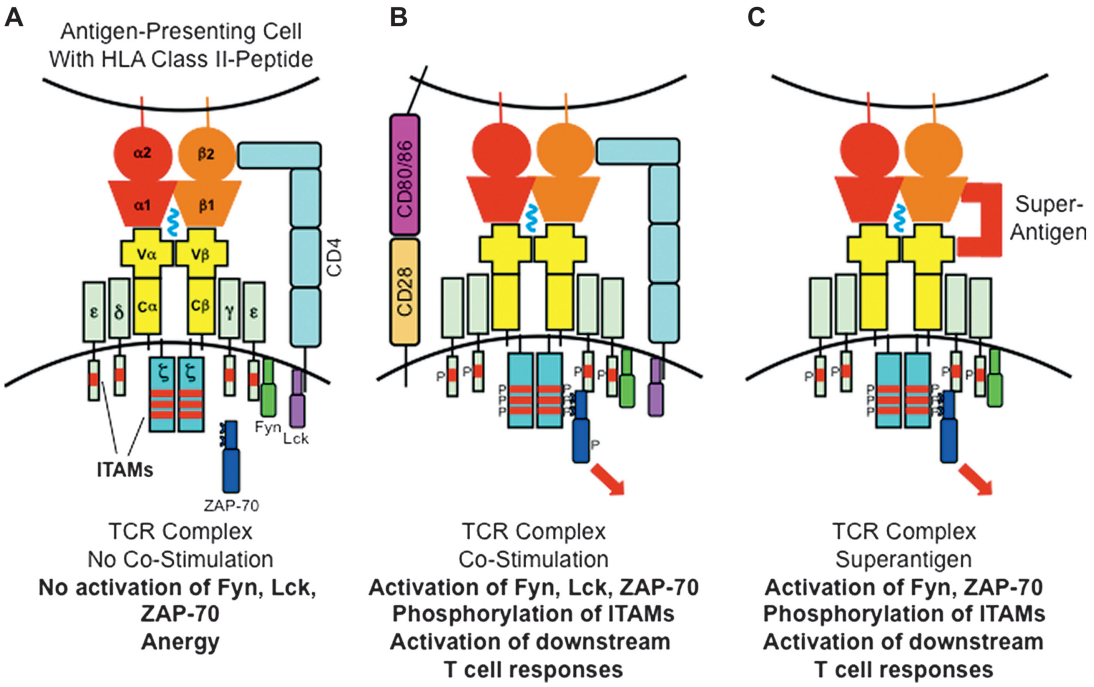

FIG 7. The TCR complex and T-cell activation. **A**, the complete TCR complex includes the rearranged TCR α and β chains and also the CD3γ, CD3δ, CD3ε, and CD3ζ chains. The CD3 chains contain ITAMs in their cytoplasmic domains that can be phosphorylated to activate the intracellular signaling cascade 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

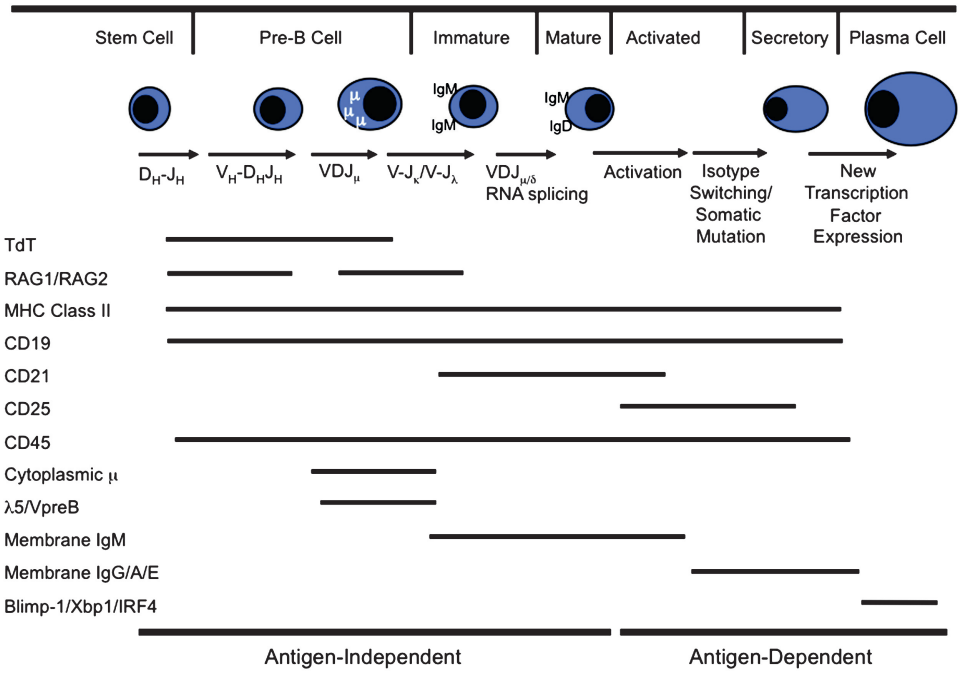

FIG 8. B-cell differentiation and development. B cells differentiate in the bone marrow from stem cells to become mature surface IgM- and IgD-expressing cells. This occurs in the absence of antigen. In peripheral lymphoid tissues the B cell can then mature further under the influence of antigen and T-cell 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]

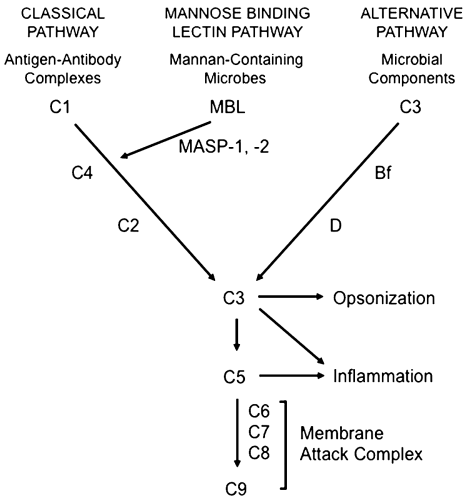

FIG 9. The activation pathways of complement. Three pathways lead to activation of complement. The classical pathway is initiated by complexes of IgM, IgG1, or IgG3 with antigens. This activates proteolysis of C1 that cleaves C4 and C2 to form the classical pathway C3 convertase. The mannose lectin pathway 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.

REFERENCES

1. Hiemstra PS. The role of epithelial beta-defensins and cathelicidins in host defense of the lung. Exp Lung Res 2007;33:537-42.
2. Holmskov U, Thiel S, Jensenius JC. Collectins and ficolins: humoral lectins of the innate immune defense. Annu Rev Immunol 2003;21:547-78.
3. Sjoberg AP, Trouw LA, Blom AM. Complement activation and inhibition: a delicate balance. Trends Immunol 2009;30:83-90.
4. Bonilla FA, Oettgen HC. Adaptive immunity. J Allergy Clin Immunol 2010;125: S33-40.
5. Schroeder HW, Cavacini L. Structure and function of immunoglobolins. J Allergy Clin Immunol 2010;125:S41-52.
6. Huston DP. The biology of the immune system. JAMA 1997;278:1804-14.
7. Robin C, Bollerot K, Mendes S, Haak E, Crisan M, Cerisoli F, et al. Human placenta is a potent hematopoietic niche containing hematopoietic stem and progenitor cells throughout development. Cell Stem Cell 2009;5:385-95.
8. Brignier AC, Gewirtz AM. Embryonic and adult stem cell therapy. J Allergy Clin Immunol 2010;125:S336-44.
9. Jonsson AH, Yokoyama WM. Natural killer cell tolerance licensing and other mechanisms. Adv Immunol 2009;101:27-79.
10. Balato A, Unutmaz D, Gaspari AA. Natural killer T cells: an unconventional T-cell subset with diverse effector and regulatory functions. J Invest Dermatol 2009;129:1628-42.
11. Kennedy AD, DeLeo FR. Neutrophil apoptosis and the resolution of infection. Immunol Res 2009;43:25-61.
12. Benoit M, Desnues B, Mege JL. Macrophage polarization in bacterial infections. J Immunol 2008;181:3733-9.
13. Gordon S. Alternative activation of macrophages. Nat Rev Immunol 2003;3: 23-35.
14. Minai-Fleminger Y, Levi-Schaffer F. Mast cells and eosinophils: the two key effector cells in allergic inflammation. Inflamm Res 2009;58:631-8.
15. Schroeder JT. Basophils: beyond effector cells of allergic inflammation. Adv Immunol 2009;101:123-61.
16. Lambrecht BN, Hammad H. Biology of lung dendritic cells at the origin of asthma. Immunity 2009;31:412-24.
17. Gilliet M, Cao W, Liu YJ. Plasmacytoid dendritic cells: sensing nucleic acids in viral infection and autoimmune diseases. Nat Rev Immunol 2008;8:594-606.
18. Colonna M, Pulendran B, Iwasaki A. Dendritic cells at the host-pathogen interface. Nat Immunol 2006;7:117-20.
19. Naik SH, Sathe P, Park HY, Metcalf D, Proietto AI, Dakic A, et al. Development of plasmacytoid and conventional dendritic cell subtypes from single precursor cells derived in vitro and in vivo. Nat Immunol 2007;8:1217-26.
20. Bjorkman PJ. MHC restriction in three dimensions: a view of T cell receptor/ligand interactions. Cell 1997;89:167-70.
21. Zinkernagel RM, Doherty PC. The discovery of MHC restriction. Immunol Today 1997;18:14-7.
22. Joshi NS, Kaech SM. Effector CD8 T cell development: a balancing act between memory cell potential and terminal differentiation. J Immunol 2008; 180:1309-15.
23. Niedermann G. Immunological functions of the proteasome. Curr Top Microbiol Immunol 2002;268:91-136.
24. Tanahashi N, Yokota K, Ahn JY, Chung CH, Fujiwara T, Takahashi E, et al. Molecular properties of the proteasome activator PA28 family proteins and gamma-interferon regulation. Genes Cells 1997;2:195-211.
25. Garbi N, Tanaka S, Momburg F, Hammerling GJ. Impaired assembly of the major histocompatibility complex class I peptide-loading complex in mice deficient in the oxidoreductase ERp57. Nat Immunol 2006;7:93-102.
26. Momburg F, Tan P. Tapasin-the keystone of the loading complex optimizing peptide binding by MHC class I molecules in the endoplasmic reticulum. Mol Immunol 2002;39:217-33.
27. Melief CJ. Mini-review: regulation of cytotoxic T lymphocyte responses by dendritic cells: peaceful coexistence of cross-priming and direct priming? Eur J Immunol 2003;33:2645-54.
28. Sigal LJ, Crotty S, Andino R, Rock KL. Cytotoxic T-cell immunity to virus-infected non-haematopoietic cells requires presentation of exogenous antigen. Nature 1999;398:77-80.
29. Konig R, Fleury S, Germain RN. The structural basis of CD4-MHC class II interactions: coreceptor contributions to T cell receptor antigen recognition and oligomerization-dependent signal transduction. Curr Top Microbiol Immunol 1996; 205:19-46.
30. Turley SJ, Inaba K, Garrett WS, Ebersold M, Unternaehrer J, Steinman RM, et al. Transport of peptide-MHC class II complexes in developing dendritic cells. Science 2000;288:522-7.
31. Van Kaer L. Accessory proteins that control the assembly of MHC molecules with peptides. Immunol Res 2001;23:205-14.
32. Sadegh-Nasseri S, Chen M, Narayan K, Bouvier M. The convergent roles of tapasin and HLA-DM in antigen presentation. Trends Immunol 2008;29:141-7.
33. Ghodke Y, Joshi K, Chopra A, Patwardhan B. HLA and disease. Eur J Epidemiol 2005;20:475-88.
34. Reveille JD. Recent studies on the genetic basis of ankylosing spondylitis. Curr Rheumatol Rep 2009;11:340-8.
35. Pociot F, McDermott MF. Genetics of type 1 diabetes mellitus. Genes Immun 2002;3:235-49.
36. Caillat-Zucman S. Molecular mechanisms of HLA association with autoimmune diseases. Tissue Antigens 2009;73:1-8.
37. Sonderstrup G, McDevitt HO. DR, DQ, and you: MHC alleles and autoimmunity. J Clin Invest 2001;107:795-6.
38. Alper CA, Kruskall MS, Marcus-Bagley D, Craven DE, Katz AJ, Brink SJ, et al. Genetic prediction of nonresponse to hepatitis B vaccine. N Engl J Med 1989; 321:708-12.
39. Poland GA, Ovsyannikova IG, Jacobson RM, Vierkant RA, Jacobsen SJ, Pankratz VS, et al. Identification of an association between HLA class II alleles and low antibody levels after measles immunization. Vaccine 2001;20:430-8.
40. Grant EP, Beckman EM, Behar SM, Degano M, Frederique D, Besra GS, et al. Fine specificity of TCR complementarity-determining region residues and lipid antigen hydrophilic moieties in the recognition of a CD1-lipid complex. J Immunol 2002;168:3933-40.
41. Prigozy TI, Naidenko O, Qasba P, Elewaut D, Brossay L, Khurana A, et al. Glycolipid antigen processing for presentation by CD1d molecules. Science 2001; 291:664-7.
42. Wu J, Groh V, Spies T. T cell antigen receptor engagement and specificity in the recognition of stress-inducible MHC class I-related chains by human epithelial gamma delta T cells. J Immunol 2002;169:1236-40.

43. Godfrey DI, MacDonald HR, Kronenberg M, Smyth MJ, Van Kaer L. NKT cells: what's in a name? Nat Rev Immunol 2004;4:231-7.
44. Gellert M. V(D)J recombination: RAG proteins, repair factors, and regulation. Annu Rev Biochem 2002;71:101-32.
45. Nguyen HH, Zemlin M, Ivanov II, Andrasi J, Zemlin C, Vu HL, et al. Heterosubtypic immunity to influenza A virus infection requires a properly diversified antibody repertoire. J Virol 2007;81:9331-8.
46. Miller JF. The discovery of thymus function and of thymus-derived lymphocytes. Immunol Rev 2002;185:7-14.
47. Nitta T, Murata S, Ueno T, Tanaka K, Takahama Y. Thymic microenvironments for T-cell repertoire formation. Adv Immunol 2008;99:59-94.
48. Mathis D, Benoist C. Aire. Annu Rev Immunol 2009;27:287-312.
49. Ishikawa H, Naito T, Iwanaga T, Takahashi-Iwanaga H, Suematsu M, Hibi T, et al. Curriculum vitae of intestinal intraepithelial T cells: their developmental and behavioral characteristics. Immunol Rev 2007;215:154-65.
50. Salmond RJ, Filby A, Qureshi I, Caserta S, Zamoyska R. T-cell receptor proximal signaling via the Src-family kinases, Lck and Fyn, influences T-cell activation, differentiation, and tolerance. Immunol Rev 2009;228:9-22.
51. Nurieva RI, Liu X, Dong C. Yin-Yang of costimulation: crucial controls of immune tolerance and function. Immunol Rev 2009;229:88-100.
52. Riley JL. PD-1 signaling in primary T cells. Immunol Rev 2009;229:114-25.
53. Notarangelo LD. Primary immunodeficiencies. J Allergy Clin Immunol 2010; 125:S182-94.
54. von Boehmer H, Kisielow P, Kishi H, Scott B, Borgulya P, Teh HS. The expression of CD4 and CD8 accessory molecules on mature T cells is not random but correlates with the specificity of the alpha beta receptor for antigen. Immunol Rev 1989;109:143-51.
55. Sakaguchi S, Ono M, Setoguchi R, Yagi H, Hori S, Fehervari Z, et al. Foxp3+ CD25+ CD4+ natural regulatory T cells in dominant self-tolerance and autoimmune disease. Immunol Rev 2006;212:8-27.
56. Nakamura K, Kitani A, Strober W. Cell contact-dependent immunosuppression by CD4(+)CD25(+) regulatory T cells is mediated by cell surface-bound transforming growth factor beta. J Exp Med 2001;194:629-44.
57. Curotto de Lafaille MA, Lafaille JJ. Natural and adaptive foxp3+ regulatory T cells: more of the same or a division of labor? Immunity 2009;30:626-35.
58. Huehn J, Polansky JK, Hamann A. Epigenetic control of FOXP3 expression: the key to a stable regulatory T-cell lineage? Nat Rev Immunol 2009;9:83-9.
59. Sallusto F, Lanzavecchia A. Heterogeneity of CD4+ memory T cells: functional modules for tailored immunity. Eur J Immunol 2009;39:2076-82.
60. Unutmaz D. RORC2: the master of human Th17 cell programming. Eur J Immunol 2009;39:1452-5.
61. Thomas MJ, MacAry PA, Noble A, Askenase PW, Kemeny DM. T cytotoxic 1 and T cytotoxic 2 CD8 T cells both inhibit IgE responses. Int Arch Allergy Immunol 2001;124:187-9.
62. Racila DM, Kline JN. Perspectives in asthma: molecular use of microbial products in asthma prevention and treatment. J Allergy Clin Immunol 2005;116:1202-5.
63. Larkin EA, Carman RJ, Krakauer T, Stiles BG. *Staphylococcus aureus*: the toxic presence of a pathogen extraordinaire. Curr Med Chem 2009;16:4003-19.
64. Thomas LR, Cobb RM, Oltz EM. Dynamic regulation of antigen receptor gene assembly. Adv Exp Med Biol 2009;650:103-15.
65. Jankovic M, Casellas R, Yannoutsos N, Wardemann H, Nussenzweig MC. RAGs and regulation of autoantibodies. Annu Rev Immunol 2004;22:485-501.
66. Khan WN. B cell receptor and BAFF receptor signaling regulation of B cell homeostasis. J Immunol 2009;183:3561-7.
67. Kurosaki T, Hikida M. Tyrosine kinases and their substrates in B lymphocytes. Immunol Rev 2009;228:132-48.
68. Carroll MC. Complement and humoral immunity. Vaccine 2008;26(suppl 8):I28-33.
69. Malisan F, Briere F, Bridon JM, Harindranath N, Mills FC, Max EE, et al. Interleukin-10 induces immunoglobulin G isotype switch recombination in human CD40-activated naive B lymphocytes. J Exp Med 1996;183:937-47.
70. Chaudhuri J, Basu U, Zarrin A, Yan C, Franco S, Perlot T, et al. Evolution of the immunoglobulin heavy chain class switch recombination mechanism. Adv Immunol 2007;94:157-214.
71. Schmidlin H, Diehl SA, Blom B. New insights into the regulation of human B-cell differentiation. Trends Immunol 2009;30:277-85.
72. Durandy A, Taubenheim N, Peron S, Fischer A. Pathophysiology of B-cell intrinsic immunoglobulin class switch recombination deficiencies. Adv Immunol 2007; 94:275-306.
73. Tangye SG, Tarlinton DM. Memory B cells: effectors of long-lived immune responses. Eur J Immunol 2009;39:2065-75.
74. Fu YX, Chaplin DD. Development and maturation of secondary lymphoid tissues. Annu Rev Immunol 1999;17:399-433.
75. Allen CD, Okada T, Cyster JG. Germinal-center organization and cellular dynamics. Immunity 2007;27:190-202.
76. Schwab SR, Cyster JG. Finding a way out: lymphocyte egress from lymphoid organs. Nat Immunol 2007;8:1295-301.
77. Fonacier LS, Dreskin SC, Leung DYM. Allergic skin diseases. J Allergy Clin Immunol 2010;125:S138-49.
78. Pesu M, Candotti F, Husa M, Hofmann SR, Notarangelo LD, O'Shea JJ. Jak3, severe combined immunodeficiency, and a new class of immunosuppressive drugs. Immunol Rev 2005;203:127-42.
79. Kovanen PE, Leonard WJ. Cytokines and immunodeficiency diseases: critical roles of the gamma(c)-dependent cytokines interleukins 2, 4, 7, 9, 15, and 21, and their signaling pathways. Immunol Rev 2004;202:67-83.
80. Chapgier A, Boisson-Dupuis S, Jouanguy E, Vogt G, Feinberg J, Prochnicka-Chalufour A, et al. Novel STAT1 alleles in otherwise healthy patients with mycobacterial disease. PLoS Genet 2006;2:e131.
81. Tangye SG, Cook MC, Fulcher DA. Insights into the role of STAT3 in human lymphocyte differentiation as revealed by the hyper-IgE syndrome. J Immunol 2009;182:21-8.
82. Kaplan MH, Sun YL, Hoey T, Grusby MJ. Impaired IL-12 responses and enhanced development of Th2 cells in Stat4-deficient mice. Nature 1996;382:174-7.
83. Medzhitov R. Recognition of microorganisms and activation of the immune response. Nature 2007;449:819-26.
84. Spiegelberg HL, Raz E. DNA-based approaches to the treatment of allergies. Curr Opin Mol Ther 2002;4:64-71.
85. von Bernuth H, Picard C, Jin Z, Pankla R, Xiao H, Ku CL, et al. Pyogenic bacterial infections in humans with MyD88 deficiency. Science 2008;321:691-6.
86. Istomin AY, Godzik A. Understanding diversity of human innate immunity receptors: analysis of surface features of leucine-rich repeat domains in NLRs and TLRs. BMC Immunol 2009;10:48.
87. Sutterwala FS, Ogura Y, Szczepanik M, Lara-Tejero M, Lichtenberger GS, Grant EP, et al. Critical role for NALP3/CIAS1/Cryopyrin in innate and adaptive immunity through its regulation of caspase-1. Immunity 2006;24:317-27.
88. Eisenbarth SC, Colegio OR, O'Connor W, Sutterwala FS, Flavell RA. Crucial role for the Nalp3 inflammasome in the immunostimulatory properties of aluminium adjuvants. Nature 2008;453:1122-6.
89. Gantner BN, Simmons RM, Underhill DM. Dectin-1 mediates macrophage recognition of *Candida albicans* yeast but not filaments. EMBO J 2005;24:1277-86.
90. Waters P, Vaid M, Kishore U, Madan T. Lung surfactant proteins A and D as pattern recognition proteins. Adv Exp Med Biol 2009;653:74-97.
91. Agrawal A, Singh PP, Bottazzi B, Garlanda C, Mantovani A. Pattern recognition by pentraxins. Adv Exp Med Biol 2009;653:98-116.
92. Sutherland TE, Maizels RM, Allen JE. Chitinases and chitinase-like proteins: potential therapeutic targets for the treatment of T-helper type 2 allergies. Clin Exp Allergy 2009;39:943-55.
93. Reese TA, Liang HE, Tager AM, Luster AD, Van Rooijen N, Voehringer D, et al. Chitin induces accumulation in tissue of innate immune cells associated with allergy. Nature 2007;447:92-6.
94. Kazakova MH, Sarafian VS. YKL-40—a novel biomarker in clinical practice? Folia Med (Plovdiv) 2009;51:5-14.
95. Ober C, Chupp GL. The chitinase and chitinase-like proteins: a review of genetic and functional studies in asthma and immune-mediated diseases. Curr Opin Allergy Clin Immunol 2009;9:401-8.
96. Cooper MA, Colonna M, Yokoyama WM. Hidden talents of natural killers: NK cells in innate and adaptive immunity. EMBO Rep 2009;10:1103-10.
97. Carroll MC. The complement system in regulation of adaptive immunity. Nat Immunol 2004;5:981-6.
98. Longhi MP, Harris CL, Morgan BP, Gallimore A. Holding T cells in check—a new role for complement regulators? Trends Immunol 2006;27:102-8.
99. Tedesco F. Inherited complement deficiencies and bacterial infections. Vaccine 2008;26(suppl 8):I3-8.
100. Harboe M, Mollnes TE. The alternative complement pathway revisited. J Cell Mol Med 2008;12:1074-84.
101. Botto M, Kirschfink M, Macor P, Pickering MC, Wurzner R, Tedesco F. Complement in human diseases: lessons from complement deficiencies. Mol Immunol 2009;46:2774-83.
102. Brodsky RA. Narrative review: paroxysmal nocturnal hemoglobinuria: the physiology of complement-related hemolytic anemia. Ann Intern Med 2008;148: 587-95.
103. Ley K, Laudanna C, Cybulsky MI, Nourshargh S. Getting to the site of inflammation: the leukocyte adhesion cascade updated. Nat Rev Immunol 2007;7:678-89.
104. Abram CL, Lowell CA. The ins and outs of leukocyte integrin signaling. Annu Rev Immunol 2009;27:339-62.
105. Commins SP, Borish L, Steinke JW. Immunologic messenger molecules: Cytokines, interferons, and chemokines. J Allergy Clin Immunol 2010;125:S53-72.
106. Trimble LA, Prince KA, Pestano GA, Daley J, Cantor H. Fas-dependent elimination of nonselected CD8 cells and lpr disease. J Immunol 2002;168:4960-7.

107. Fortner KA, Budd RC. The death receptor Fas (CD95/APO-1) mediates the deletion of T lymphocytes undergoing homeostatic proliferation. J Immunol 2005;175:4374-82.
108. Strasser A, Jost PJ, Nagata S. The many roles of FAS receptor signaling in the immune system. Immunity 2009;30:180-92.
109. Hardie WD, Glasser SW, Hagood JS. Emerging concepts in the pathogenesis of lung fibrosis. Am J Pathol 2009;175:3-16.
110. Guarino M, Tosoni A, Nebuloni M. Direct contribution of epithelium to organ fibrosis: epithelial-mesenchymal transition. Hum Pathol 2009;40:1365-76.
111. Annunziato F, Cosmi L, Liotta F, Maggi E, Romagnani S. Type 17 T helper cells-origins, features and possible roles in rheumatic disease. Nat Rev Rheumatol 2009;5:325-31.
112. Joseph A, Brasington R, Kahl L, Ranganathan P, Cheng TP, Atkinson J. Immunologic rheumatic disorders. J Allergy Clin Immunol 2010;125:S204-15.
113. Ono SJ. Molecular genetics of allergic diseases. Annu Rev Immunol 2000;18:347-66.
114. Zhou Z, Song X, Li B, Greene MI. FOXP3 and its partners: structural and biochemical insights into the regulation of FOXP3 activity. Immunol Res 2008;42:19-28.
115. Rudwaleit M, Andermann B, Alten R, Sorensen H, Listing J, Zink A, et al. Atopic disorders in ankylosing spondylitis and rheumatoid arthritis. Ann Rheum Dis 2002;61:968-74.
116. Simpson CR, Anderson WJ, Helms PJ, Taylor MW, Watson L, Prescott GJ, et al. Coincidence of immune-mediated diseases driven by Th1 and Th2 subsets suggests a common aetiology. A population-based study using computerized general practice data. Clin Exp Allergy 2002;32:37-42.
117. Rabin RL, Levinson AI. The nexus between atopic disease and autoimmunity: a review of the epidemiological and mechanistic literature. Clin Exp Immunol 2008;153:19-30.
118. Burlingham WJ. A lesson in tolerance—maternal instruction to fetal cells. N Engl J Med 2009;360:1355-7.
119. Mold JE, Michaelsson J, Burt TD, Muench MO, Beckerman KP, Busch MP, et al. Maternal alloantigens promote the development of tolerogenic fetal regulatory T cells in utero. Science 2008;322:1562-5.
120. Blois SM, Ilarregui JM, Tometten M, Garcia M, Orsal AS, Cordo-Russo R, et al. A pivotal role for galectin-1 in fetomaternal tolerance. Nat Med 2007;1:1450-7.
121. Li DJ, Guo PF. The regulatory role of thymic stromal lymphopoietin (TSLP) in maternal-fetal immune tolerance during early human pregnancy. J Reprod Immunol 2009;83:106-8.

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.

S. E. T. is supported by a Chaim Roifman Scholar Award from the Canadian Immunodeficiency Society and a Career Development Award from the Canadian Child Health Clinician Scientist Program (CCHCSP)-a CIHR Strategic Training Program and operating grants from the Canadian Cystic Fibrosis Foundation and the CIHR Team in Mutagenesis and Infectious Diseases. D.H.B. is supported by NIH grants AI 038425, AI072115 and AI070535.

Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

Received for publication May 14, 2009; revised July 3, 2009; accepted for publication July 8, 2009.

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.

0091-6749/$36.00

doi:10.1016/j.jaci.2009.07.016

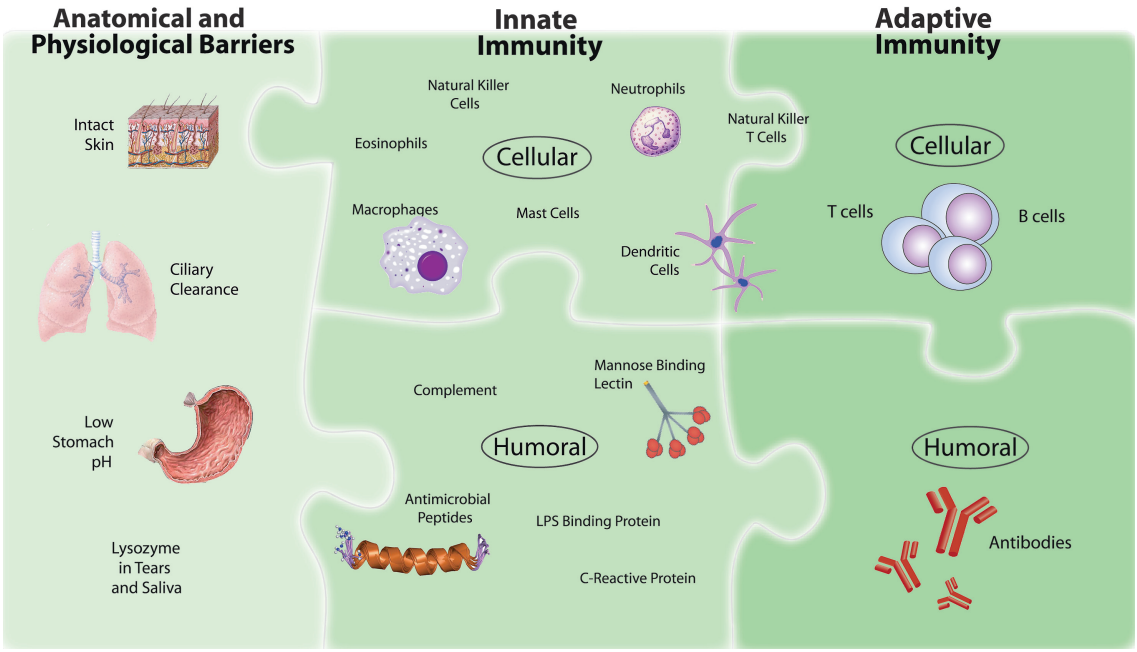

FIG 1. Integrated human immune system. The human microbial defense system can be simplistically viewed as consisting of 3 levels: (1) anatomic and physiologic barriers; (2) innate immunity; and (3) adaptive immunity. In common with many classification systems, some elements are difficult to categorize. 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

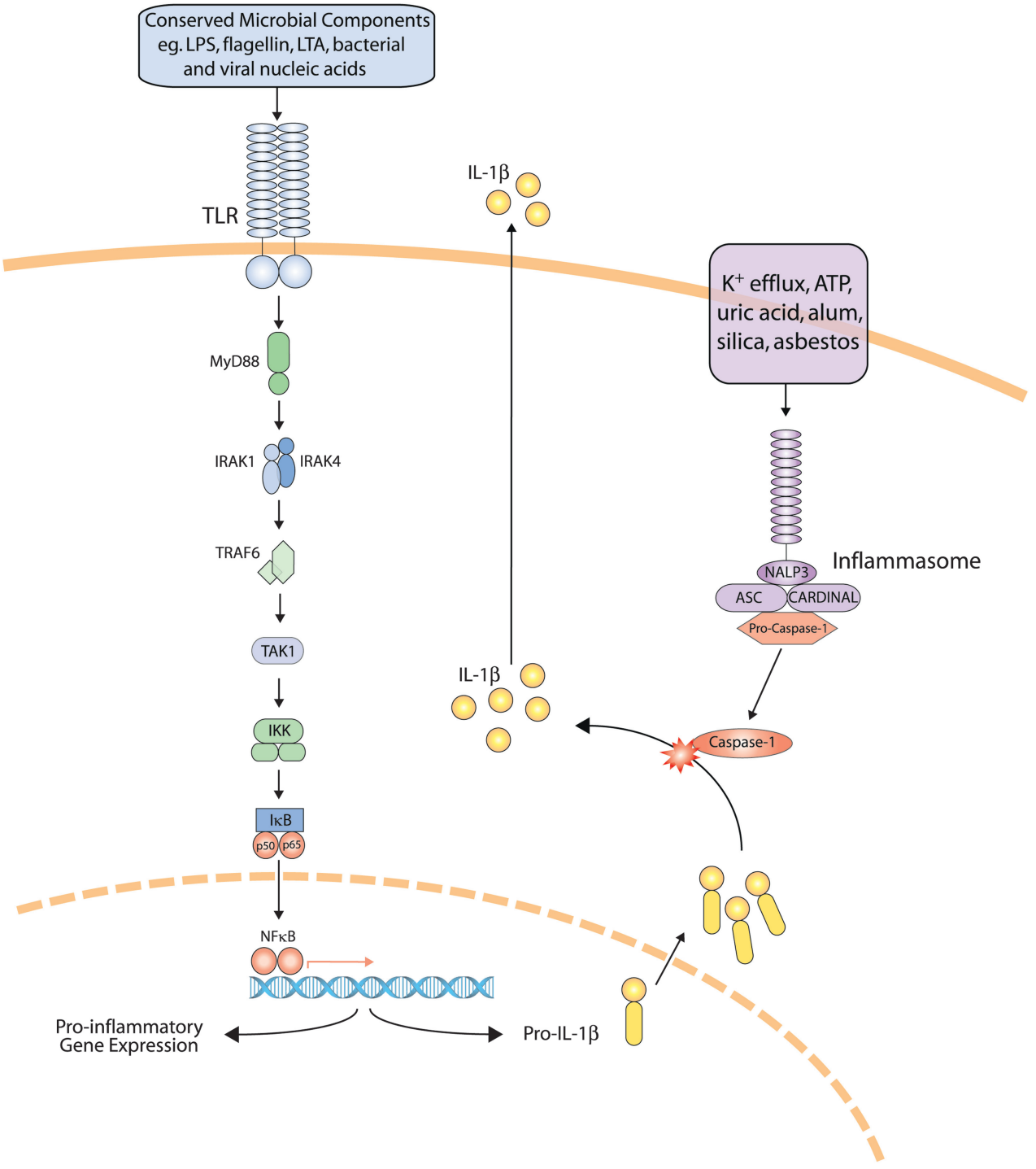

FIG 2. Overview of TLR signaling and the NLRP3 inflammasome. TLR ligation initiates a signaling cascade that culminates in the translocation of the transcription factor nuclear factor κB *(NF-κB)* and others to the nucleus, generating an acute inflammatory response. The NLRP3 (or NALP3) inflammasome 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.

We thank the members of the UBC Center for Understanding and Preventing Infections in Children for constructive input and Rachel Victor for creating our high-quality figures.

REFERENCES

1. Frank MM. Complement disorders and hereditary angioedema. J Allergy Clin Immunol 2010;125:S262-71.
2. Janeway CA Jr, Medzhitov R. Innate immune recognition. Annu Rev Immunol 2002;20:197-216.
3. Pancer Z, Cooper MD. The evolution of adaptive immunity. Annu Rev Immunol 2006;24:497-518.
4. Dempsey PW, Allison ME, Akkaraju S, Goodnow CC, Fearon DT. C3d of complement as a molecular adjuvant: bridging innate and acquired immunity. Science 1996;271:348-50.
5. Kabelitz D. Expression and function of Toll-like receptors in T lymphocytes. Curr Opin Immunol 2007;19:39-45.
6. Beutler B. Innate immunity: an overview. Mol Immunol 2004;40:845-59.
7. Janeway CA Jr. Approaching the asymptote? Evolution and revolution in immunology. Cold Spring Harb Symp Quant Biol 1989;54:1-13.
8. Bianchi ME. DAMPs, PAMPs and alarmins: all we need to know about danger. J Leukoc Biol 2007;81:1-5.
9. Joncker NT, Raulet DH. Regulation of NK cell responsiveness to achieve self-tolerance and maximal responses to diseased target cells. Immunol Rev 2008;224:85-97.

10. Lemaitre B, Nicolas E, Michaut L, Reichhart JM, Hoffmann JA. The dorsoventral regulatory gene cassette spatzle/Toll/cactus controls the potent antifungal response in *Drosophila* adults. Cell 1996;86:973-83.
11. Takeda K, Kaisho T, Akira S. Toll-like receptors. Annu Rev Immunol 2003;21: 335-76.
12. Akira S, Takeda K. Toll-like receptor signalling. Nat Rev Immunol 2004;4:499-511.
13. Ho J, Li Y, Hirschfeld AF, Mansouri D, Turvey SE. Advances in innate immunity: the role of Toll-like receptor signaling in human disease. J Respir Dis Thorac Surg Intensive Care Tuberc 2004;3:7-14.
14. Turvey SE, Hawn TR. Towards subtlety: understanding the role of Toll-like receptor signaling in susceptibility to human infections. Clin Immunol 2006;120:1-9.
15. Picard C, Puel A, Bonnet M, Ku CL, Bustamante J, Yang K, et al. Pyogenic bacterial infections in humans with IRAK-4 deficiency. Science 2003;299:2076-9.
16. von Bernuth H, Picard C, Jin Z, Pankla R, Xiao H, Ku CL, et al. Pyogenic bacterial infections in humans with MyD88 deficiency. Science 2008;321:691-6.
17. Yang K, Puel A, Zhang S, Eidenschenk C, Ku CL, Casrouge A, et al. Human TLR-7-, -8-, and -9-mediated induction of IFN-alpha/beta and -lambda is IRAK-4 dependent and redundant for protective immunity to viruses. Immunity 2005;23: 465-78.
18. Notarangelo L, Casanova JL, Fischer A, Puck J, Rosen F, Seger R, et al. Primary immunodeficiency diseases: an update. J Allergy Clin Immunol 2004;114:677-87.
19. Goldstein DB, Cavalleri GL. Genomics: understanding human diversity. Nature 2005;437:1241-2.
20. Hawn TR, Verbon A, Lettinga KD, Zhao LP, Li SS, Laws RJ, et al. A common dominant TLR5 stop codon polymorphism abolishes flagellin signaling and is associated with susceptibility to Legionnaires' disease. J Exp Med 2003;198: 1563-72.
21. Khor CC, Chapman SJ, Vannberg FO, Dunne A, Murphy C, Ling EY, et al. A Mal functional variant is associated with protection against invasive pneumococcal disease, bacteremia, malaria and tuberculosis. Nat Genet 2007;39:523-8.
22. Martinez FD. CD14, endotoxin, and asthma risk: actions and interactions. Proc Am Thorac Soc 2007;4:221-5.
23. Simpson A, John SL, Jury F, Niven R, Woodcock A, Ollier WE, et al. Endotoxin exposure, CD14, and allergic disease: an interaction between genes and the environment. Am J Respir Crit Care Med 2006;174:386-92.
24. Nejentsev S, Thye T, Szeszko JS, Stevens H, Balabanova Y, Chinbuah AM, et al. Analysis of association of the TIRAP (MAL) S180L variant and tuberculosis in three populations. Nat Genet 2008;40:261-2; author reply 262-3.
25. Chen G, Shaw MH, Kim Y-G, Nuñez G. NOD-like receptors: role in innate immunity and inflammatory disease. Annu Rev Pathol 2009;4:365-98.
26. Benko S, Philpott DJ, Girardin SE. The microbial and danger signals that activate Nod-like receptors. Cytokine 2008;43:368-73.
27. Martinon F, Mayor A, Tschopp Jr. The inflammasomes: guardians of the body. Annu Rev Immunol 2009;27:229-65.
28. Ting JPY, Lovering RC, Alnemri ES, Bertin J, Boss JM, Davis BK, et al. The NLR gene family: a standard nomenclature. Immunity 2008;28:285-7.
29. Miao EA, Andersen-Nissen E, Warren SE, Aderem A. TLR5 and Ipaf: dual sensors of bacterial flagellin in the innate immune system. Semin Immunopathol 2007;29: 275-88.
30. Franchi L, Eigenbrod T, Munoz-Planillo R, Nunez G. The inflammasome: a caspase-1-activation platform that regulates immune responses and disease pathogenesis. Nat Immunol 2009;10:241-7.
31. Masters SL, Simon A, Aksentijevich I, Kastner DL. Horror autoinflammaticus: the molecular pathophysiology of autoinflammatory disease. Annu Rev Immunol 2009;27:621-68.
32. Aksentijevich I, Nowak M, Mallah M, Chae JJ, Watford WT, Hofmann SR, et al. De novo CIAS1 mutations, cytokine activation, and evidence for genetic heterogeneity in patients with neonatal-onset multisystem inflammatory disease (NOMID): a new member of the expanding family of pyrin-associated autoinflammatory diseases. Arthritis Rheum 2002;46:3340-8.
33. Hoffman HM, Rosengren S, Boyle DL, Cho JY, Nayar J, Mueller JL, et al. Prevention of cold-associated acute inflammation in familial cold autoinflammatory syndrome by interleukin-1 receptor antagonist. Lancet 2004;364:1779-85.
34. Goldbach-Mansky R, Dailey NJ, Canna SW, Gelabert A, Jones J, Rubin BI, et al. Neonatal-onset multisystem inflammatory disease responsive to interleukin-1beta inhibition. N Engl J Med 2006;355:581-92.
35. Hugot JP, Chamaillard M, Zouali H, Lesage S, Cezard JP, Belaiche J, et al. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. Nature 2001;411:599-603.
36. Ogura Y, Bonen DK, Inohara N, Nicolae DL, Chen FF, Ramos R, et al. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. Nature 2001;411:603-6.
37. Noguchi E, Homma Y, Kang X, Netea MG, Ma X. A Crohn's disease-associated NOD2 mutation suppresses transcription of human IL10 by inhibiting activity of the nuclear ribonucleoprotein hnRNP-A1. Nat Immunol 2009;10:471-9.
38. Strober W, Kitani A, Fuss I, Asano N, Watanabe T. The molecular basis of NOD2 susceptibility mutations in Crohn's disease. Mucosal Immunol 2008;1(suppl 1):S5-9.
39. Franchi L, Nunez G. The Nlrp3 inflammasome is critical for aluminium hydroxide-mediated IL-1beta secretion but dispensable for adjuvant activity. Eur J Immunol 2008;38:2085-9.
40. Li H, Willingham SB, Ting JP, Re F. Cutting edge: inflammasome activation by alum and alum's adjuvant effect are mediated by NLRP3. J Immunol 2008;181:17-21.
41. Eisenbarth SC, Colegio OR, O'Connor W, Sutterwala FS, Flavell RA. Crucial role for the Nalp3 inflammasome in the immunostimulatory properties of aluminium adjuvants. Nature 2008;453:1122-6.
42. Horner AA, Redecke V, Raz E. Toll-like receptor ligands: hygiene, atopy and therapeutic implications. Curr Opin Allergy Clin Immunol 2004;4:555-61.
43. Broide D, Schwarze J, Tighe H, Gifford T, Nguyen MD, Malek S, et al. Immunostimulatory DNA sequences inhibit IL-5, eosinophilic inflammation, and airway hyperresponsiveness in mice. J Immunol 1998;161:7054-62.
44. Fanucchi MV, Schelegle ES, Baker GL, Evans MJ, McDonald RJ, Gershwin LJ, et al. Immunostimulatory oligonucleotides attenuate airways remodeling in allergic monkeys. Am J Respir Crit Care Med 2004;170:1153-7.
45. Gauvreau GM, Hessel EM, Boulet LP, Coffman RL, O'Byrne PM. Immunostimulatory sequences regulate interferon-inducible genes but not allergic airway responses. Am J Respir Crit Care Med 2006;174:15-20.
46. Casale TB, Kessler J, Romero FA. Safety of the intranasal toll-like receptor 4 agonist CRX-675 in allergic rhinitis. Ann Allergy Asthma Immunol 2006;97:454-6.
47. Shirota H, Sano K, Hirasawa N, Terui T, Ohuchi K, Hattori T, et al. Novel roles of CpG oligodeoxynucleotides as a leader for the sampling and presentation of CpG-tagged antigen by dendritic cells. J Immunol 2001;167:66-74.
48. Tulic MK, Fiset PO, Christodoulopoulos P, Vaillancourt P, Desrosiers M, Lavigne F, et al. Amb a 1-immunostimulatory oligodeoxynucleotide conjugate immunotherapy decreases the nasal inflammatory response. J Allergy Clin Immunol 2004;113:235-41.
49. Creticos PS, Schroeder JT, Hamilton RG, Balcer-Whaley SL, Khattignavong AP, Lindblad R, et al. Immunotherapy with a ragweed-toll-like receptor 9 agonist vaccine for allergic rhinitis. N Engl J Med 2006;355:1445-55.
50. Pulendran B, Ahmed R. Translating innate immunity into immunological memory: implications for vaccine development. Cell 2006;124:849-63.
51. Casella CR, Mitchell TC. Putting endotoxin to work for us: monophosphoryl lipid A as a safe and effective vaccine adjuvant. Cell Mol Life Sci 2008;65:3231-40.
52. Schwarz TF. AS04-adjuvanted human papillomavirus-16/18 vaccination: recent advances in cervical cancer prevention. Expert Rev Vaccines 2008;7:1465-73.
53. Kong NCT, Beran J, Kee SA, Miguel JL, Sanchez C, Bayas JM, et al. A new adjuvant improves the immune response to hepatitis B vaccine in hemodialysis patients. Kidney Int 2007;73:856-62.

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.

Disclosure of potential conflict of interest: F. A. Bonilla is an editor and author for UpToDate, Inc; is a speaker for CSL Behring and Baxter International; and has received research support from Talecris Biotherapeutics. H. C. Oettgen is a consultant/scientific advisor for Schering-Plough and a consultant for Genentech and has received research support from Novartis, although not for areas related to the current article.

Received for publication May 26, 2009; revised September 7, 2009; accepted for publication September 11, 2009.

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

0091-6749/$36.00

doi:10.1016/j.jaci.2009.09.017

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

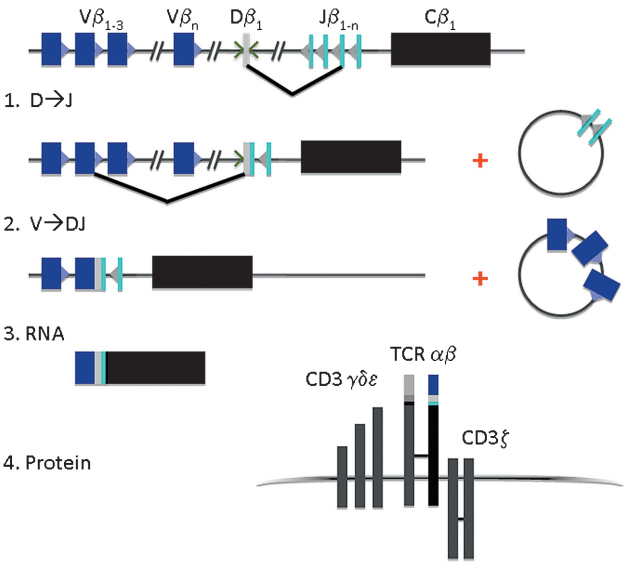

FIG 1. Sequential recombination of a random assortment of gene fragments dictates TCR structure and specificity. This schematic depiction of the TCR $V\beta_1$ locus indicates the relative locations of the Vβ, Dβ, and Jβ segments upstream of $C\beta_1$. *1,* The V(D)J recombinase recognizes signal sequences *(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

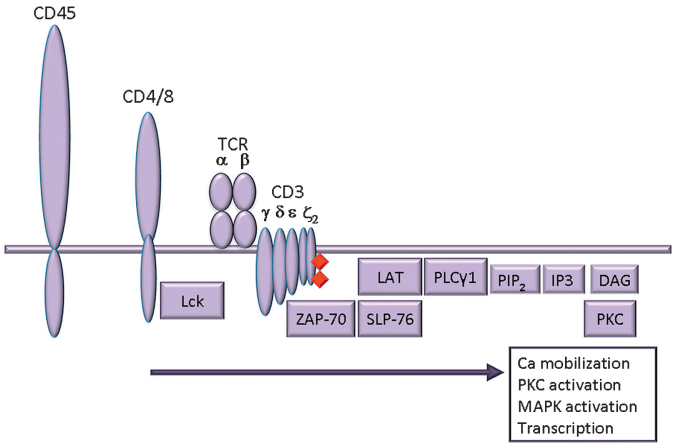

FIG 2. Signaling molecules in T-cell activation. The TCR α and β chains recognize peptide/MHC complexes expressed on APCs, an interaction that is stabilized by the simultaneous binding of T-cell CD8 to MHC class I or CD4 to MHC class II. Signaling is initiated by the CD3 chains (γ, δ, ϵ, and ζ) through 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

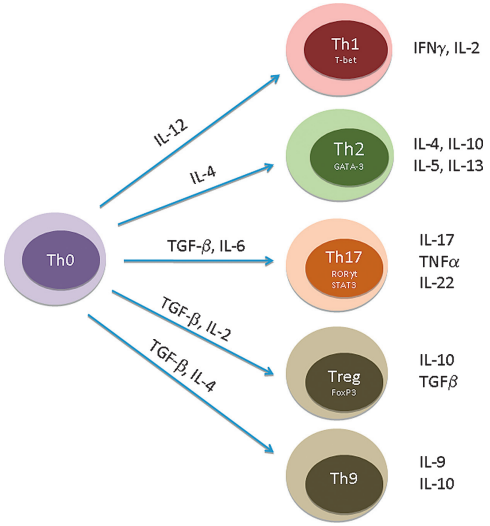

FIG 3. $CD4^+$ T_H cell subsets. Antigen-specific naive T_H0 T cells are stimulated to expand on interaction with APCs expressing MHC class II/peptide complexes. Depending on the type of APC and the cytokine milieu *(arrows)* at the site of antigen encounter, T_H0 cells can be driven down one of several 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

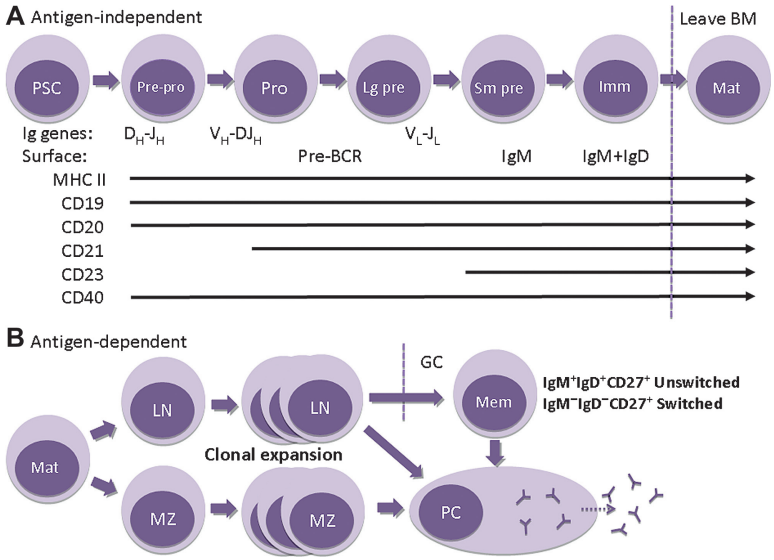

FIG 4. A, Antigen-independent B-cell development in the bone marrow. The earliest recognized committed stage is the pre-/pro-B cell, where immunoglobulin D_H and J_H genes rearrange. In the pro-B cell stage, a V_H segment is joined to the DJ_H unit. At this point, if heavy chain gene rearrangement on 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

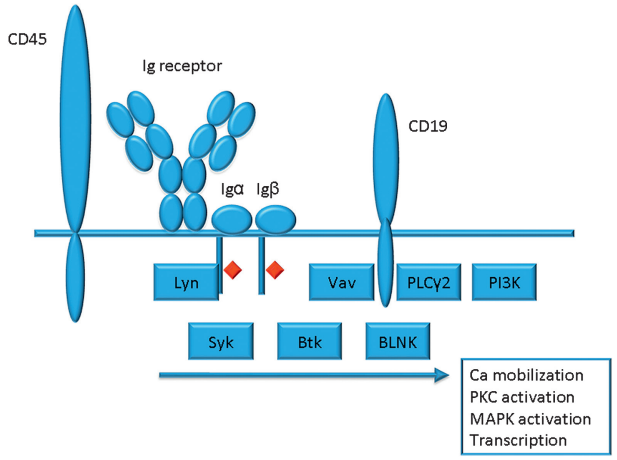

FIG 5. Signaling molecules in B-cell activation. The immunoglobulin receptor contains 2 signaling molecules called Ig-α and Ig-β (encoded by the *CD79A* and *CD79B* genes, respectively). The cytoplasmic domains of these molecules contain ITAMs *(red diamonds)*, which recruit signaling molecules to clusters 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).

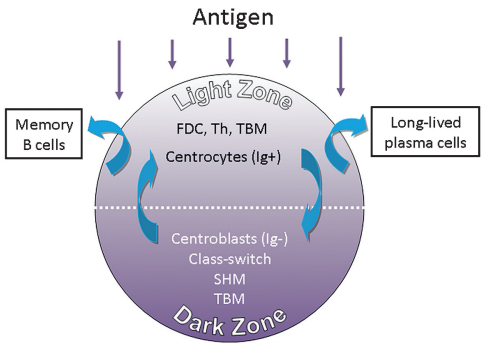

FIG 6. Diagram of a germinal center. Cells (and antigens) enter the light zone, which is positioned to facilitate exposure to sources of antigen (eg, intestinal lumen, splenic arterioles, and subcapsular sinus in lymph nodes). This area has a high concentration of follicular dendritic cells *(FDC)*, 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.

REFERENCES

1. Cooper MD, Alder MN. The evolution of adaptive immune systems. Cell 2006; 124:815-22.
2. Hedrick SM. Thymus lineage commitment: a single switch. Immunity 2008;28: 297-9.
3. Jenkinson EJ, Jenkinson WE, Rossi SW, Anderson G. The thymus and T-cell commitment: the right niche for Notch? Nat Rev Immunol 2006;6:551-5.
4. Takahama Y. Journey through the thymus: stromal guides for T-cell development and selection. Nat Rev Immunol 2006;6:127-35.
5. Oltz EM, Osipovich O. Targeting V(D)J recombinase: putting a PHD to work. Immunity 2007;27:539-41.
6. Chan K, Puck JM. Development of population-based newborn screening for severe combined immunodeficiency. J Allergy Clin Immunol 2005;115:391-8.
7. Mathis D, Benoist C. Aire. Annu Rev Immunol 2009;27:287-312.
8. Dustin ML. The cellular context of T cell signaling. Immunity 2009;30:482-92.
9. Feske S. Calcium signalling in lymphocyte activation and disease. Nat Rev Immunol 2007;7:690-702.
10. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol 1986;136:2348-57.
11. Kaminuma O, Kitamura F, Miyatake S, Yamaoka K, Miyoshi H, Inokuma S, et al. T-box 21 transcription factor is responsible for distorted T(H)2 differentiation in human peripheral CD4 + T cells. J Allergy Clin Immunol 2009;123:813-23, e3.
12. Steinman L. A brief history of T(H)17, the first major revision in the T(H)1/T(H)2 hypothesis of T cell-mediated tissue damage. Nat Med 2007;13:139-45.
13. Wang H, Lee CH, Qi C, Tailor P, Feng J, Abbasi S, et al. IRF8 regulates B-cell lineage specification, commitment, and differentiation. Blood 2008;112: 4028-38.
14. Dardalhon V, Awasthi A, Kwon H, Galileos G, Gao W, Sobel RA, et al. IL-4 inhibits TGF-beta-induced Foxp3 + T cells and, together with TGF-beta, generates IL-9 + IL-10 + Foxp3(-) effector T cells. Nat Immunol 2008;9:1347-55.
15. Veldhoen M, Uyttenhove C, van Snick J, Helmby H, Westendorf A, Buer J, et al. Transforming growth factor-beta "reprograms" the differentiation of T helper 2 cells and promotes an interleukin 9-producing subset. Nat Immunol 2008;9: 1341-6.
16. Chatila TA. Role of regulatory T cells in human diseases. J Allergy Clin Immunol 2005;116:949-60.
17. Meyer EH, DeKruyff RH, Umetsu DT. T cells and NKT cells in the pathogenesis of asthma. Annu Rev Med 2008;59:281-92.
18. Fuxa M, Skok JA. Transcriptional regulation in early B cell development. Curr Opin Immunol 2007;19:129-36.
19. LeBien TW, Tedder TF. B lymphocytes: how they develop and function. Blood 2008;112:1570-80.
20. Wang YH, Liu YJ. The IL-17 cytokine family and their role in allergic inflammation. Curr Opin Immunol 2008;20:697-702.
21. Conley ME, Dobbs AK, Farmer DM, Kilic S, Paris K, Grigoriadou S, et al. Primary B cell immunodeficiencies: comparisons and contrasts. Annu Rev Immunol 2009; 27:199-227.
22. Dorshkind K, Montecino-Rodriguez E. Fetal B-cell lymphopoiesis and the emergence of B-1-cell potential. Nat Rev Immunol 2007;7:213-9.
23. Steiniger B, Timphus EM, Barth PJ. The splenic marginal zone in humans and rodents: an enigmatic compartment and its inhabitants. Histochem Cell Biol 2006; 126:641-8.
24. Wehr C, Kivioja T, Schmitt C, Ferry B, Witte T, Eren E, et al. The EUROclass trial: defining subgroups in common variable immunodeficiency. Blood 2008;111:77-85.
25. Richards S, Watanabe C, Santos L, Craxton A, Clark EA. Regulation of B-cell entry into the cell cycle. Immunol Rev 2008;224:183-200.
26. Vos Q, Lees A, Wu ZQ, Snapper CM, Mond JJ. B-cell activation by T-cell-independent type 2 antigens as an integral part of the humoral immune response to pathogenic microorganisms. Immunol Rev 2000;176:154-70.
27. He B, Qiao X, Cerutti A. CpG DNA induces IgG class switch DNA recombination by activating human B cells through an innate pathway that requires TLR9 and cooperates with IL-10. J Immunol 2004;173:4479-91.
28. Lee JJ, Ozcan E, Rauter I, Geha RS. Transmembrane activator and calcium-modulator and cyclophilin ligand interactor mutations in common variable immunodeficiency. Curr Opin Allergy Clin Immunol 2008;8:520-6.
29. Batista FD, Harwood NE. The who, how and where of antigen presentation to B cells. Nat Rev Immunol 2009;9:15-27.
30. Allen CD, Okada T, Cyster JG. Germinal-center organization and cellular dynamics. Immunity 2007;27:190-202.
31. Stavnezer J, Guikema JE, Schrader CE. Mechanism and regulation of class switch recombination. Annu Rev Immunol 2008;26:261-92.
32. Oettgen HC. Regulation of the IgE isotype switch: new insights on cytokine signals and the functions of epsilon germline transcripts. Curr Opin Immunol 2000;12: 618-23.
33. Johansen FE, Brandtzaeg P. Transcriptional regulation of the mucosal IgA system. Trends Immunol 2004;25:150-7.
34. Steele EJ. Mechanism of somatic hypermutation: critical analysis of strand biased mutation signatures at A:T and G:C base pairs. Mol Immunol 2009;46:305-20.
35. Peled JU, Kuang FL, Iglesias-Ussel MD, Roa S, Kalis SL, Goodman MF, et al. The biochemistry of somatic hypermutation. Annu Rev Immunol 2008;26:481-511.

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.

Disclosure of potential conflict of interest: H. W. Schroeder receives research support from the National Institutes of Health/National Institute of Allergy and Infectious Diseases, the United States Immunodeficiency Network, and NABI Pharmaceuticals and is a councilor for the Henry Kunkel Society. L. Cavacini is a consultant for GTC Biotherapeutics and receives research support from the National Institutes of Health.

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.

0091-6749/$36.00

doi:10.1016/j.jaci.2009.09.046

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.

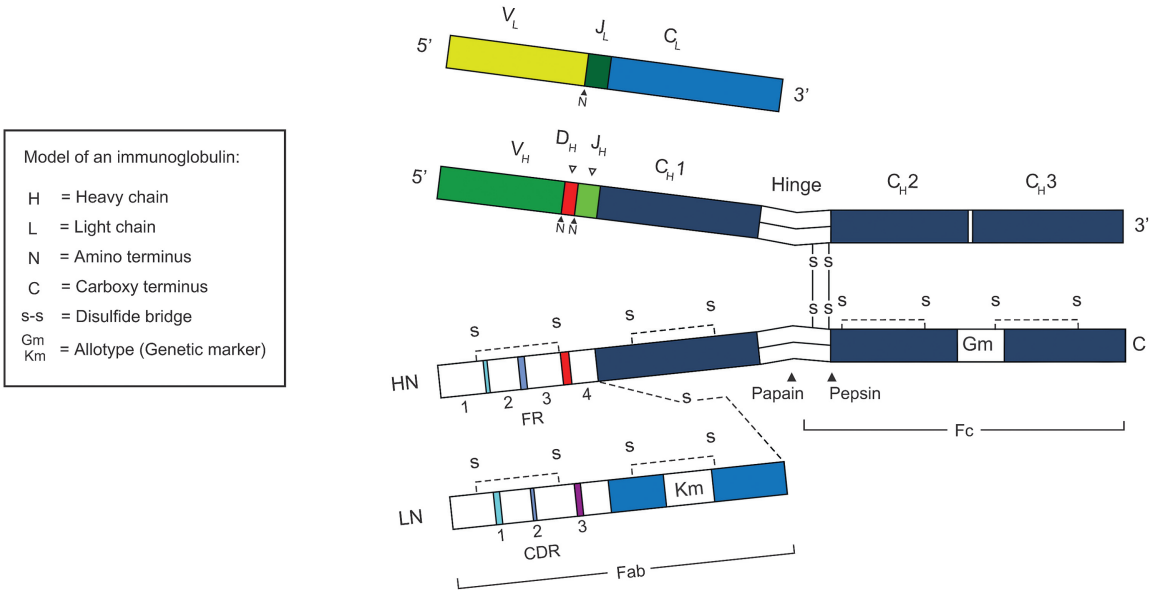

FIG 1. Two-dimensional model of an IgG molecule. The H and L chains at the top deconstruct the antibody at a nucleotide level. The chains at the bottom deconstruct the protein sequence. See the text for further details.

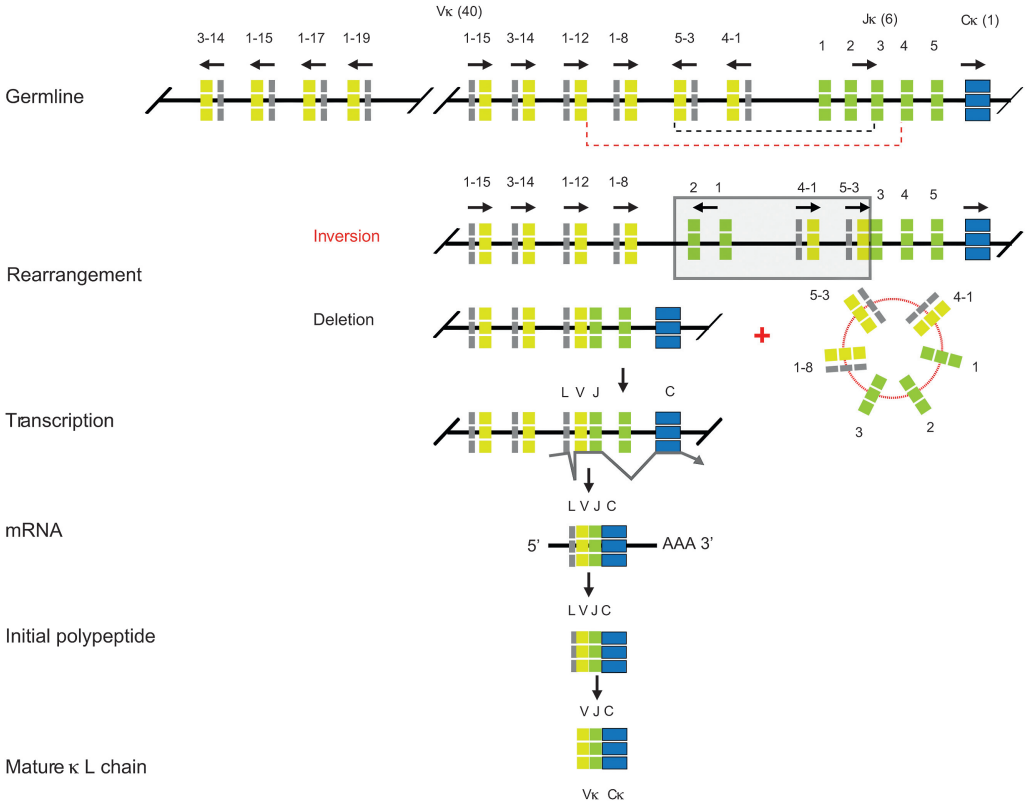

FIG 2. Rearrangement events in the human κ locus. See the text for further details.

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.

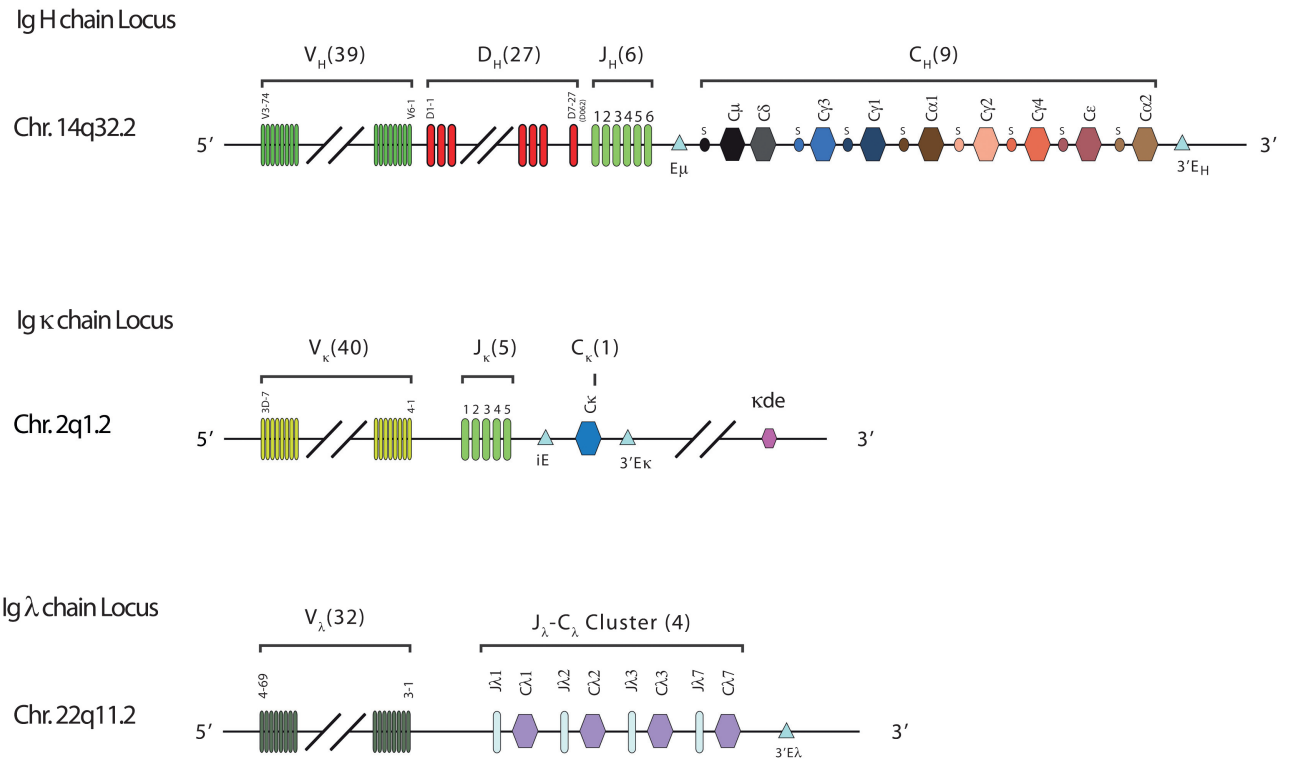

FIG 3. Representation of the chromosomal organization of the immunoglobulin H, κ, and λ gene clusters. The typical numbers of functional gene segments are shown. The κ gene cluster includes a κ-deleting element that can rearrange to sequences upstream of Cκ in cells that express λ chains, reducing the 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 *Vκ* and *Jκ* gene segments (Fig 2), whereas the κ C domains are encoded by a single *Cκ* exon. The locus contains 5 *Jκ* and 75 *Vκ* gene segments upstream of *Cκ* (Fig 3). One third of the *Vκ* 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 *Vκ* 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 *Vκ*.

Each active *Vκ* gene segment has the potential to rearrange to any of the 5 *Jκ* elements, generating a potential "combinatorial" repertoire of more than 140 distinct *VJ* combinations. The *Vκ* gene segment contains FR1, FR2, and FR3; CDR1 and CDR2; and the amino-terminal portion of CDR3. The *Jκ* 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 *Vκ* or *Jκ* 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 *Cλ* exons, each of which is associated with its own *Jλ* (Fig 3). *Vλ* genes are arranged in 3 distinct clusters, each containing members of different *Vλ* families.[16] Depending on the individual haplotype, there are approximately 30 to 36 potentially functional *Vλ* 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

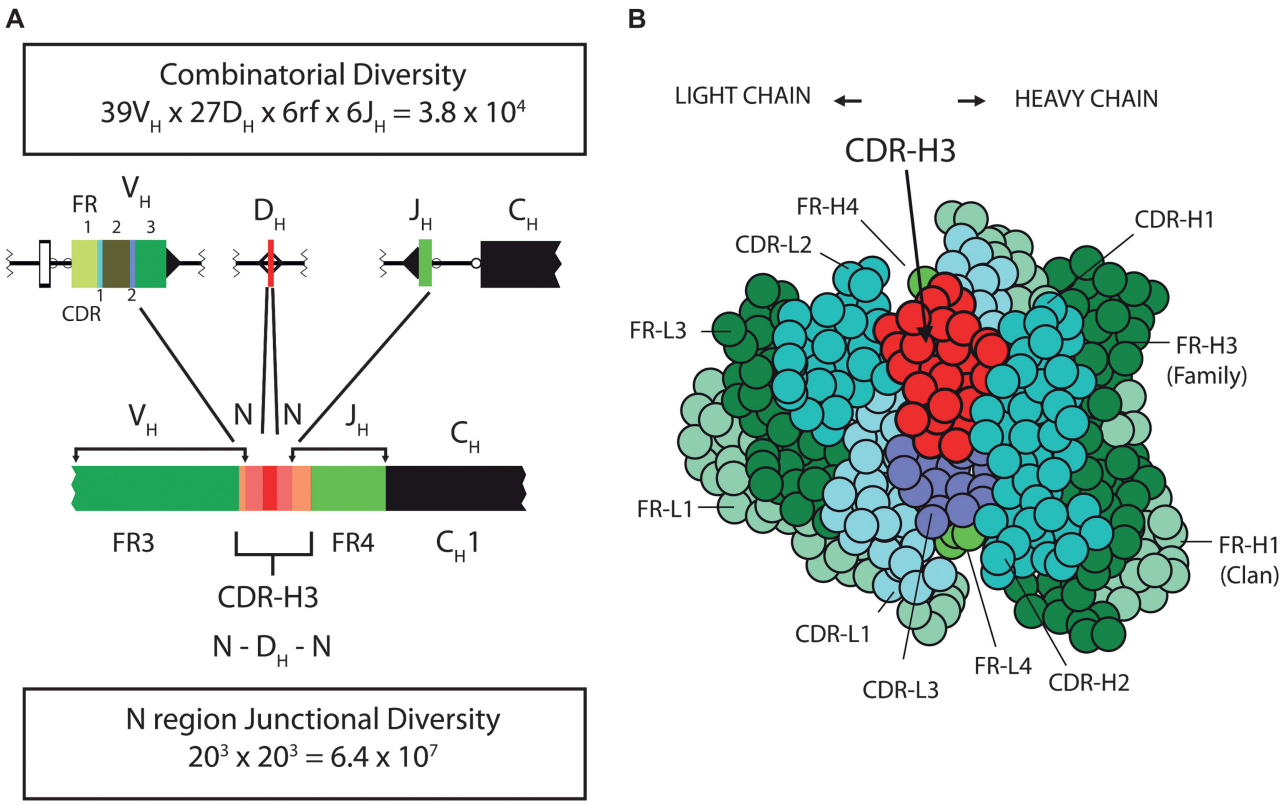

FIG 4. The antigen-binding site is the product of a nested gradient of diversity. **A**, H chain rearrangement can yield as many as 38,000 different VDJ combinations. The addition of 9 N nucleotides on either side of the D gene segment can yield up to 64,000,000 different CDR-H3 junctional sequences. **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

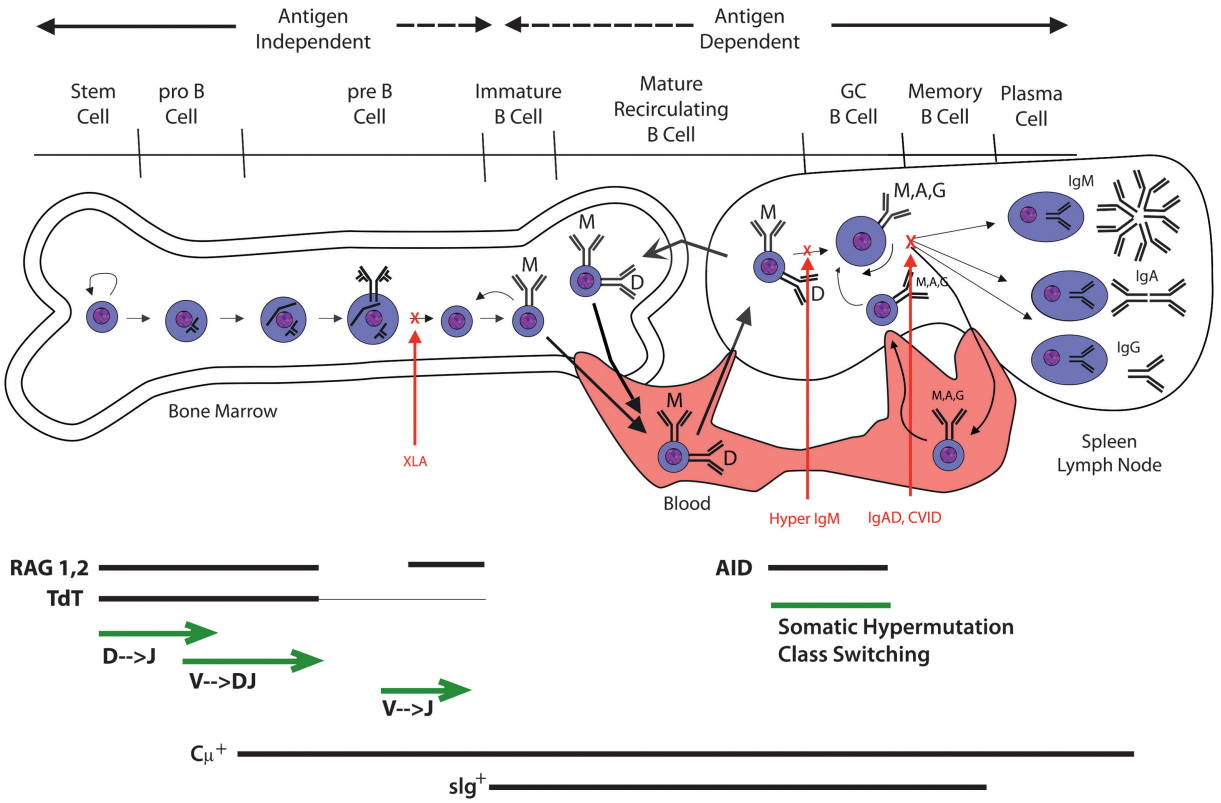

FIG 5. Immunoglobulin diversification and B-cell development. B-cell development as a function of immunoglobulin rearrangement and modification is shown. After birth, B-cell development begins in the bone marrow and is independent of antigen stimulation. The pre–B cell is defined by the presence of cytoplasmic 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]

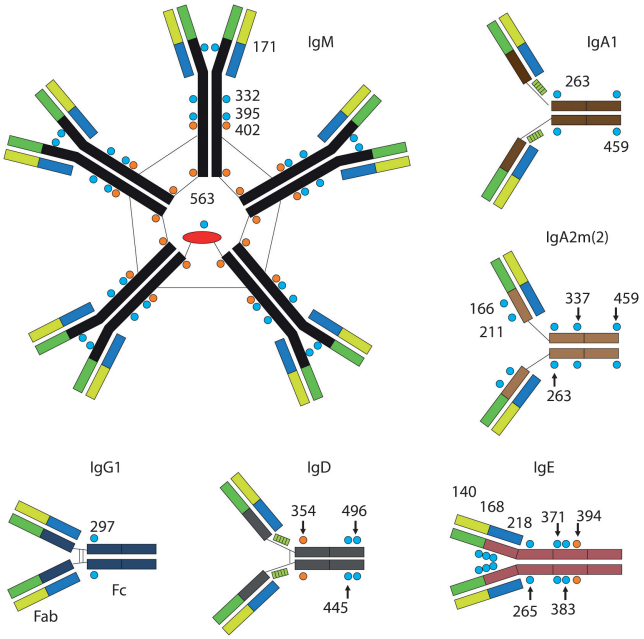

FIG 6. Structural and glycosylation properties of immunoglobulins. Depiction of the structure and glycosylation sites (indicated by amino acid location) for human IgM, IgG, IgD, IgE, IgA2, and IgA2. Adapted from Arnold et al.[28]

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.

We thank Ms Elena M. Schroeder for her help in creating the figures for this manuscript.

REFERENCES

1. Williams AF, Barclay AN. The immunoglobulin superfamily—domains for cell surface recognition. Annu Rev Immunol 1988;6:381-405.
2. Harpaz Y, Chothia C. Many of the immunoglobulin superfamily domains in cell adhesion molecules and surface receptors belong to a new structural set which is close to that containing variable domains. J Mol Biol 1994;238:528-39.
3. Torres RM, Imboden J, Schroeder HW Jr. Antigen receptor genes, gene products, and co-receptors. In: Rich RR, Fleisher TA, Shearer WT, Schroeder HW Jr, Frew AJ, Weyand CM, editors. Clinical immunology: principles and practice. 3rd ed London: Mosby Elsevier; 2008. p. 53-77.
4. Smith KA, Nelson PN, Warren P, Astley SJ, Murray PG, Greenman J. Demystified…recombinant antibodies. J Clin Pathol 2004;57:912-7.
5. Hamers-Casterman C, Atarhouch T, Muyldermans S, Robinson G, Hamers C, Songa EB, et al. Naturally occurring antibodies devoid of light chains. Nature 1993;363:446-8.
6. Roux KH, Greenberg AS, Greene L, Strelets L, Avila D, McKinney EC, et al. Structural analysis of the nurse shark (new) antigen receptor (NAR): molecular convergence of NAR and unusual mammalian immunoglobulins. Proc Natl Acad Sci U S A 1998;95:11804-9.
7. Vincke C, Loris R, Saerens D, Martinez-Rodriguez S, Muyldermans S, Conrath K. General strategy to humanize a camelid single-domain antibody and identification of a universal humanized nanobody scaffold. J Biol Chem 2009;284:3273-84.
8. Jazwinska EC, Dunckley H, Propert DN, Gatenby PA, Serjeantson SW. GM typing by immunoglobulin heavy chain gene RFLP analysis. Am J Hum Genet 1988;43:175-81.
9. Leder P. The genetics of antibody diversity. Sci Am 1982;246:102-15.
10. Tonegawa S. Somatic generation of antibody diversity. Nature 1983;302:575-81.
11. Dudley DD, Chaudhuri J, Bassing CH, Alt FW. Mechanism and control of V(D)J recombination versus class switch recombination: similarities and differences. Adv Immunol 2005;86:43-112.
12. Zachau HG. The human immunoglobulin kappa genes. In: Honjo T, Alt FW, Rabbitts PH, editors. Immunoglobulin genes. 2nd ed London: Academic Press; 1995. p. 173.
13. Brodeur PH, Riblet RJ. The immunoglobulin heavy chain variable region (IgH-V) locus in the mouse. I. One hundred Igh-V genes comprise seven families of homologous genes. Eur J Immunol 1984;14:922-30.
14. Kirkham PM, Schroeder HW Jr. Antibody structure and the evolution of immunoglobulin V gene segments. Semin Immunol 1994;6:347-60.
15. Lee SK, Bridges SL Jr, Koopman WJ, Schroeder HW Jr. The immunoglobulin kappa light chain repertoire expressed in the synovium of a patient with rheumatoid arthritis. Arthritis Rheum 1992;35:905-13.
16. Kawasaki K, Minoshima S, Nakato E, Shibuya K, Shintani A, Schmeits JL, et al. One-megabase sequence analysis of the human immunoglobulin lambda gene locus. PCR Methods Appl 1997;7:250-61.
17. Bankovich AJ, Raunser S, Juo ZS, Walz T, Davis MM, Garcia KC. Structural insight into pre-B cell receptor function. Science 2007;316:291-4.
18. Matsuda F, Ishii K, Bourvagnet P, Kuma K, Hayashida H, Miyata T, et al. The complete nucleotide sequence of the human immunoglobulin heavy chain variable region locus. J Exp Med 1998;188:2151-62.
19. Corbett SJ, Tomlinson IM, Sonnhammer EL, Buck D, Winter G. Sequence of the human immunoglobulin diversity (D) segment locus: a systematic analysis provides no evidence for the use of DIR segments, inverted D segments, "minor" D segments or D-D recombination. J Mol Biol 1997;270:587-97.
20. Honjo T. Immunoglobulin genes. Annu Rev Immunol 1983;1:499-528.
21. Dorner T, Foster SJ, Farner NL, Lipsky PE. Somatic hypermutation of human immunoglobulin heavy chain genes: targeting of RGYW motifs on both DNA strands. Eur J Immunol 1998;28:3384-96.

22. Rada C, Ehrenstein MR, Neuberger MS, Milstein C. Hot spot focusing of somatic hypermutation in MSH2-deficient mice suggests two stages of mutational targeting. Immunity 1998;9:135-41.
23. Neuberger MS. Antibody diversification by somatic mutation: from Burnet onwards. Immunol Cell Biol 2008;86:124-32.
24. Muramatsu M, Nagaoka H, Shinkura R, Begum NA, Honjo T. Discovery of activation-induced cytidine deaminase, the engraver of antibody memory. Adv Immunol 2007;94:1-36.
25. Matthias P, Rolink AG. Transcriptional networks in developing and mature B cells. Nat Rev Immunol 2005;5:497-508.
26. Normansell DE. Human immunoglobulin subclasses. Diagn Clin Immunol 1987;5: 115-28.
27. Torres M, Casadevall A. The immunoglobulin constant region contributes to affinity and specificity. Trends Immunol 2008;29:91-7.
28. Arnold JN, Wormald MR, Sim RB, Rudd PM, Dwek RA. The impact of glycosylation on the biological function and structure of human immunoglobulins. Annu Rev Immunol 2007;25:21-50.
29. Liu H, Bulseco G-G, Sun J. Effect of posttranslational modifications on the thermal stability of a recombinant monoclonal antibody. Immunol Lett 2006;106:144-53.
30. Siberil S, de Romeuf C, Bihoreau N, Fernandez N, Meterreau JL, Regenman A, et al. Selection of a human anti-RhD monoclonal antibody for therapeutic use: impact of IgG glycosylation on activating and inhibitory Fc gamma R functions. Clin Immunol 2006;118:170-9.
31. Sazinsky SL, Ott RG, Silver NW, Tidor B, Ravetch JV, Wittrup KD. Aglycosylated immunoglobulin G1 variants productively engage activating Fc receptors. Proc Natl Acad Sci U S A 2008;105:20167-72.
32. Kanda Y, Yamada T, Mori K, Okazaki A, Inoue M, Kitajima-Miyama K, et al. Comparison of biological activity among nonfucosylated therapeutic IgG1 antibodies with three different N-linked Fc oligosaccharides: the high mannose, hybrid and complex types. Glycobiology 2006;17:104-18.
33. Natsume A, Wakitani M, Yamane-Ohnuki N, Shoji-Hosaka E, Niwa R, Uchida K, et al. Fucose removal from complex-type oligosaccharide enhances the antibody-dependent cellular cytotoxicity of single-gene-encoded antibody comprising a single-chain antibody linked the antibody constant region. J Immunol Methods 2005; 306:93-103.
34. Arnold JN, Dwek RA, Rudd PM, Sim RB. Mannan binding lectin and its interaction with immunoglobulins in health and in disease. Immunol Lett 2006;106:103-10.
35. Albert H, Collin M, Dudziak D, Ravetch JV, Nimmerjahn F. In vivo enzymatic modulation of IgG glycosylation inhibits autoimmune disease in an IgG subclass-dependent manner. Proc Natl Acad Sci U S A 2009;105:15005-9.
36. Anthony RM, Wermeling F, Karlsson MC, Ravetch JV. Identification of a receptor required for the anti-inflammatory activity of IVIG. Proc Natl Acad Sci U S A 2008;105:19571-8.
37. Miranda LR, Duval M, Doherty H, Seaman MS, Posner MR, Cavacini LA. The neutralization properties of a HIV-specific antibody are markedly altered by glycosylation events outside the antigen-binding domain. J Immunol 2007;178:7132-8.
38. Collin M, Shannon O, Bjorck L. IgG glycan hydrolysis by a bacterial enzyme as a therapy against autoimmune conditions. Proc Natl Acad Sci U S A 2008;105: 4265-70.
39. Boes M. Role of natural and immune IgM antibodies in immune responses. Mol Immunol 2000;37:1141-9.
40. Riesbeck K, Nordstrom T. Structure and immunological action of the human pathogen *Moraxella catarrhalis* IgD-binding protein. Crit Rev Immunol 2006;26: 353-76.
41. Geisberger R, Lamers M, Achatz G. The riddle of the dual expression of IgM and IgD. Immunology 2006;118:429-37.
42. Parlowsky T, Welzel J, Amagai M, Zillikens D, Wygold T. Neonatal pemphigus vulgaris: IgG4 autoantibodies to desmoglein 3 induce skin blisters in newborns. J Am Acad Dermatol 2003;48:623-5.
43. Yeh SW, Cavacini LA, Bhol KC, Lin MS, Kumar M, Duval M, et al. Pathogenic human monoclonal antibody against desmoglein 3. J Immunol 2006; 120:68-75.
44. Cavacini LA, Kuhrt D, Duval M, Mayer K, Posner M. Binding and neutralization activity of IgG1 and IgG3 from serum of HIV infected individuals. AIDS Res Hum Retroviruses 2003;19:785-92.
45. Scharf O, Golding H, King LR, Eller N, Frazier D, Golding B, et al. Immunoglobin G3 from polyclonal human immunodeficiency virus (HIV) immune globulin is more potent than other subclasses in neutralizing HIV type 1. J Virol 2001;75: 6558-65.
46. Woof JM, Mestecky J. Mucosal immunoglobulins. Immunol Rev 2005;206:64-82.
47. Stubbe H, Berdoz J, Kraehenbuhl JP, Corthesy B. Polymeric IgA is superior to monomeric IgA and IgG carrying the same variable domain in preventing *Clostridium difficile* toxin A damaging of T84 monolayers. J Immunol 2000;164:1952-60.
48. Corthesy B. Roundtrip ticket for secretory IgA: role in mucosal homeostasis? J Immunol 2007;178:27-32.
49. Chang TW, Wu PC, Hsu CL, Hung AF. Anti-IgE antibodies for the treatment of IgE-mediated allergic diseases. Adv Immunol 2007;93:63-119.
50. Nimmerjahn F, Ravetch JV. FC-receptors are regulators of immunity. Adv Immunol 2007;96:179-204.
51. Hinton PR, Johlfs MG, Xiong JM, Hanestad K, Ong KC, Bullock C, et al. Engineered human IgG antibodies with longer serum half-lives in primates. J Biol Chem 2004;279:6213-6.
52. Petkova SB, Akilesh S, Sproule TJ, Christianson GJ, Al Khabbaz H, Brown AC, et al. Enhanced half-life of genetically engineered human IgG1 antibodies in a humanized FcRn mouse model: potential application in humorally mediated autoimmune disease. Int Immunol 2006;18:1759-69.

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.

Supported by National Institutes of Health grant R01 AI-057438 and AI-50989 (L.B. and J.W.S.), and by an American Academy of Allergy, Asthma & Immunology/GlaxoSmithKline career development award and T32 AI-007496-14 (S.P.C.).

Disclosure of potential conflict S. P. Commins has received research support from GlaxoSmithKline. L. Borish has received research support from Merck and Genentech. J. W. Steinke has received research support from the National Institutes of Health.

Received for publication June 15, 2009; revised July 9, 2009; accepted for publication July 10, 2009.

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.

0091-6749/$36.00

doi:10.1016/j.jaci.2009.07.008

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

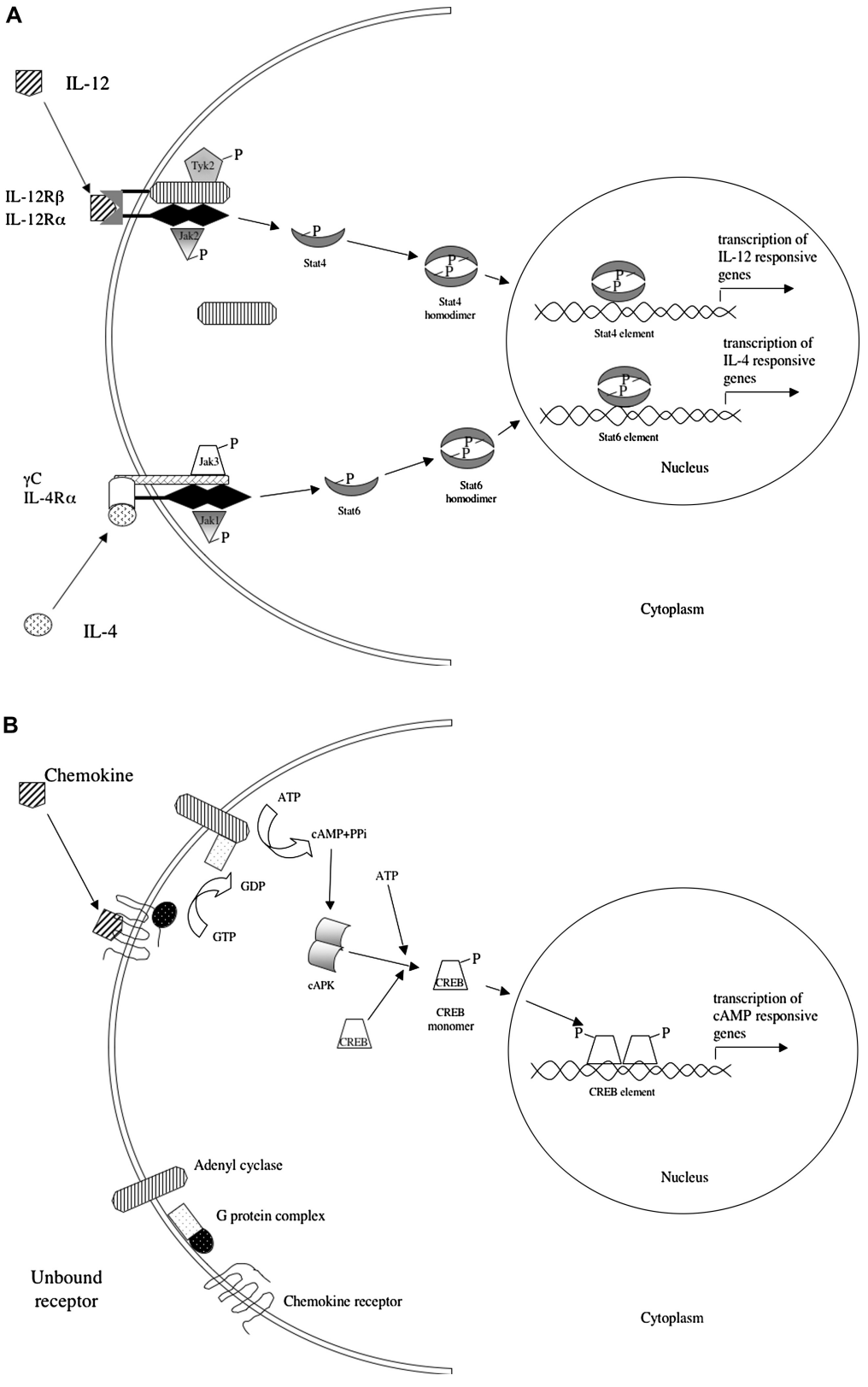

FIG 1. Comparison of cytokine and chemokine signaling. **A,** Generalized cytokine signaling: a model of intracellular signaling pathways leading to transcription modulation by IL-4 and IL-12 (see text for details). **B,** Generalized chemokine signaling: a model of chemokine binding and activation of 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. 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.

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.

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.

REFERENCES

1. Beutler B, Cerami A. The biology of cachectin/TNF—a primary mediator of the host response. Annu Rev Immunol 1989;7:625-55.
2. Perez C, Albert I, DeFay K, Zachariades N, Gooding L, Kriegler M. A nonsecretable cell surface mutant of tumor necrosis factor (TNF) kills by cell-to-cell contact. Cell 1990;63:251-8.
3. Tartaglia LA, Goeddel DV. Two TNF receptors. Immunol Today 1992;13:151-3.
4. Tracey KJ, Fong Y, Hesse DG, Manogue KR, Lee AT, Kuo GC, et al. Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteraemia. Nature 1987;330:662-4.
5. Dinarello CA, Wolff SM. The role of interleukin-1 in disease. N Engl J Med 1993;328:106-13.
6. Sims JE, Gayle MA, Slack JL, Alderson MR, Bird TA, Giri JG, et al. Interleukin 1 signaling occurs exclusively via the type I receptor. Proc Natl Acad Sci U S A 1993;90:6155-9.
7. Arend WP. Interleukin-1 receptor antagonist. Adv Immunol 1993;54:167-227.
8. Cerretti DP, Kozlosky CJ, Mosley B, Nelson N, Van Ness K, Greenstreet TA, et al. Molecular cloning of the interleukin-1 beta converting enzyme. Science 1992;256:97-100.
9. Akira S, Taga T, Kishimoto T. Interleukin-6 in biology and medicine. Adv Immunol 1993;54:1-78.
10. Muller-Newen G, Kuster A, Hemmann U, Keul R, Horsten U, Martens A, et al. Soluble IL-6 receptor potentiates the antagonistic activity of soluble gp130 on IL-6 responses. J Immunol 1998;161:6347-55.
11. Heinrich PC, Behrmann I, Haan S, Hermanns HM, Muller-Newen G, Schaper F. Principles of interleukin (IL)-6-type cytokine signalling and its regulation. Biochem J 2003;374:1-20.
12. Brunda MJ. Interleukin-12. J Leukoc Biol 1994;55:280-8.
13. Oppmann B, Lesley R, Blom B, Timans JC, Xu Y, Hunte B, et al. Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. Immunity 2000;13:715-25.
14. Dinarello CA. Interleukin-18, a proinflammatory cytokine. Eur Cytokine Netw 2000;11:483-6.
15. Grabstein KH, Eisenman J, Shanebeck K, Rauch C, Srinivasan S, Fung V, et al. Cloning of a T cell growth factor that interacts with the beta chain of the interleukin-2 receptor. Science 1994;264:965-8.
16. Larousserie F, Bardel E, Pflanz S, Arnulf B, Lome-Maldonado C, Hermine O, et al. Analysis of interleukin-27 (EBI3/p28) expression in Epstein-Barr virus- and human T-cell leukemia virus type 1-associated lymphomas: heterogeneous expression of EBI3 subunit by tumoral cells. Am J Pathol 2005;166:1217-28.
17. Pflanz S, Hibbert L, Mattson J, Rosales R, Vaisberg E, Bazan JF, et al. WSX-1 and glycoprotein 130 constitute a signal-transducing receptor for IL-27. J Immunol 2004;172:2225-31.
18. Kim SH, Han SY, Azam T, Yoon DY, Dinarello CA. Interleukin-32: a cytokine and inducer of TNFalpha. Immunity 2005;22:131-42.
19. Netea MG, Azam T, Ferwerda G, Girardin SE, Walsh M, Park JS, et al. IL-32 synergizes with nucleotide oligomerization domain (NOD) 1 and NOD2 ligands for IL-1beta and IL-6 production through a caspase 1-dependent mechanism. Proc Natl Acad Sci U S A 2005;102:16309-14.
20. Minshall E, Chakir J, Laviolette M, Molet S, Zhu Z, Olivenstein R, et al. IL-11 expression is increased in severe asthma: association with epithelial cells and eosinophils. J Allergy Clin Immunol 2000;105:232-8.
21. Tang W, Geba GP, Zheng T, Ray P, Homer RJ, Kuhn C 3rd, et al. Targeted expression of IL-11 in the murine airway causes lymphocytic inflammation, bronchial remodeling, and airways obstruction. J Clin Invest 1996;98:2845-53.
22. Adolf GR. Monoclonal antibodies and enzyme immunoassays specific for human interferon (IFN) omega 1: evidence that IFN-omega 1 is a component of human leukocyte IFN. Virology 1990;175:410-7.
23. Adolf GR, Maurer-Fogy I, Kalsner I, Cantell K. Purification and characterization of natural human interferon omega 1. Two alternative cleavage sites for the signal peptidase. J Biol Chem 1990;265:9290-5.
24. Flores I, Mariano TM, Pestka S. Human interferon omega binds to the alpha/beta receptor. J Biol Chem 1991;266:19875-7.
25. Sheppard P, Kindsvogel W, Xu W, Henderson K, Schlutsmeyer S, Whitmore TE, et al. IL-28, IL-29 and their class II cytokine receptor IL-28R. Nat Immunol 2003;4:63-8.
26. Kotenko SV, Gallagher G, Baurin VV, Lewis-Antes A, Shen M, Shah NK, et al. IFN-lambdas mediate antiviral protection through a distinct class II cytokine receptor complex. Nat Immunol 2003;4:69-77.
27. Mennechet FJ, Uze G. Interferon-lambda-treated dendritic cells specifically induce proliferation of FOXP3-expressing suppressor T cells. Blood 2006;107:4417-23.
28. Konforte D, Simard N, Paige CJ. IL-21: an executor of B cell fate. J Immunol 2009;182:1781-7.
29. Parrish-Novak J, Dillon SR, Nelson A, Hammond A, Sprecher C, Gross JA, et al. Interleukin 21 and its receptor are involved in NK cell expansion and regulation of lymphocyte function. Nature 2000;408:57-63.
30. Gross JA, Johnston J, Mudri S, Enselman R, Dillon SR, Madden K, et al. TACI and BCMA are receptors for a TNF homologue implicated in B-cell autoimmune disease. Nature 2000;404:995-9.
31. Thompson JS, Bixler SA, Qian F, Vora K, Scott ML, Cachero TG, et al. BAFF-R, a newly identified TNF receptor that specifically interacts with BAFF. Science 2001;293:2108-11.
32. Castigli E, Wilson SA, Garibyan L, Rachid R, Bonilla F, Schneider L, et al. TACI is mutant in common variable immunodeficiency and IgA deficiency. Nat Genet 2005;37:829-34.
33. Salzer U, Chapel HM, Webster AD, Pan-Hammarstrom Q, Schmitt-Graeff A, Schlesier M, et al. Mutations in TNFRSF13B encoding TACI are associated with common variable immunodeficiency in humans. Nat Genet 2005;37:820-8.
34. Finkelman FD, Holmes J, Katona IM, Urban JF Jr, Beckmann MP, Park LS, et al. Lymphokine control of in vivo immunoglobulin isotype selection. Annu Rev Immunol 1990;8:303-33.
35. Thornton AM, Donovan EE, Piccirillo CA, Shevach EM. Cutting edge: IL-2 is critically required for the in vitro activation of CD4 + CD25 + T cell suppressor function. J Immunol 2004;172:6519-23.
36. Farrar MA, Schreiber RD. The molecular cell biology of interferon-gamma and its receptor. Annu Rev Immunol 1993;11:571-611.
37. Newport MJ, Huxley CM, Huston S, Hawrylowicz CM, Oostra BA, Williamson R, et al. A mutation in the interferon-gamma-receptor gene and susceptibility to mycobacterial infection. N Engl J Med 1996;335:1941-9.
38. Bach EA, Aguet M, Schreiber RD. The IFN gamma receptor: a paradigm for cytokine receptor signaling. Annu Rev Immunol 1997;15:563-91.
39. Cruikshank WW, Center DM, Nisar N, Wu M, Natke B, Theodore AC, et al. Molecular and functional analysis of a lymphocyte chemoattractant factor: association of biologic function with CD4 expression. Proc Natl Acad Sci U S A 1994;91:5109-13.
40. Wilson KC, Center DM, Cruikshank WW. The effect of interleukin-16 and its precursor on T lymphocyte activation and growth. Growth Factors 2004;22:97-104.
41. Kawaguchi M, Adachi M, Oda N, Kokubu F, Huang SK. IL-17 cytokine family. J Allergy Clin Immunol 2004;114:1265-74.
42. Molet S, Hamid Q, Davoine F, Nutku E, Taha R, Page N, et al. IL-17 is increased in asthmatic airways and induces human bronchial fibroblasts to produce cytokines. J Allergy Clin Immunol 2001;108:430-8.
43. Ishigame H, Kakuta S, Nagai T, Kadoki M, Nambu A, Komiyama Y, et al. Differential roles of interleukin-17A and -17F in host defense against mucoepithelial bacterial infection and allergic responses. Immunity 2009;30:108-19.
44. Milner JD, Brenchley JM, Laurence A, Freeman AF, Hill BJ, Elias KM, et al. Impaired T(H)17 cell differentiation in subjects with autosomal dominant hyper-IgE syndrome. Nature 2008;452:773-6.
45. Ma CS, Chew GY, Simpson N, Priyadarshi A, Wong M, Grimbacher B, et al. Deficiency of Th17 cells in hyper IgE syndrome due to mutations in STAT3. J Exp Med 2008;205:1551-7.
46. Minegishi Y, Saito M, Nagasawa M, Takada H, Hara T, Tsuchiya S, et al. Molecular explanation for the contradiction between systemic Th17 defect and localized bacterial infection in hyper-IgE syndrome. J Exp Med 2009;206:1291-301.
47. Lin H, Lee E, Hestir K, Leo C, Huang M, Bosch E, et al. Discovery of a cytokine and its receptor by functional screening of the extracellular proteome. Science 2008;320:807-11.
48. Paul WE, Ohara J. B-cell stimulatory factor-1/interleukin 4. Annu Rev Immunol 1987;5:429-59.
49. Coffman RL, Ohara J, Bond MW, Carty J, Zlotnik A, Paul WE. B cell stimulatory factor-1 enhances the IgE response of lipopolysaccharide-activated B cells. J Immunol 1986;136:4538-41.
50. Romagnani S. Regulation and deregulation of human IgE synthesis. Immunol Today 1990;11:316-21.
51. Vella A, Teague TK, Ihle J, Kappler J, Marrack P. Interleukin 4 (IL-4) or IL-7 prevents death of resting T cells: Stat-6 is probably not required for the effect of IL-4. J Exp Med 1997;186:325-30.
52. Enelow R, Baramki DF, Borish LC. Inhibition of effector T lymphocytes mediated through antagonism of IL-4. J Allergy Clin Immunol 2004;113:560-2.
53. Schleimer RP, Sterbinsky SA, Kaiser J, Bickel CA, Klunk DA, Tomioka K, et al. IL-4 induces adherence of human eosinophils and basophils but not neutrophils to endothelium. Association with expression of VCAM-1. J Immunol 1992;148:1086-92.
54. Hsieh FH, Lam BK, Penrose JF, Austen KF, Boyce JA. T helper cell type 2 cytokines coordinately regulate immunoglobulin E-dependent cysteinyl leukotriene

production by human cord blood-derived mast cells: profound induction of leukotriene C(4) synthase expression by interleukin 4. J Exp Med 2001;193:123-33.
55. Izuhara K, Shirakawa T. Signal transduction via the interleukin-4 receptor and its correlation with atopy. Int J Mol Med 1999;3:3-10.
56. Steinke JW, Negri J, Enelow R, Baramki DF, Borish L. Proinflammatory effects of IL-4 antagonism. J Allergy Clin Immunol 2006;118:756-8.
57. Zurawski G, de Vries JE. Interleukin 13, an interleukin 4-like cytokine that acts on monocytes and B cells, but not on T cells. Immunol Today 1994;15: 19-26.
58. Donaldson DD, Whitters MJ, Fitz LJ, Neben TY, Finnerty H, Henderson SL, et al. The murine IL-13 receptor alpha 2: molecular cloning, characterization, and comparison with murine IL-13 receptor alpha 1. J Immunol 1998;161:2317-24.
59. Zhu Z, Homer RJ, Wang Z, Chen Q, Geba GP, Wang J, et al. Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. J Clin Invest 1999; 103:779-88.
60. Clutterbuck EJ, Hirst EM, Sanderson CJ. Human interleukin-5 (IL-5) regulates the production of eosinophils in human bone marrow cultures: comparison and interaction with IL-1, IL-3, IL-6, and GMCSF. Blood 1989;73:1504-12.
61. Mould AW, Ramsay AJ, Matthaei KI, Young IG, Rothenberg ME, Foster PS. The effect of IL-5 and eotaxin expression in the lung on eosinophil trafficking and degranulation and the induction of bronchial hyperreactivity. J Immunol 2000;164: 2142-50.
62. Rothenberg ME, Petersen J, Stevens RL, Silberstein DS, McKenzie DT, Austen KF, et al. IL-5-dependent conversion of normodense human eosinophils to the hypodense phenotype uses 3T3 fibroblasts for enhanced viability, accelerated hypodensity, and sustained antibody-dependent cytotoxicity. J Immunol 1989;143: 2311-6.
63. Haldar P, Brightling CE, Hargadon B, Gupta S, Monteiro W, Sousa A, et al. Mepolizumab and exacerbations of refractory eosinophilic asthma. N Engl J Med 2009;360:973-84.
64. Nair P, Pizzichini MM, Kjarsgaard M, Inman MD, Efthimiadis A, Pizzichini E, et al. Mepolizumab for prednisone-dependent asthma with sputum eosinophilia. N Engl J Med 2009;360:985-93.
65. Kitamura T, Sato N, Arai K, Miyajima A. Expression cloning of the human IL-3 receptor cDNA reveals a shared beta subunit for the human IL-3 and GM-CSF receptors. Cell 1991;66:1165-74.
66. Rothenberg ME, Owen WF Jr, Silberstein DS, Woods J, Soberman RJ, Austen KF, et al. Human eosinophils have prolonged survival, enhanced functional properties, and become hypodense when exposed to human interleukin 3. J Clin Invest 1988;81:1986-92.
67. Owen WF Jr, Rothenberg ME, Silberstein DS, Gasson JC, Stevens RL, Austen KF, et al. Regulation of human eosinophil viability, density, and function by granulocyte/macrophage colony-stimulating factor in the presence of 3T3 fibroblasts. J Exp Med 1987;166:129-41.
68. Ochs M, Knudsen L, Allen L, Stumbaugh A, Levitt S, Nyengaard JR, et al. GM-CSF mediates alveolar epithelial type II cell changes, but not emphysema-like pathology, in SP-D-deficient mice. Am J Physiol Lung Cell Mol Physiol 2004;287: L1333-41.
69. Guth AM, Janssen WJ, Bosio CM, Crouch EC, Henson PM, Dow SW. Lung environment determines unique phenotype of alveolar macrophages. Am J Physiol Lung Cell Mol Physiol 2009;296:L936-46.
70. Anderson DM, Lyman SD, Baird A, Wignall JM, Eisenman J, Rauch C, et al. Molecular cloning of mast cell growth factor, a hematopoietin that is active in both membrane bound and soluble forms. Cell 1990;63:235-43.
71. Matsuda H, Kannan Y, Ushio H, Kiso Y, Kanemoto T, Suzuki H, et al. Nerve growth factor induces development of connective tissue-type mast cells in vitro from murine bone marrow cells. J Exp Med 1991;174:7-14.
72. Yanagida M, Fukamachi H, Ohgami K, Kuwaki T, Ishii H, Uzumaki H, et al. Effects of T-helper 2-type cytokines, interleukin-3 (IL-3), IL-4, IL-5, and IL-6 on the survival of cultured human mast cells. Blood 1995;86:3705-14.
73. Tsuji K, Koike K, Komiyama A, Miyajima A, Arai K, Nakahata T. Synergistic action of interleukin-10 (IL-10) with IL-3, IL-4 and stem cell factor on colony formation from murine mast cells in culture. Int J Hematol 1995;61:51-60.
74. Godfraind C, Louahed J, Faulkner H, Vink A, Warnier G, Grencis R, et al. Intraepithelial infiltration by mast cells with both connective tissue-type and mucosal-type characteristics in gut, trachea, and kidneys of IL-9 transgenic mice. J Immunol 1998;160:3989-96.
75. Gebhardt T, Sellge G, Lorentz A, Raab R, Manns MP, Bischoff SC. Cultured human intestinal mast cells express functional IL-3 receptors and respond to IL-3 by enhancing growth and IgE receptor-dependent mediator release. Eur J Immunol 2002;32:2308-16.
76. Hultner L, Druez C, Moeller J, Uyttenhove C, Schmitt E, Rude E, et al. Mast cell growth-enhancing activity (MEA) is structurally related and functionally identical to the novel mouse T cell growth factor P40/TCGFIII (interleukin 9). Eur J Immunol 1990;20:1413-6.
77. Veldhoen M, Uyttenhove C, van Snick J, Helmby H, Westendorf A, Buer J, et al. Transforming growth factor-beta "reprograms" the differentiation of T helper 2 cells and promotes an interleukin 9-producing subset. Nat Immunol 2008;9: 1341-6.
78. Dardalhon V, Awasthi A, Kwon H, Galileos G, Gao W, Sobel RA, et al. IL-4 inhibits TGF-beta-induced Foxp3 + T cells and, together with TGF-beta, generates IL-9 + IL-10 + Foxp3(-) effector T cells. Nat Immunol 2008;9: 1347-55.
79. Fort MM, Cheung J, Yen D, Li J, Zurawski SM, Lo S, et al. IL-25 induces IL-4, IL-5, and IL-13 and Th2-associated pathologies in vivo. Immunity 2001;15: 985-95.
80. Dillon SR, Sprecher C, Hammond A, Bilsborough J, Rosenfeld-Franklin M, Presnell SR, et al. Interleukin 31, a cytokine produced by activated T cells, induces dermatitis in mice. Nat Immunol 2004;5:752-60.
81. Neis MM, Peters B, Dreuw A, Wenzel J, Bieber T, Mauch C, et al. Enhanced expression levels of IL-31 correlate with IL-4 and IL-13 in atopic and allergic contact dermatitis. J Allergy Clin Immunol 2006;118:930-7.
82. Sonkoly E, Muller A, Lauerma AI, Pivarcsi A, Soto H, Kemeny L, et al. IL-31: a new link between T cells and pruritus in atopic skin inflammation. J Allergy Clin Immunol 2006;117:411-7.
83. Sporn MB, Roberts AB. Transforming growth factor-beta: recent progress and new challenges. J Cell Biol 1992;119:1017-21.
84. Chen W, Frank ME, Jin W, Wahl SM. TGF-beta released by apoptotic T cells contributes to an immunosuppressive milieu. Immunity 2001;14:715-25.
85. Sonoda E, Matsumoto R, Hitoshi Y, Ishii T, Sugimoto M, Araki S, et al. Transforming growth factor beta induces IgA production and acts additively with interleukin 5 for IgA production. J Exp Med 1989;170:1415-20.
86. Kay AB, Phipps S, Robinson DS. A role for eosinophils in airway remodelling in asthma. Trends Immunol 2004;25:477-82.
87. Del Prete G, De Carli M, Almerigogna F, Giudizi MG, Biagiotti R, Romagnani S. Human IL-10 is produced by both type 1 helper (Th1) and type 2 helper (Th2) T cell clones and inhibits their antigen-specific proliferation and cytokine production. J Immunol 1993;150:353-60.
88. Ding L, Linsley PS, Huang LY, Germain RN, Shevach EM. IL-10 inhibits macrophage costimulatory activity by selectively inhibiting the up-regulation of B7 expression. J Immunol 1993;151:1224-34.
89. Taylor A, Akdis M, Joss A, Akkoc T, Wenig R, Colonna M, et al. IL-10 inhibits CD28 and ICOS costimulations of T cells via src homology 2 domain-containing protein tyrosine phosphatase 1. J Allergy Clin Immunol 2007;120:76-83.
90. Borish L, Aarons A, Rumbyrt J, Cvietusa P, Negri J, Wenzel S. Interleukin-10 regulation in normal subjects and patients with asthma. J Allergy Clin Immunol 1996;97:1288-96.
91. Conti P, Kempuraj D, Frydas S, Kandere K, Boucher W, Letourneau R, et al. IL-10 subfamily members: IL-19, IL-20, IL-22, IL-24 and IL-26. Immunol Lett 2003;88:171-4.
92. Liao SC, Cheng YC, Wang YC, Wang CW, Yang SM, Yu CK, et al. IL-19 induced Th2 cytokines and was up-regulated in asthma patients. J Immunol 2004;173: 6712-8.
93. Blumberg H, Conklin D, Xu WF, Grossmann A, Brender T, Carollo S, et al. Interleukin 20: discovery, receptor identification, and role in epidermal function. Cell 2001;104:9-19.
94. Wolk K, Kunz S, Witte E, Friedrich M, Asadullah K, Sabat R. IL-22 increases the innate immunity of tissues. Immunity 2004;21:241-54.
95. Wolk K, Witte E, Hoffmann U, Doecke WD, Endesfelder S, Asadullah K, et al. IL-22 induces lipopolysaccharide-binding protein in hepatocytes: a potential systemic role of IL-22 in Crohn's disease. J Immunol 2007;178:5973-81.
96. Ikeuchi H, Kuroiwa T, Hiramatsu N, Kaneko Y, Hiromura K, Ueki K, et al. Expression of interleukin-22 in rheumatoid arthritis: potential role as a proinflammatory cytokine. Arthritis Rheum 2005;52:1037-46.
97. Whittington HA, Armstrong L, Uppington KM, Millar AB. Interleukin-22: a potential immunomodulatory molecule in the lung. Am J Respir Cell Mol Biol 2004;31:220-6.
98. Mumm JB, Ekmekcioglu S, Poindexter NJ, Chada S, Grimm EA. Soluble human MDA-7/IL-24: characterization of the molecular form(s) inhibiting tumor growth and stimulating monocytes. J Interferon Cytokine Res 2006;26:877-86.
99. Zheng M, Bocangel D, Doneske B, Mhashilkar A, Ramesh R, Hunt KK, et al. Human interleukin 24 (MDA-7/IL-24) protein kills breast cancer cells via the IL-20 receptor and is antagonized by IL-10. Cancer Immunol Immunother 2007;56: 205-15.
100. Hor S, Pirzer H, Dumoutier L, Bauer F, Wittmann S, Sticht H, et al. The T-cell lymphokine interleukin-26 targets epithelial cells through the interleukin-20 receptor 1 and interleukin-10 receptor 2 chains. J Biol Chem 2004;279:33343-51.

101. Niedbala W, Wei XQ, Cai B, Hueber AJ, Leung BP, McInnes IB, et al. IL-35 is a novel cytokine with therapeutic effects against collagen-induced arthritis through the expansion of regulatory T cells and suppression of Th17 cells. Eur J Immunol 2007;37:3021-9.
102. Collison LW, Workman CJ, Kuo TT, Boyd K, Wang Y, Vignali KM, et al. The inhibitory cytokine IL-35 contributes to regulatory T-cell function. Nature 2007;450:566-9.
103. Mosmann TR, Coffman RL. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu Rev Immunol 1989;7: 145-73.
104. Manetti R, Parronchi P, Giudizi MG, Piccinni MP, Maggi E, Trinchieri G, et al. Natural killer cell stimulatory factor (interleukin 12 [IL-12]) induces T helper type 1 (Th1)-specific immune responses and inhibits the development of IL-4-producing Th cells. J Exp Med 1993;177:1199-204.
105. Villarino AV, Huang E, Hunter CA. Understanding the pro- and anti-inflammatory properties of IL-27. J Immunol 2004;173:715-20.
106. Seder RA, Paul WE, Davis MM. Fazekas de St Groth B. The presence of interleukin 4 during in vitro priming determines the lymphokine-producing potential of CD4 + T cells from T cell receptor transgenic mice. J Exp Med 1992;176:1091-8.
107. Bilenki L, Yang J, Fan Y, Wang S, Yang X. Natural killer T cells contribute to airway eosinophilic inflammation induced by ragweed through enhanced IL-4 and eotaxin production. Eur J Immunol 2004;34:345-54.
108. Akbari O, Faul JL, Hoyte EG, Berry GJ, Wahlstrom J, Kronenberg M, et al. CD4+ invariant T-cell-receptor+ natural killer T cells in bronchial asthma. N Engl J Med 2006;354:1117-29.
109. Gallagher G, Eskdale J, Jordan W, Peat J, Campbell J, Boniotto M, et al. Human interleukin-19 and its receptor: a potential role in the induction of Th2 responses. Int Immunopharmacol 2004;4:615-26.
110. Tamachi T, Maezawa Y, Ikeda K, Kagami S, Hatano M, Seto Y, et al. IL-25 enhances allergic airway inflammation by amplifying a TH2 cell-dependent pathway in mice. J Allergy Clin Immunol 2006;118:606-14.
111. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. Immunity 2005;23:479-90.
112. Coyle AJ, Lloyd C, Tian J, Nguyen T, Erikkson C, Wang L, et al. Crucial role of the interleukin 1 receptor family member T1/ST2 in T helper cell type 2-mediated lung mucosal immune responses. J Exp Med 1999;190:895-902.
113. Xu D, Chan WL, Leung BP, Huang F, Wheeler R, Piedrafita D, et al. Selective expression of a stable cell surface molecule on type 2 but not type 1 helper T cells. J Exp Med 1998;187:787-94.
114. Lohning M, Stroehmann A, Coyle AJ, Grogan JL, Lin S, Gutierrez-Ramos JC, et al. T1/ST2 is preferentially expressed on murine Th2 cells, independent of interleukin 4, interleukin 5, and interleukin 10, and important for Th2 effector function. Proc Natl Acad Sci U S A 1998;95:6930-5.
115. Wang YH, Ito T, Homey B, Watanabe N, Martin R, Barnes CJ, et al. Maintenance and polarization of human TH2 central memory T cells by thymic stromal lymphopoietin-activated dendritic cells. Immunity 2006;24:827-38.
116. Al-Shami A, Spolski R, Kelly J, Keane-Myers A, Leonard WJ. A role for TSLP in the development of inflammation in an asthma model. J Exp Med 2005;202: 829-39.
117. Zhou B, Comeau MR, De Smedt T, Liggitt HD, Dahl ME, Lewis DB, et al. Thymic stromal lymphopoietin as a key initiator of allergic airway inflammation in mice. Nat Immunol 2005;6:1047-53.
118. Yoo J, Omori M, Gyarmati D, Zhou B, Aye T, Brewer A, et al. Spontaneous atopic dermatitis in mice expressing an inducible thymic stromal lymphopoietin transgene specifically in the skin. J Exp Med 2005;202:541-9.
119. Soumelis V, Reche PA, Kanzler H, Yuan W, Edward G, Homey B, et al. Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. Nat Immunol 2002;3:673-80.
120. Dong C. Diversification of T-helper-cell lineages: finding the family root of IL-17-producing cells. Nat Rev Immunol 2006;6:329-33.
121. Iwakura Y, Ishigame H. The IL-23/IL-17 axis in inflammation. J Clin Invest 2006; 116:1218-22.
122. Mangan PR, Harrington LE, O'Quinn DB, Helms WS, Bullard DC, Elson CO, et al. Transforming growth factor-beta induces development of the T(H)17 lineage. Nature 2006;441:231-4.
123. Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature 2006;441:235-8.
124. Holland SM, DeLeo FR, Elloumi HZ, Hsu AP, Uzel G, Brodsky N, et al. STAT3 mutations in the hyper-IgE syndrome. N Engl J Med 2007;357:1608-19.
125. Minegishi Y, Saito M, Tsuchiya S, Tsuge I, Takada H, Hara T, et al. Dominant-negative mutations in the DNA-binding domain of STAT3 cause hyper-IgE syndrome. Nature 2007;448:1058-62.
126. Sakaguchi S. Regulatory T cells: key controllers of immunologic self-tolerance. Cell 2000;101:455-8.
127. Akdis CA, Blesken T, Akdis M, Wuthrich B, Blaser K. Role of interleukin 10 in specific immunotherapy. J Clin Invest 1998;102:98-106.
128. Francis JN, Till SJ, Durham SR. Induction of IL-10 + CD4 + CD25 + T cells by grass pollen immunotherapy. J Allergy Clin Immunol 2003;111:1255-61.
129. Darnell JE Jr, Kerr IM, Stark GR. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science 1994; 264:1415-21.
130. Ihle JN, Witthuhn BA, Quelle FW, Yamamoto K, Thierfelder WE, Kreider B, et al. Signaling by the cytokine receptor superfamily: JAKs and STATs. Trends Biochem Sci 1994;19:222-7.
131. Ghislain JJ, Wong T, Nguyen M, Fish EN. The interferon-inducible Stat2:Stat1 heterodimer preferentially binds in vitro to a consensus element found in the promoters of a subset of interferon-stimulated genes. J Interferon Cytokine Res 2001; 21:379-88.
132. Dumoutier L, Lejeune D, Hor S, Fickenscher H, Renauld JC. Cloning of a new type II cytokine receptor activating signal transducer and activator of transcription (STAT)1, STAT2 and STAT3. Biochem J 2003;370:391-6.
133. Dumoutier L, Tounsi A, Michiels T, Sommereyns C, Kotenko SV, Renauld JC. Role of the interleukin (IL)-28 receptor tyrosine residues for antiviral and antiproliferative activity of IL-29/interferon-lambda 1: similarities with type I interferon signaling. J Biol Chem 2004;279:32269-74.
134. Takeda K, Tanaka T, Shi W, Matsumoto M, Minami M, Kashiwamura S, et al. Essential role of Stat6 in IL-4 signalling. Nature 1996;380:627-30.
135. Losman JA, Chen XP, Hilton D, Rothman P. Cutting edge: SOCS-1 is a potent inhibitor of IL-4 signal transduction. J Immunol 1999;162:3770-4.
136. Moser B, Loetscher P. Lymphocyte traffic control by chemokines. Nat Immunol 2001;2:123-8.
137. Zlotnik A, Yoshie O. Chemokines: a new classification system and their role in immunity. Immunity 2000;12:121-7.
138. Kelner GS, Kennedy J, Bacon KB, Kleyensteuber S, Largaespada DA, Jenkins NA, et al. Lymphotactin: a cytokine that represents a new class of chemokine. Science 1994;266:1395-9.
139. Bazan JF, Bacon KB, Hardiman G, Wang W, Soo K, Rossi D, et al. A new class of membrane-bound chemokine with a CX3C motif. Nature 1997;385:640-4.
140. Charo IF, Myers SJ, Herman A, Franci C, Connolly AJ, Coughlin SR. Molecular cloning and functional expression of two monocyte chemoattractant protein-1 receptors reveals alternative splicing of the carboxy-terminal tails. Proc Natl Acad Sci U S A 1994;91:2752-6.
141. Ponath DP, Qin S, Post TW, Wang J, Wu L, Gerard NP, et al. Molecular cloning and characterization of a human eotaxin receptor expressed selectively on eosinophils. J Exp Med 1996;183:2437-48.
142. Daugherty BL, Siciliano SJ, DeMartino JA, Malkowitz L, Sirotina A, Springer MS. Cloning, expression, and characterization of the human eosinophil eotaxin receptor. J Exp Med 1996;183:2349-54.
143. Isik N, Hereld D, Jin T. Fluorescence resonance energy transfer imaging reveals that chemokine-binding modulates heterodimers of CXCR4 and CCR5 receptors. PLoS ONE 2008;3:e3424.
144. Thelen M. Dancing to the tune of chemokines. Nat Immunol 2001;2:129-34.
145. Ma YC, Huang J, Ali S, Lowry W, Huang XY. Src tyrosine kinase is a novel direct effector of G proteins. Cell 2000;102:635-46.
146. Baggiolini M, Dewald B, Moser B. Human chemokines: an update. Annu Rev Immunol 1997;15:675-705.
147. Li Z, Jiang H, Xie W, Zhang Z, Smrcka A, Wu D. Roles of PLC-b2 and -b3 and PI3Kg in chemoattractant-mediated signal transduction. Science 2000;287:1046-9.
148. Lopez-Ilasaca M, Crespo P, Pellici PG, Gutkind JS, Wetzker R. Linkage of G protein-coupled receptors to the MAPK signaling pathway through PI3-kinase γ. Science 1997;275:394-7.
149. Balakin KV, Ivanenkov YA, Tkachenko SE, Kiselyov AS, Ivachtchenko AV. Regulators of chemokine receptor activity as promising anticancer therapeutics. Curr Cancer Drug Targets 2008;8:299-340.
150. Pruenster M, Muddle L, Bombosi P, Dimitrova S, Zsak M, Middleton J, et al. The Duffy antigen receptor for chemokines transports chemokines and supports their promigratory activity. Nat Immunol 2009;10:101-8.
151. Zarbock A, Ley K. Neutrophil adhesion and activation under flow. Microcirculation 2009;16:31-42.
152. Constantin G, Majeed M, Giagulli C, Piccio L, Kim JY, Butcher EC, et al. Chemokines trigger immediate beta2 integrin affinity and mobility changes: differential regulation and roles in lymphocyte arrest under flow. Immunity 2000;13: 759-69.
153. Luther SA, Cyster JG. Chemokines as regulators of T cell differentiation. Nat Immunol 2001;2:102-7.

154. Mackay CR. Chemokines: immunology's high impact factors. Nat Immunol 2001;2:95-101.
155. Breitfeld D, Ohl L, Kremmer E, Ellwart J, Sallusto F, Lipp M, et al. Follicular B helper T cells express CXC chemokine receptor 5, localize to B cell follicles, and support immunoglobulin production. J Exp Med 2000;192:1545-52.
156. Schaerli P, Willimann K, Lang AB, Lipp M, Loetscher P, Moser B. CXC chemokine receptor 5 expression defines follicular homing T cells with B cell helper function. J Exp Med 2000;192:1553-62.
157. von Vietinghoff S, Ley K. Homeostatic regulation of blood neutrophil counts. J Immunol 2008;181:5183-8.
158. Ying S, Merg Q, Zeibecoglou K, Robinson DS, Macfarlane A, Humbert M, et al. Eosinophil chemotactic chemokines (eotaxin, eotaxin-2, RANTES, monocyte chemoattractant protein-3 (MCP-3), and MCP-4), and C-C chemokine receptor 3 expression in bronchial biopsies from atopic and nonatopic (intrinsic) asthmatics. J Immunol 1999;163:6321-9.
159. Miotto D, Christodoulopoulos P, Olivenstein R, Taha R, Cameron L, Tsicopoulos A, et al. Expression of IFN-gamma-inducible protein; monocyte chemotactic proteins 1, 3, and 4; and eotaxin in TH1- and TH2-mediated lung diseases. J Allergy Clin Immunol 2001;107:664-70.
160. Medoff BD, Sauty A, Tager AM, Maclean JA, Smith RN, Mathew A, et al. IFN-γ-inducible protein 10 (CXCL10) contributes to airway hyperreactivity and airway inflammation in a mouse model of asthma. J Immunol 2002;168:5278-86.
161. Gonzalo JA, Lloyd CM, Peled A, Delaney T, Coyle AJ, Gutierrez-Ramos JC. Critical involvement of the chemotactic axis CXCR4/stromal cell-derived factor-1a in the inflammatory component of allergic airway disease. J Immunol 2000;165:499-508.
162. Gerard C, Rollins BJ. Chemokines and disease. Nat Immunol 2001;2:108-15.
163. Venge J, Lampinen M, Hakansson L, Rak S, Venge P. Identification of IL-5 and RANTES as the major eosinophil chemoattractants in the asthmatic lung. J Allergy Clin Immunol 1996;97:1110-5.
164. Meurer R, Van Riper G, Feeney W, Cunningham P, Hora D, Springer MS, et al. Formation of eosinophilic and monocytic intradermal inflammatory sites in the dog by injection of human RANTES but not human monocyte chemoattractant protein 1, human macrophage inflammatory protein 1 alpha, or human interleukin 8. J Exp Med 1993;178:1913-21.
165. Terada N, Nomura T, Kim WJ, Otsuka Y, Takahashi R, Kishi H, et al. Expression of C-C chemokine TARC in human nasal mucosa and its regulation by cytokines. Clin Exp Allergy 2001;31:1923-31.
166. Hartl D, Latzin P, Zissel G, Krane M, Krauss-Etschmann S, Griese M. Chemokines indicate allergic bronchopulmonary aspergillosis in patients with cystic fibrosis. Am J Respir Crit Care Med 2006;173:1370-6.
167. Oppenheim JJ, Zachariae CO, Mukaida N, Matsushima K. Properties of the novel proinflammatory supergene "intercrine" cytokine family. Annu Rev Immunol 1991;9:617-48.
168. Horuk R. The interleukin-8-receptor family: from chemokines to malaria. Immunol Today 1994;15:169-74.
169. Leonard EJ, Yoshimura T. Neutrophil attractant/activation protein-1 (NAP-1 [interleukin-8]). Am J Respir Crit Care Med 1990;2:479-86.
170. Fulkerson PC, Zimmermann N, Hassman LM, Finkelman FD, Rothenberg ME. Pulmonary chemokine expression is coordinately regulated by STAT1, STAT6, and IFN-gamma. J Immunol 2004;173:7565-74.
171. Reefer AJ, Satinover SM, Solga MD, Lannigan JA, Nguyen JT, Wilson BB, et al. Analysis of CD25hiCD4+ "regulatory" T-cell subtypes in atopic dermatitis reveals a novel T(H)2-like population. J Allergy Clin Immunol 2008;121:415-22, e3.
172. Nguyen KD, Fohner A, Booker JD, Dong C, Krensky AM, Nadeau KC. XCL1 enhances regulatory activities of CD4 + CD25(high) CD127(low/-) T cells in human allergic asthma. J Immunol 2008;181:5386-95.

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

From the Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health.

Supported by the Intramural Research Program of the National Institutes of Health, National Institute of Allergy and Infectious Diseases.

Disclosure of potential conflict of interest: K. D. Stone is an employee of the National Institutes of Health, National Institute of Allergy and Infectious Diseases, Division of Intramural Research. C. Prussin and D. D. Metcalfe are employees of the National Institutes of Health, Department of Health and Human Services.

Received for publication June 9, 2009; revised November 9, 2009; accepted for publication November 11, 2009.

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.

0091-6749/$00.00

Published by Elsevier, Inc on behalf of the American Academy of Allergy, Asthma & Immunology

doi:10.1016/j.jaci.2009.11.017

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.

REFERENCES

1. Smith P, Ownby DR. Clinical significance of IgE. In: Adkinson N, Bochner BS, Busse WW, Holgate ST, Lemanske RF, Simons FER, editors. Middleton's allergy: principles and practice. 7th ed St Louis: Mosby Elsevier; 2009.
2. Vercelli D. Immunobiology of IgE. In: Adkinson N, Bochner BS, Busse WW, Holgate ST, Lemanske RF, Simons FER, editors. Middleton's allergy: principles and practice. 7th ed St Louis: Mosby Elsevier; 2009.
3. Gould HJ, Sutton BJ. IgE in allergy and asthma today. Nat Rev Immunol 2008;8:205-17.
4. MacGlashan D Jr. IgE receptor and signal transduction in mast cells and basophils. Curr Opin Immunol 2008;20:717-23.
5. Gomez G, Jogie-Brahim S, Shima M, Schwartz LB. Omalizumab reverses the phenotypic and functional effects of IgE-enhanced Fc epsilonRI on human skin mast cells. J Immunol 2007;179:1353-61.
6. Rivera J, Fierro NA, Olivera A, Suzuki R. New insights on mast cell activation via the high affinity receptor for IgE. Adv Immunol 2008;98:85-120.
7. Gilfillan AM, Rivera J. The tyrosine kinase network regulating mast cell activation. Immunol Rev 2009;228:149-69.
8. Rosenwasser LJ, Meng J. Anti-CD23. Clin Rev Allergy Immunol 2005;29:61-72.
9. Weskamp G, Ford JW, Sturgill J, Martin S, Docherty AJ, Swendeman S, et al. ADAM10 is a principal "sheddase" of the low-affinity immunoglobulin E receptor CD23. Nat Immunol 2006;7:1293-8.
10. Tantisira KG, Silverman ES, Mariani TJ, Xu J, Richter BG, Klanderman BJ, et al. FCER2: a pharmacogenetic basis for severe exacerbations in children with asthma. J Allergy Clin Immunol 2007;120:1285-91.
11. Poole JA, Meng J, Reff M, Spellman MC, Rosenwasser LJ. Anti-CD23 monoclonal antibody, lumiliximab, inhibited allergen-induced responses in antigen-presenting cells and T cells from atopic subjects. J Allergy Clin Immunol 2005;116:780-8.

12. Hamilton R. Assessment of human allergic diseases. In: Rich R, Fleisher TA, Shearer WT, Schroeder HW, Frew AJ, Weyand CM, editors. Clinical immunology: principles and practice. 3rd ed St Louis: Mosby Elsevier; 2008.
13. Hamilton RG. Accuracy of US Food and Drug Administration-cleared IgE antibody assays in the presence of anti-IgE (omalizumab). J Allergy Clin Immunol 2006;117:759-66.
14. Hamilton RG, Marcotte GV, Saini SS. Immunological methods for quantifying free and total serum IgE levels in allergy patients receiving omalizumab (Xolair) therapy. J Immunol Methods 2005;303:81-91.
15. Wang J, Godbold JH, Sampson HA. Correlation of serum allergy (IgE) tests performed by different assay systems. J Allergy Clin Immunol 2008;121:1219-24.
16. Christensen LH, Holm J, Lund G, Riise E, Lund K. Several distinct properties of the IgE repertoire determine effector cell degranulation in response to allergen challenge. J Allergy Clin Immunol 2008;122:298-304.
17. Pien GC, Orange JS. Evaluation and clinical interpretation of hypergammaglobulinemia E: differentiating atopy from immunodeficiency. Ann Allergy Asthma Immunol 2008;100:392-5.
18. Ozcan E, Notarangelo LD, Geha RS. Primary immune deficiencies with aberrant IgE production. J Allergy Clin Immunol 2008;122:1054-64.
19. Casale TB, Stokes J. Anti-IgE therapy: clinical utility beyond asthma. J Allergy Clin Immunol 2009;123:770-1,e1.
20. Cox L, Platts-Mills TA, Finegold I, Schwartz LB, Simons FE, Wallace DV. American Academy of Allergy, Asthma & Immunology/American College of Allergy, Asthma and Immunology Joint Task Force Report on omalizumab-associated anaphylaxis. J Allergy Clin Immunol 2007;120:1373-7.
21. Hsu F, Boyce JA. Biology of mast cells and their mediators. In: Adkinson N, Bochner BS, Busse WW, Holgate ST, Lemanske RF, Simons FER, editors. Middleton's allergy: principles and practice. 7th ed St Louis: Mosby Elsevier; 2009.
22. Metz M, Brockow K, Metcalfe DD, Galli SJ. Mast cells, basophils, and mastocytosis. In: Rich R, Fleisher TA, Shearer WT, Schroeder HW, Frew AJ, Weyand CM, editors. Clinical immunology: principles and practice. 3rd ed St Louis: Mosby Elsevier; 2008.
23. Zhao W, Schwartz LB. Mast cells. In: Greaves M, Kaplan AP, editors. Urticaria and angioedema. New York: Marcel Dekker; 2009.
24. Metcalfe DD. Mast cells and mastocytosis. Blood 2008;112:946-56.
25. Horny H-P, Metcalfe DD, Bennett JM, Bain BJ, Akin C, Escribano L, editors. WHO classification of tumours of hematopoietic and lymphoid tissues. Lyon: International Agency for Research on Cancer; 2008. p. 54-63.
26. Akin C, Scott LM, Kocabas CN, Kushnir-Sukhov N, Brittain E, Noel P, et al. Demonstration of an aberrant mast-cell population with clonal markers in a subset of patients with "idiopathic" anaphylaxis. Blood 2007;110:2331-3.
27. Bonadonna P, Perbellini O, Passalacqua G, Caruso B, Colarossi S, Dal Fior D, et al. Clonal mast cell disorders in patients with systemic reactions to Hymenoptera stings and increased serum tryptase levels. J Allergy Clin Immunol 2009;123:680-6.
28. Sonneck K, Florian S, Mullauer L, Wimazal F, Fodinger M, Sperr WR, et al. Diagnostic and subdiagnostic accumulation of mast cells in the bone marrow of patients with anaphylaxis: Monoclonal mast cell activation syndrome. Int Arch Allergy Immunol 2007;142:158-64.
29. Karasuyama H, Mukai K, Tsujimura Y, Obata K. Newly discovered roles for basophils: a neglected minority gains new respect. Nat Rev Immunol 2009;9:9-13.
30. Min B. Basophils: what they "can do" versus what they "actually do. Nat Immunol 2008;9:1333-9.
31. Sullivan BM, Locksley RM. Basophils: a nonredundant contributor to host immunity. Immunity 2009;30:12-20.
32. Schroeder J. Biology of basophils. In: Adkinson N, Bochner BS, Busse WW, Holgate ST, Lemanske RF, Simons FER, editors. Middleton's allergy: principles and practice. 7th ed St Louis: Mosby Elsevier; 2009.
33. MacGlashan D. Biochemical events in basophil/mast cell activation and mediator release. In: Adkinson N, Bochner BS, Busse WW, Holgate ST, Lemanske RF, Simons FER, editors. Middleton's allergy: principles and practice. 7th ed St Louis: Mosby Elsevier; 2009.
34. Pecaric-Petkovic T, Didichenko SA, Kaempfer S, Spiegl N, Dahinden CA. Human basophils and eosinophils are the direct target leukocytes of the novel IL-1 family member IL-33. Blood 2009;113:1526-34.
35. Suzukawa M, Iikura M, Koketsu R, Nagase H, Tamura C, Komiya A, et al. An IL-1 cytokine member, IL-33, induces human basophil activation via its ST2 receptor. J Immunol 2008;181:5981-9.
36. Tschopp CM, Spiegl N, Didichenko S, Lutmann W, Julius P, Virchow JC, et al. Granzyme B, a novel mediator of allergic inflammation: its induction and release in blood basophils and human asthma. Blood 2006;108:2290-9.
37. Perrigoue JG, Saenz SA, Siracusa MC, Allenspach EJ, Taylor BC, Giacomin PR, et al. MHC class II-dependent basophil-CD4 + T cell interactions promote T(H)2 cytokine-dependent immunity. Nat Immunol 2009;10:697-705.
38. Sokol CL, Chu NQ, Yu S, Nish SA, Laufer TM, Medzhitov R. Basophils function as antigen-presenting cells for an allergen-induced T helper type 2 response. Nat Immunol 2009;10:713-20.
39. Yoshimoto T, Yasuda K, Tanaka H, Nakahira M, Imai Y, Fujimori Y, et al. Basophils contribute to T(H)2-IgE responses in vivo via IL-4 production and presentation of peptide-MHC class II complexes to CD4 + T cells. Nat Immunol 2009;10:706-12.
40. Blanchard C, Rothenberg ME. Biology of the eosinophil. Adv Immunol 2009;101:81-121.
41. Hogan SP, Rosenberg HF, Moqbel R, Phipps S, Foster PS, Lacy P, et al. Eosinophils: biological properties and role in health and disease. Clin Exp Allergy 2008;38:709-50.
42. Moqbel R, Lacy P, Adamko DJ, Odemuyiwa SO. Biology of eosinophils. In: Adkinson N, Bochner BS, Busse WW, Holgate ST, Lemanske RF, Simons FER, editors. Middleton's allergy: principles and practice. 7th ed St Louis: Mosby Elsevier; 2009.
43. Munitz A, Levi-Schaffer F. Inhibitory receptors on eosinophils: a direct hit to a possible Achilles heel? J Allergy Clin Immunol 2007;119:1382-7.
44. Rosenberg HF, Phipps S, Foster PS. Eosinophil trafficking in allergy and asthma. J Allergy Clin Immunol 2007;119:1303-12.
45. Weller P. Eosinophilia and eosinophil-related disorders. In: Adkinson N, Bochner BS, Busse WW, Holgate ST, Lemanske RF, Simons FER, editors. Middleton's allergy: principles and practice. 7th ed St Louis: Mosby Elsevier; 2009.
46. Wenzel SE. Asthma: defining of the persistent adult phenotypes. Lancet 2006;368:804-13.
47. Haldar P, Brightling CE, Hargadon B, Gupta S, Monteiro W, Sousa A, et al. Mepolizumab and exacerbations of refractory eosinophilic asthma. N Engl J Med 2009;360:973-84.
48. Nair P, Pizzichini MM, Kjarsgaard M, Inman MD, Efthimiadis A, Pizzichini E, et al. Mepolizumab for prednisone-dependent asthma with sputum eosinophilia. N Engl J Med 2009;360:985-93.
49. Gleich GJ, Leiferman KM. The hypereosinophilic syndromes: current concepts and treatments. Br J Haematol 2009;145:271-85.
50. Gotlib J, Cools J. Five years since the discovery of FIP1L1-PDGFRA: what we have learned about the fusion and other molecularly defined eosinophilias. Leukemia 2008;22:1999-2010.
51. Klion AD, Bochner BS, Gleich GJ, Nutman TB, Rothenberg ME, Simon HU, et al. Approaches to the treatment of hypereosinophilic syndromes: a workshop summary report. J Allergy Clin Immunol 2006;117:1292-302.
52. Rothenberg ME, Klion AD, Roufosse FE, Kahn JE, Weller PF, Simon HU, et al. Treatment of patients with the hypereosinophilic syndrome with mepolizumab. N Engl J Med 2008;358:1215-28.

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.

Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

Received for publication July 29, 2009; revised October 12, 2009; accepted for publication October 14, 2009.

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.

0091-6749/$36.00

doi:10.1016/j.jaci.2009.10.071

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.

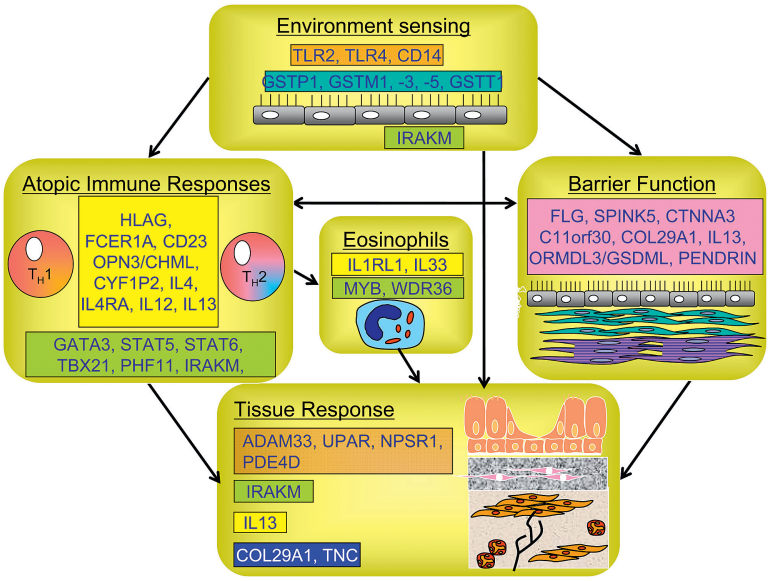

FIG 1. Susceptibility genes for allergic disease. Group 1: sensing the environment. The group of genes encodes molecules that directly modulate the effect of environmental risk factors for allergic disease. For example, genes such as *TLR2, TLR4,* and *CD14,* encoding components of the innate immune 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.

REFERENCES

1. Salter HH. On asthma: its pathology and treatment. London: John Churchil & Sons; 1860.
2. Chavanas S, Bodemer C, Rochat A, Hamel-Teillac D, Ali M, Irvine AD, et al. Mutations in SPINK5, encoding a serine protease inhibitor, cause Netherton syndrome. Nat Genet 2000;25:141-2.
3. Milner JD, Brenchley JM, Laurence A, Freeman AF, Hill BJ, Elias KM, et al. Impaired T(H)17 cell differentiation in subjects with autosomal dominant hyper-IgE syndrome. Nature 2008;452:773-6.
4. Minegishi Y, Saito M, Tsuchiya S, Tsuge I, Takada H, Hara T, et al. Dominant-negative mutations in the DNA-binding domain of STAT3 cause hyper-IgE syndrome. Nature 2007;448:1058-62.
5. Smith FJ, Irvine AD, Terron-Kwiatkowski A, Sandilands A, Campbell LE, Zhao Y, et al. Loss-of-function mutations in the gene encoding filaggrin cause ichthyosis vulgaris. Nat Genet 2006;38:337-42.
6. Skadhauge LR, Christensen K, Kyvik KO, Sigsgaard T. Genetic and environmental influence on asthma: a population-based study of 11,688 Danish twin pairs. Eur Respir J 1999;13:8-14.
7. Hanson B, McGue M, Roitman-Johnson B, Segal NL, Bouchard TJ Jr, Blumenthal MN. Atopic disease and immunoglobulin E in twins reared apart and together. Am J Hum Genet 1991;48:873-9.
8. Lawrence S, Beasley R, Doull I, Begishvili B, Lampe F, Holgate ST, et al. Genetic analysis of atopy and asthma as quantitative traits and ordered polychotomies. Ann Hum Genet 1994;58:359-68.
9. Burney P, Chinn S, Luczynska C, Jarvis D, Vermeire P, Bousquet J, et al. Genes for asthma? An analysis of the European Community Respiratory Health Survey. Am J Respir Crit Care Med 1997;156:1773-80.
10. Vercelli D. Discovering susceptibility genes for asthma and allergy. Nat Rev Immunol 2008;8:169-82.
11. Cookson WO, Sharp PA, Faux JA, Hopkin JM. Linkage between immunoglobulin E responses underlying asthma and rhinitis and chromosome 11q. Lancet 1989;1:1292-5.
12. Ober C, Hoffjan S. Asthma genetics 2006: the long and winding road to gene discovery. Genes Immun 2006;7:95-100.
13. Cardon LR, Bell JI. Association study designs for complex disease. Nat Rev Genet 2001;2:91-9.
14. Holloway JW, Koppelman GH. Identifying novel genes contributing to asthma pathogenesis. Curr Opin Allergy Clin Immunol 2007;7:69-74.
15. Rogers AJ, Raby BA, Lasky-Su JA, Murphy A, Lazarus R, Klanderman BJ, et al. Assessing the reproducibility of asthma candidate gene associations using genome-wide data. Am J Respir Crit Care Med 2009;179:1084-90.
16. Ober C. Susceptibility genes in asthma and allergy. Curr Allergy Asthma Rep 2001;1:174-9.
17. Van Eerdewegh P, Little RD, Dupuis J, Del Mastro RG, Falls K, Simon J, et al. Association of the ADAM33 gene with asthma and bronchial hyperresponsiveness. Nature 2002;418:426-30.
18. Ober C, Tan Z, Sun Y, Possick JD, Pan L, Nicolae R, et al. Effect of variation in CHI3L1 on serum YKL-40 level, risk of asthma, and lung function. N Engl J Med 2008;358:1682-91.
19. Allen M, Heinzmann A, Noguchi E, Abecasis G, Broxholme J, Ponting CP, et al. Positional cloning of a novel gene influencing asthma from chromosome 2q14. Nat Genet 2003;35:258-63.
20. Nicolae D, Cox NJ, Lester LA, Schneider D, Tan Z, Billstrand C, et al. Fine mapping and positional candidate studies identify HLA-G as an asthma susceptibility gene on chromosome 6p21. Am J Hum Genet 2005;76:349-57.
21. Zhang Y, Leaves NI, Anderson GG, Ponting CP, Broxholme J, Holt R, et al. Positional cloning of a quantitative trait locus on chromosome 13q14 that influences immunoglobulin E levels and asthma. Nat Genet 2003;34:181-6.
22. Oguma T, Palmer LJ, Birben E, Sonna LA, Asano K, Lilly CM. Role of prostanoid DP receptor variants in susceptibility to asthma. N Engl J Med 2004;351:1752-63.
23. Barton SJ, Koppelman GH, Vonk JM, Browning CA, Nolte IM, Stewart CE, et al. PLAUR polymorphisms are associated with asthma, PLAUR levels, and lung function decline. J Allergy Clin Immunol 2009;123:1391-400, e17.
24. Koppelman GH, Meyers DA, Howard TD, Zheng SL, Hawkins GA, Ampleford EJ, et al. Identification of PCDH1 as a novel susceptibility gene for bronchial hyperresponsiveness. Am J Respir Crit Care Med 2009;180:929-35.
25. Soderhall C, Marenholz I, Kerscher T, Ruschendorf F, Esparza-Gordillo J, Worm M, et al. Variants in a novel epidermal collagen gene (COL29A1) are associated with atopic dermatitis. PLoS Biol 2007;5:e242.
26. Blakey JD, Sayers I, Ring SM, Strachan DP, Hall IP. Positionally cloned asthma susceptibility gene polymorphisms and disease risk in the British 1958 Birth Cohort. Thorax 2009;64:381-7.
27. Daley D, Lemire M, Akhabir L, Chan-Yeung M, He JQ, McDonald T, et al. Analyses of associations with asthma in four asthma population samples from Canada and Australia. Hum Genet 2009;125:445-59.
28. Hersh CP, Raby BA, Soto-Quiros ME, Murphy AJ, Avila L, Lasky-Su J, et al. Comprehensive testing of positionally cloned asthma genes in two populations. Am J Respir Crit Care Med 2007;176:849-57.
29. Denham S, Koppelman GH, Blakey J, Wjst M, Ferreira MA, Hall IP, et al. Meta-analysis of genome-wide linkage studies of asthma and related traits. Respir Res 2008;9:38.
30. Altshuler D, Brooks LD, Chakravarti A, Collins FS, Daly MJ, Donnelly P. A haplotype map of the human genome. Nature 2005;437:1299-320.
31. Altshuler D, Daly MJ, Lander ES. Genetic mapping in human disease. Science 2008;322:881-8.
32. McCarthy MI, Abecasis GR, Cardon LR, Goldstein DB, Little J, Ioannidis JP, et al. Genome-wide association studies for complex traits: consensus, uncertainty and challenges. Nat Rev Genet 2008;9:356-69.
33. Hindorff LA, Junkins HA, Mehta JP, Manolio TA. A catalog of published genome-wide association studies. Available at: http://www.genome.gov/gwastudies. Accessed October 1, 2009.
34. Moffatt MF, Kabesch M, Liang L, Dixon AL, Strachan D, Heath S, et al. Genetic variants regulating ORMDL3 expression contribute to the risk of childhood asthma. Nature 2007;448:470-3.
35. Bouzigon E, Corda E, Aschard H, Dizier MH, Boland A, Bousquet J, et al. Effect of 17q21 variants and smoking exposure in early-onset asthma. N Engl J Med 2008;359:1985-94.
36. Leung TF, Sy HY, Ng MC, Chan IH, Wong GW, Tang NL, et al. Asthma and atopy are associated with chromosome 17q21 markers in Chinese children. Allergy 2009;64:621-8.
37. Bisgaard H, Bonnelykke K, Sleiman PM, Brasholt M, Chawes B, Kreiner-Moller E, et al. Chromosome 17q21 gene variants are associated with asthma and exacerbations but not atopy in early childhood. Am J Respir Crit Care Med 2009;179:179-85.
38. Wu H, Romieu I, Sienra-Monge JJ, Li H, del Rio-Navarro BE, London SJ. Genetic variation in ORM1-like 3 (ORMDL3) and gasdermin-like (GSDML) and childhood asthma. Allergy 2009;64:629-35.
39. Himes BE, Hunninghake GM, Baurley JW, Rafaels NM, Sleiman P, Strachan DP, et al. Genome-wide association analysis identifies PDE4D as an asthma-susceptibility gene. Am J Hum Genet 2009;84:581-93.
40. Hancock DB, Romieu I, Shi M, Sienra-Monge JJ, Wu H, Chiu GY, et al. Genome-wide association study implicates chromosome 9q21.31 as a susceptibility locus for asthma in Mexican children. PLoS Genet 2009;5:e1000623.
41. Gudbjartsson DF, Bjornsdottir US, Halapi E, Helgadottir A, Sulem P, Jonsdottir GM, et al. Sequence variants affecting eosinophil numbers associate with asthma and myocardial infarction. Nat Genet 2009;41:342-7.
42. Weidinger S, Gieger C, Rodriguez E, Baurecht H, Mempel M, Klopp N, et al. Genome-wide scan on total serum IgE levels identifies FCER1A as novel susceptibility locus. PLoS Genet 2008;4:e1000166.
43. Kim SH, Cho BY, Park CS, Shin ES, Cho EY, Yang EM, et al. Alpha-T-catenin (CTNNA3) gene was identified as a risk variant for toluene diisocyanate-induced asthma by genome-wide association analysis. Clin Exp Allergy 2009;39:203-12.
44. Esparza-Gordillo J, Weidinger S, Folster-Holst R, Bauerfeind A, Ruschendorf F, Patone G, et al. A common variant on chromosome 11q13 is associated with atopic dermatitis. Nat Genet 2009;41:596-601.
45. Holloway JW, Koppelman GH. 17q21 variants and asthma—questions and answers. N Engl J Med 2008;359:2043-5.

46. Galanter J, Choudhry S, Eng C, Nazario S, Rodriguez-Santana JR, Casal J, et al. ORMDL3 gene is associated with asthma in three ethnically diverse populations. Am J Respir Crit Care Med 2008;177:1194-200.
47. Tavendale R, Macgregor DF, Mukhopadhyay S, Palmer CN. A polymorphism controlling ORMDL3 expression is associated with asthma that is poorly controlled by current medications. J Allergy Clin Immunol 2008;121:860-3.
48. Shimizu M, Matsuda A, Yanagisawa K, Hirota T, Akahoshi M, Inomata N, et al. Functional SNPs in the distal promoter of the ST2 gene are associated with atopic dermatitis. Hum Mol Genet 2005;14:2919-27.
49. Reijmerink NE, Postma DS, Bruinenberg M, Nolte IM, Meyers DA, Bleecker ER, et al. Association of IL1RL1, IL18R1, and IL18RAP gene cluster polymorphisms with asthma and atopy. J Allergy Clin Immunol 2008;122:651-4, e8.
50. Choi JH, Nahm DH, Kim SH, Kim YS, Suh CH, Park HS, et al. Increased levels of IgG to cytokeratin 19 in sera of patients with toluene diisocyanate-induced asthma. Ann Allergy Asthma Immunol 2004;93:293-8.
51. Maher B. Personal genomes: the case of the missing heritability. Nature 2008; 456:18-21.
52. Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, Hunter DJ, et al. Finding the missing heritability of complex diseases. Nature 2009;461:747-53.
53. Frazer KA, Murray SS, Schork NJ, Topol EJ. Human genetic variation and its contribution to complex traits. Nat Rev Genet 2009;10:241-51.
54. 1000 genomes: A deep catalog of human genetic variation. Available at: http://www.1000genomes.org/page.php. Accessed October 1, 2009.
55. McCarroll SA, Kuruvilla FG, Korn JM, Cawley S, Nemesh J, Wysoker A, et al. Integrated detection and population-genetic analysis of SNPs and copy number variation. Nat Genet 2008;40:1166-74.
56. Yang IA, Holloway JW. Asthma: advancing gene-environment studies. Clin Exp Allergy 2007;37:1264-6.
57. Smit LA, Siroux V, Bouzigon E, Oryszczyn MP, Lathrop M, Demenais F, et al. CD14 and toll-like receptor gene polymorphisms, country living, and asthma in adults. Am J Respir Crit Care Med 2009;179:363-8.
58. Bieli C, Eder W, Frei R, Braun-Fahrlander C, Klimecki W, Waser M, et al. A polymorphism in CD14 modifies the effect of farm milk consumption on allergic diseases and CD14 gene expression. J Allergy Clin Immunol 2007;120:1308-15.
59. Sharma S, Raby BA, Hunninghake GM, Soto-Quiros M, Avila L, Murphy AJ, et al. Variants in TGFB1, dust mite exposure, and disease severity in children with asthma. Am J Respir Crit Care Med 2009;179:356-62.
60. Hunninghake GM, Soto-Quiros ME, Lasky-Su J, Avila L, Ly NP, Liang C, et al. Dust mite exposure modifies the effect of functional IL10 polymorphisms on allergy and asthma exacerbations. J Allergy Clin Immunol 2008;122:93-8, e1-5.
61. Kim Y, Park CS, Shin HD, Choi JW, Cheong HS, Park BL, et al. A promoter nucleotide variant of the dendritic cell-specific DCNP1 associates with serum IgE levels specific for dust mite allergens among the Korean asthmatics. Genes Immun 2007;8:369-78.
62. Yang IA, Fong KM, Zimmerman PV, Holgate ST, Holloway JW. Genetic susceptibility to the respiratory effects of air pollution. Thorax 2008;63:555-63.
63. Salam MT, Islam T, Gauderman WJ, Gilliland FD. Roles of arginase variants, atopy, and ozone in childhood asthma. J Allergy Clin Immunol 2009;123:596-602, e1-8.
64. Islam T, Berhane K, McConnell R, Gauderman WJ, Avol E, Peters JM, et al. Glutathione-S-transferase (GST) P1, GSTM1, exercise, ozone and asthma incidence in school children. Thorax 2009;64:197-202.
65. Schroer KT, Biagini Myers JM, Ryan PH, Lemasters GK, Bernstein DI, Villareal M, et al. Associations between multiple environmental exposures and glutathione S-transferase P1 on persistent wheezing in a birth cohort. J Pediatr 2008;154: 401-8, e1.
66. Wu H, Romieu I, Sienra-Monge JJ, del Rio-Navarro BE, Anderson DM, Dunn EW, et al. Parental smoking modifies the relation between genetic variation in tumor necrosis factor-alpha (TNF) and childhood asthma. Environ Health Perspect 2007;115:616-22.
67. Davey Smith G, Ebrahim S. What can Mendelian randomisation tell us about modifiable behavioural and environmental exposures? BMJ 2005;330:1076-9.
68. Sheehan NA, Didelez V, Burton PR, Tobin MD. Mendelian randomisation and causal inference in observational epidemiology. PLoS Med 2008;5:e177.
69. Obermayer-Pietsch BM, Bonelli CM, Walter DE, Kuhn RJ, Fahrleitner-Pammer A, Berghold A, et al. Genetic predisposition for adult lactose intolerance and relation to diet, bone density, and bone fractures. J Bone Miner Res 2004;19:42-7.
70. Palmer CN, Irvine AD, Terron-Kwiatkowski A, Zhao Y, Liao H, Lee SP, et al. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. Nat Genet 2006;38:441-6.
71. Yang IA, Savarimuthu S, Kim ST, Holloway JW, Bell SC, Fong KM. Gene-environmental interaction in asthma. Curr Opin Allergy Clin Immunol 2007;7: 75-82.
72. London SJ, Romieu I. Gene by environment interaction in asthma (*). Annu Rev Public Health 2009;30:55-80.
73. Breton CV, Vora H, Salam MT, Islam T, Wenten M, Gauderman WJ, et al. Variation in the GST mu locus and tobacco smoke exposure as determinants of childhood lung function. Am J Respir Crit Care Med 2009;179:601-7.
74. Kabesch M, Schedel M, Carr D, Woitsch B, Fritzsch C, Weiland SK, et al. IL-4/IL-13 pathway genetics strongly influence serum IgE levels and childhood asthma. J Allergy Clin Immunol 2006;117:269-74.
75. Suttner K, Rosenstiel P, Depner M, Schedel M, Pinto LA, Ruether A, et al. TBX21 gene variants increase childhood asthma risk in combination with HLX1 variants. J Allergy Clin Immunol 2009;123:1062-8, e1-8.
76. Pykalainen M, Kinos R, Valkonen S, Rydman P, Kilpelainen M, Laitinen LA, et al. Association analysis of common variants of STAT6, GATA3, and STAT4 to asthma and high serum IgE phenotypes. J Allergy Clin Immunol 2005;115:80-7.
77. Balaci L, Spada MC, Olla N, Sole G, Loddo L, Anedda F, et al. IRAK-M is involved in the pathogenesis of early-onset persistent asthma. Am J Hum Genet 2007;80:1103-14.
78. Martinez FD. CD14, endotoxin, and asthma risk: actions and interactions. Proc Am Thorac Soc 2007;4:221-5.
79. Yang IA, Fong KM, Holgate ST, Holloway JW. The role of Toll-like receptors and related receptors of the innate immune system in asthma. Curr Opin Allergy Clin Immunol 2006;6:23-8.
80. Bierbaum S, Nickel R, Koch A, Lau S, Deichmann KA, Wahn U, et al. Polymorphisms and haplotypes of acid mammalian chitinase are associated with bronchial asthma. Am J Respir Crit Care Med 2005;172:1505-9.
81. Sutherland TE, Maizels RM, Allen JE. Chitinases and chitinase-like proteins: potential therapeutic targets for the treatment of T-helper type 2 allergies. Clin Exp Allergy 2009;39:943-55.
82. Holgate ST, Davies DE, Powell RM, Howarth PH, Haitchi HM, Holloway JW. Local genetic and environmental factors in asthma disease pathogenesis: chronicity and persistence mechanisms. Eur Respir J 2007;29:793-803.
83. Presland RB, Coulombe PA, Eckert RL, Mao-Qiang M, Feingold KR, Elias PM. Barrier function in transgenic mice overexpressing K16, involucrin, and filaggrin in the suprabasal epidermis. J Invest Dermatol 2004;123:603-6.
84. Brown SJ, McLean WH. Eczema genetics: current state of knowledge and future goals. J Invest Dermatol 2009;129:543-52.
85. Sandilands A, Sutherland C, Irvine AD, McLean WH. Filaggrin in the frontline: role in skin barrier function and disease. J Cell Sci 2009;122:1285-94.
86. Baurecht H, Irvine AD, Novak N, Illig T, Buhler B, Ring J, et al. Toward a major risk factor for atopic eczema: meta-analysis of filaggrin polymorphism data. J Allergy Clin Immunol 2007;120:1406-12.
87. Henderson J, Northstone K, Lee SP, Liao H, Zhao Y, Pembrey M, et al. The burden of disease associated with filaggrin mutations: a population-based, longitudinal birth cohort study. J Allergy Clin Immunol 2008;121:872-7, e9.
88. Palmer CNA, Ismail T, Lee SP, Terron-Kwiatkowski A, Zhao Y, Liao H, et al. Filaggrin null mutations are associated with increased asthma severity in children and young adults. J Allergy Clin Immunol 2007;120:64-8.
89. Weidinger S, O'Sullivan M, Illig T, Baurecht H, Depner M, Rodriguez E, et al. Filaggrin mutations, atopic eczema, hay fever, and asthma in children. J Allergy Clin Immunol 2008;121:1203-9, e1.
90. Marenholz I, Nickel R, Ruschendorf F, Schulz F, Esparza-Gordillo J, Kerscher T, et al. Filaggrin loss-of-function mutations predispose to phenotypes involved in the atopic march. J Allergy Clin Immunol 2006;118:866-71.
91. Fallon PG, Sasaki T, Sandilands A, Campbell LE, Saunders SP, Mangan NE, et al. A homozygous frameshift mutation in the mouse Flg gene facilitates enhanced percutaneous allergen priming. Nat Genet 2009;41:602-8.
92. Smith M, Tourigny MR, Noakes P, Thornton CA, Tulic MK, Prescott SL. Children with egg allergy have evidence of reduced neonatal CD4(+)CD25(+)CD127(lo/-) regulatory T cell function. J Allergy Clin Immunol 2008;121:1460-6, e1-7.
93. Dezateux C, Stocks J. Lung development and early origins of childhood respiratory illness. Br Med Bull 1997;53:40-57.
94. Haland G, Carlsen KC, Sandvik L, Devulapalli CS, Munthe-Kaas MC, Pettersen M, et al. Reduced lung function at birth and the risk of asthma at 10 years of age. N Engl J Med 2006;355:1682-9.
95. Palmer LJ, Rye PJ, Gibson NA, Burton PR, Landau LI, Lesouef PN. Airway responsiveness in early infancy predicts asthma, lung function, and respiratory symptoms by school age. Am J Respir Crit Care Med 2001;163:37-42.
96. Lucas JS, Inskip HM, Godfrey KM, Foreman CT, Warner JO, Gregson RK, et al. Small size at birth and greater postnatal weight gain: relationships to diminished infant lung function. Am J Respir Crit Care Med 2004;170:534-40.
97. Lowe L, Murray CS, Custovic A, Simpson BM, Kissen PM, Woodcock A. Specific airway resistance in 3-year-old children: a prospective cohort study. Lancet 2002;359:1904-8.
98. Haitchi HM, Powell RM, Shaw TJ, Howarth PH, Wilson SJ, Wilson DI, et al. ADAM33 expression in asthmatic airways and human embryonic lungs. Am J Respir Crit Care Med 2005;171:958-65.

99. Simpson A, Maniatis N, Jury F, Cakebread JA, Lowe LA, Holgate ST, et al. Polymorphisms in a disintegrin and metalloprotease 33 (ADAM33) predict impaired early-life lung function. Am J Respir Crit Care Med 2005;172:55-60.
100. Henrichsen CN, Chaignat E, Reymond A. Copy number variants, diseases and gene expression. Hum Mol Genet 2009;18:R1-8.
101. McCarthy MI, Hirschhorn JN. Genome-wide association studies: potential next steps on a genetic journey. Hum Mol Genet 2008;17:R156-65.
102. Jirtle RL, Skinner MK. Environmental epigenomics and disease susceptibility. Nat Rev Genet 2007;8:253-62.
103. Benn CS, Jeppesen DL, Hasselbalch H, Olesen AB, Bjorksten B, Lisse I, et al. Thymus size and head circumference at birth and the development of allergic disease. Clin Exp Allergy 2001;31:1862-6.
104. Fergusson DM, Crane J, Beasley R, Horwood LJ. Perinatal factors and atopic disease in childhood. Clin Exp Allergy 2006;27:1394-401.
105. Godfrey KM, Barker DJ, Osmond C. Disproportionate fetal growth and raised IgE concentration in adult life. Clin Exp Allergy 2006;24:641-8.
106. Baccarelli A, Wright RO, Bollati V, Tarantini L, Litonjua AA, Suh HH, et al. Rapid DNA methylation changes after exposure to traffic particles. Am J Respir Crit Care Med 2009;179:572-8.
107. Li Y, Langholz B, Salam MT, Gilliand FD. Maternal and grandmaternal smoking patterns are associated with early childhood asthma. Chest 2005;127:1232-41.
108. Hollingsworth JW, Maruoka S, Boon K, Garantziotis S, Li Z, Tomfohr J, et al. In utero supplementation with methyl donors enhances allergic airway disease in mice. J Clin Invest 2008;118:3462-9.
109. Miller RL, Ho SM. Environmental epigenetics and asthma: current concepts and call for studies. Am J Respir Crit Care Med 2008;177:567-73.
110. Burke W, Fesinmeyer M, Reed K, Hampson L, Carlsten C. Family history as a predictor of asthma risk. Am J Prev Med 2003;24:160-9.
111. Koppelman GH, te Meerman GJ, Postma DS. Genetic testing for asthma. Eur Respir J 2008;32:775-82.
112. Janssens AC, Aulchenko YS, Elefante S, Borsboom GJ, Steyerberg EW, van Duijn CM. Predictive testing for complex diseases using multiple genes: fact or fiction? Genet Med 2006;8:395-400.
113. Lyssenko V, Jonsson A, Almgren P, Pulizzi N, Isomaa B, Tuomi T, et al. Clinical risk factors, DNA variants, and the development of type 2 diabetes. N Engl J Med 2008;359:2220-32.
114. Meigs JB, Shrader P, Sullivan LM, McAteer JB, Fox CS, Dupuis J, et al. Genotype score in addition to common risk factors for prediction of type 2 diabetes. N Engl J Med 2008;359:2208-19.
115. Narayan KM, Weber MB. Clinical risk factors, DNA variants, and the development of type 2 diabetes. N Engl J Med 2009;360:136-1.
116. Holloway JW, Yang IA, Holgate ST. Interpatient variability in rates of asthma progression: can genetics provide an answer? J Allergy Clin Immunol 2008; 121:573-9.
117. Marenholz I, Kerscher T, Bauerfeind A, Esparza-Gordillo J, Nickel R, Keil T, et al. An interaction between filaggrin mutations and early food sensitization improves the prediction of childhood asthma. J Allergy Clin Immunol 2009;123:911-6.
118. Busse WW, Israel E, Nelson HS, Baker JW, Charous BL, Young DY, et al. Daclizumab improves asthma control in patients with moderate to severe persistent asthma: a randomized, controlled trial. Am J Respir Crit Care Med 2008;178:1002-8.
119. Nair P, Pizzichini MM, Kjarsgaard M, Inman MD, Efthimiadis A, Pizzichini E, et al. Mepolizumab for prednisone-dependent asthma with sputum eosinophilia. N Engl J Med 2009;360:985-93.
120. Flood-Page P, Swenson C, Faiferman I, Matthews J, Williams M, Brannick L, et al. A study to evaluate safety and efficacy of mepolizumab in patients with moderate persistent asthma. Am J Respir Crit Care Med 2007;176:1062-71.
121. Wenzel SE, Barnes PJ, Bleecker ER, Bousquet J, Busse W, Dahlen SE, et al. A randomized, double-blind, placebo-controlled study of tumor necrosis factor-alpha blockade in severe persistent asthma. Am J Respir Crit Care Med 2009; 179:549-58.
122. Anderson GP. Endotyping asthma: new insights into key pathogenic mechanisms in a complex, heterogeneous disease. Lancet 2008;372:1107-19.
123. Haldar P, Pavord ID, Shaw DE, Berry MA, Thomas M, Brightling CE, et al. Cluster analysis and clinical asthma phenotypes. Am J Respir Crit Care Med 2008; 178:218-24.
124. Wenzel SE, Busse WW. Severe asthma: lessons from the Severe Asthma Research Program. J Allergy Clin Immunol 2007;119:14-23.
125. Lima JJ, Blake KV, Tantisira KG, Weiss ST. Pharmacogenetics of asthma. Curr Opin Pulm Med 2009;15:57-62.
126. Wechsler ME, Lehman E, Lazarus SC, Lemanske RF Jr, Boushey HA, Deykin A, et al. β-Adrenergic receptor polymorphisms and response to salmeterol. Am J Respir Crit Care Med 2006;173:519-26.
127. Bleecker ER, Postma DS, Lawrance RM, Meyers DA, Ambrose HJ, Goldman M. Effect of ADRB2 polymorphisms on response to longacting beta2-agonist therapy: a pharmacogenetic analysis of two randomised studies. Lancet 2007;370:2118-25.
128. Bleecker ER, Yancey SW, Baitinger LA, Edwards LD, Klotsman M, Anderson WH, et al. Salmeterol response is not affected by beta2-adrenergic receptor genotype in subjects with persistent asthma. J Allergy Clin Immunol 2006;118:809-16.
129. Hawkins GA, Weiss ST, Bleecker ER. Clinical consequences of ADRbeta2 polymorphisms. Pharmacogenomics 2008;9:349-58.
130. Litonjua AA, Lasky-Su J, Schneiter K, Tantisira KG, Lazarus R, Klanderman B, et al. ARG1 is a novel bronchodilator response gene: screening and replication in four asthma cohorts. Am J Respir Crit Care Med 2008;178:688-94.
131. Tantisira KG, Lake S, Silverman ES, Palmer LJ, Lazarus R, Silverman EK, et al. Corticosteroid pharmacogenetics: association of sequence variants in CRHR1 with improved lung function in asthmatics treated with inhaled corticosteroids. Hum Mol Genet 2004;13:1353-9.
132. Dijkstra A, Koppelman GH, Vonk JM, Bruinenberg M, Schouten JP, Postma DS. Pharmacogenomics and outcome of asthma: no clinical application for long-term steroid effects by CRHR1 polymorphisms. J Allergy Clin Immunol 2008;121:1510-3.
133. Hawkins GA, Lazarus R, Smith RS, Tantisira KG, Meyers DA, Peters SP, et al. The glucocorticoid receptor heterocomplex gene STIP1 is associated with improved lung function in asthmatic subjects treated with inhaled corticosteroids. J Allergy Clin Immunol 2009;123:1376-83, e7.
134. Duroudier NP, Tulah AS, Sayers I. Leukotriene pathway genetics and pharmacogenetics in allergy. Allergy 2009;64:823-39.
135. Lima JJ, Zhang S, Grant A, Shao L, Tantisira KG, Allayee H, et al. Influence of leukotriene pathway polymorphisms on response to montelukast in asthma. Am J Respir Crit Care Med 2006;173:379-85.
136. Klotsman M, York TP, Pillai SG, Vargas-Irwin C, Sharma SS, van den Oord EJ, et al. Pharmacogenetics of the 5-lipoxygenase biosynthetic pathway and variable clinical response to montelukast. Pharmacogenet Genomics 2007; 17:189-96.
137. Tantisira KG, Lima J, Sylvia J, Klanderman B, Weiss ST. 5-lipoxygenase pharmacogenetics in asthma: overlap with Cys-leukotriene receptor antagonist loci. Pharmacogenet Genomics 2009;19:244-7.

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.
(J Allergy Clin Immunol 2010;125:S95-102.)

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.
Supported by National Institutes of Health grants 1P01HL70831-01, HL56396, and AI50500.
Disclosure of potential conflict of interest: R. F. Lemanske is a Speaker for Merck, Doembecher Children's Hospital, Washington University, Medicus Group, Park Nicolet Institute, ACAAI, LA Allergy Society, Michigan Allergy/Asthma Society, Medical College of Wisconsin, Fund for Medical Research and Education (Detroit), Children's Hospital of Minnesota, Toronto Allergy Society, AAAAI, Beaumont Hospital, University of Illinois, Canadian Society of Allergy and Clinical Immunology, and New York Presbyterian; is a consultant and speaker for AstraZeneca; is a consultant for Map Pharmaceuticals, Gray Consulting, Smith Research, Inc., Merck Childhood Asthma Network, Novartis, Quintiles/Innovax, RC Horowitz & Co, Inc., International Meetings and Science, and Scienomics; is an author for Up-to-Date; and is an Editor for Elsevier, Inc. W. W. Busse is on the Advisory Board for Altair, Amgen, Abbott Laboratories, Asthmatx, Bristol-Meyer Squibb, Centocor, Genentech, GlaxoSmithKline, Merck, Pfizer, Schering-Plough, and Wyeth; is a speaker for Merck, and a consultant for Alexion, AstraZeneca, Boehringer Ingelheim, Dainippon Sumitomo, Funxional Therapeutics Ltd, Novartis, and TEVA; and has received research support from Novartis, Centocor, GlaxoSmithKline, MedImmune, Ception, and the National Institutes of Health–National Institute of Allergy and Infectious Diseases, and the National Heart, Lung, and Blood Institute.
Received for publication August 26, 2009; revised October 16, 2009; accepted for publication October 20, 2009.
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.
0091-6749/$36.00

doi:10.1016/j.jaci.2009.10.047

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

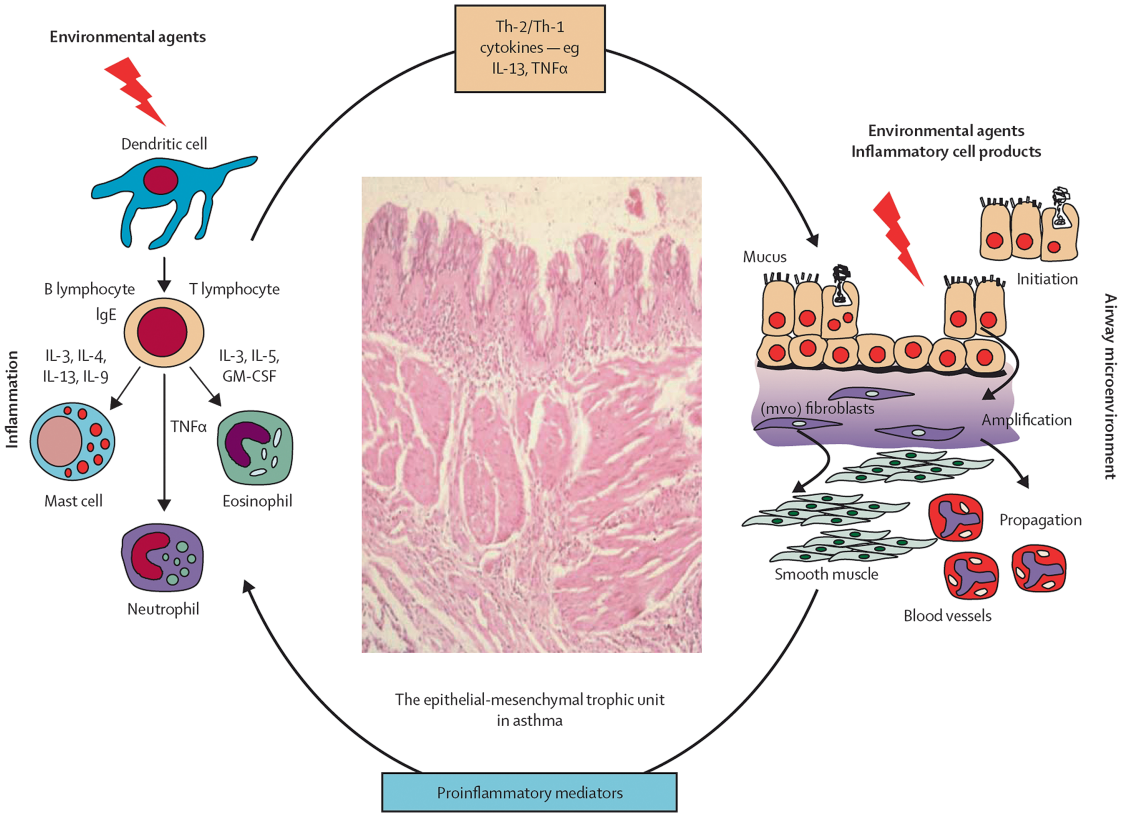

FIG 1. Inflammatory and remodeling responses in asthma with activation of the epithelial mesenchymal trophic unit. The epithelial mesenchymal trophic unit has been defined as bidirectional interaction between the epithelium and underlying mesenchyme involving the release of selective growth factors and 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.

REFERENCES

1. Jackson DJ, Gangnon RE, Evans MD, Roberg KA, Anderson EL, Pappas TE, et al. Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children. Am J Respir Crit Care Med 2008;178:667-72.
2. Kusel MM, de Klerk NH, Kebadze T, Vohma V, Holt PG, Johnston SL, et al. Early-life respiratory viral infections, atopic sensitization, and risk of subsequent development of persistent asthma. J Allergy Clin Immunol 2007;119:1105-10.
3. Bush RK, Peden DB. Advances in environmental and occupational disorders in 2008. J Allergy Clin Immunol 2009;123:575-8.
4. Busse WW, Lemanske RF Jr. Expert panel report 3: moving forward to improve asthma care. J Allergy Clin Immunol 2007;120:1012-4.
5. Rabe KF, Adachi M, Lai CK, Soriano JB, Vermeire PA, Weiss KB, et al. Worldwide severity and control of asthma in children and adults: the global asthma insights and reality surveys. J Allergy Clin Immunol 2004;114:40-7.
6. Stern DA, Morgan WJ, Halonen M, Wright AL, Martinez FD. Wheezing and bronchial hyper-responsiveness in early childhood as predictors of newly diagnosed asthma in early adulthood: a longitudinal birth-cohort study. Lancet 2008;372:1058-64.
7. Sly PD, Boner AL, Bjorksten B, Bush A, Custovic A, Eigenmann PA, et al. Early identification of atopy in the prediction of persistent asthma in children. Lancet 2008;372:1100-6.
8. Spahn JD, Covar R. Clinical assessment of asthma progression in children and adults. J Allergy Clin Immunol 2008;121:548-57.
9. Sears MR, Greene JM, Willan AR, Wiecek EM, Taylor DR, Flannery EM, et al. A longitudinal, population-based, cohort study of childhood asthma followed to adulthood. N Engl J Med 2003;349:1414-22.
10. Wenzel SE, Busse WW. Severe asthma: lessons from the Severe Asthma Research Program. J Allergy Clin Immunol 2007;119:14-21.
11. Taussig LM, Wright AL, Holberg CJ, Halonen M, Morgan WJ, Martinez FD. Tucson children's respiratory study: 1980 to present. J Allergy Clin Immunol 2003;111:661-75.
12. Borrego LM, Stocks J, Leiria-Pinto P, Peralta I, Romeira AM, Neuparth N, et al. Lung function and clinical risk factors for asthma in infants and young children with recurrent wheeze. Thorax 2009;64:203-9.
13. Xuan W, Peat JK, Toelle BG, Marks GB, Berry G, Woolcock AJ. Lung function growth and its relation to airway hyperresponsiveness and recent wheeze. Results from a longitudinal population study. Am J Respir Crit Care Med 2000;161:1820-4.
14. Lange P, Parner J, Vestbo J, Schnohr P, Jensen GA. 15-year follow-up study of ventilatory function in adults with asthma. N Engl J Med 1998;339:1194-200.
15. Covar RA, Spahn JD, Murphy JR, Szefler SJ. Progression of asthma measured by lung function in the childhood asthma management program. Am J Respir Crit Care Med 2004;170:234-41.
16. Krawiec ME, Westcott JY, Chu HW, Balzar S, Trudeau JB, Schwartz LB, et al. Persistent wheezing in very young children is associated with lower respiratory inflammation. Am J Respir Crit Care Med 2001;163:1338-43.
17. Saglani S, Malmstrom K, Pelkonen AS, Malmberg LP, Lindahl H, Kajosaari M, et al. Airway remodeling and inflammation in symptomatic infants with reversible airflow obstruction. Am J Respir Crit Care Med 2005;171:722-7.

18. Barbato A, Turato G, Baraldo S, Bazzan E, Calabrese F, Panizzolo C, et al. Epithelial damage and angiogenesis in the airways of children with asthma. Am J Respir Crit Care Med 2006;174:975-81.
19. Payne DN, Rogers AV, Adelroth E, Bandi V, Guntupalli KK, Bush A, et al. Early thickening of the reticular basement membrane in children with difficult asthma. Am J Respir Crit Care Med 2003;167:78-82.
20. Saglani S, Papaioannou G, Khoo L, Ujita M, Jeffery PK, Owens C, et al. Can HRCT be used as a marker of airway remodelling in children with difficult asthma? Respir Res 2006;7:46.
21. Payne DN, Qiu Y, Zhu J, Peachey L, Scallan M, Bush A, et al. Airway inflammation in children with difficult asthma: relationships with airflow limitation and persistent symptoms. Thorax 2004;59:862-9.
22. Malmberg LP, Pelkonen AS, Haahtela T, Turpeinen M. Exhaled nitric oxide rather than lung function distinguishes preschool children with probable asthma. Thorax 2003;58:494-9.
23. Zeiger RS, Szefler SJ, Phillips BR, Schatz M, Martinez FD, Chinchilli VM, et al. Response profiles to fluticasone and montelukast in mild-to-moderate persistent childhood asthma. J Allergy Clin Immunol 2006;117:45-52.
24. Zacharasiewicz A, Wilson N, Lex C, Erin EM, Li AM, Hansel T, et al. Clinical use of noninvasive measurements of airway inflammation in steroid reduction in children. Am J Respir Crit Care Med 2005;171:1077-82.
25. Szefler SJ, Mitchell H, Sorkness CA, Gergen PJ, O'Connor GT, Morgan WJ, et al. Management of asthma based on exhaled nitric oxide in addition to guideline-based treatment for inner-city adolescents and young adults: a randomised controlled trial. Lancet 2008;372:1065-72.
26. Hunt J. Exhaled breath condensate: an evolving tool for noninvasive evaluation of lung disease. J Allergy Clin Immunol 2002;110:28-34.
27. Corradi M, Folesani G, Andreoli R, Manini P, Bodini A, Piacentini G, et al. Aldehydes and glutathione in exhaled breath condensate of children with asthma exacerbation. Am J Respir Crit Care Med 2003;167:395-9.
28. Zanconato S, Carraro S, Corradi M, Alinovi R, Pasquale MF, Piacentini G, et al. Leukotrienes and 8-isoprostane in exhaled breath condensate of children with stable and unstable asthma. J Allergy Clin Immunol 2004;113:257-63.
29. Moss MH, Gern JE, Lemanske RF Jr. Asthma in infancy and childhood. In: Adkinson NF, Yunginger JW, Busse WW, Bochner BS, Holgate ST, editors. Middleton's allergy: principles and practice. 5th ed. Philadelphia: Mosby; 2003. p. 1225-55.
30. van Schaik SM, Tristram DA, Nagpal IS, Hintz KM, Welliver CII, Welliver RC. Increased production of IFN-γ and cysteinyl leukotrienes in virus-induced wheezing. J Allergy Clin Immunol 1999;103:630-6.
31. Barnes PJ. The cytokine network in asthma and chronic obstructive pulmonary disease. J Clin Invest 2008;118:3546-56.
32. Bousquet J, Jeffery PK, Busse WW, Johnson M, Vignola AM. Asthma. From bronchoconstriction to airways inflammation and remodeling. Am J Respir Crit Care Med 2000;161:1720-45.
33. Holgate ST, Polosa R. The mechanisms, diagnosis, and management of severe asthma in adults. Lancet 2006;368:780-93.
34. Barnes PJ. Immunology of asthma and chronic obstructive pulmonary disease. Nat Rev Immunol 2008;8:183-92.
35. Bradding P, Walls AF, Holgate ST. The role of the mast cell in the pathophysiology of asthma. J Allergy Clin Immunol 2006;117:1277-84.
36. Jacobsen EA, Taranova AG, Lee NA, Lee JJ. Eosinophils: singularly destructive effector cells or purveyors of immunoregulation? J Allergy Clin Immunol 2007;119:1313-20.
37. Holgate ST, Davies DE, Lackie PM, Wilson SJ, Puddicombe SM, Lordan JL. Epithelial-mesenchymal interactions in the pathogenesis of asthma. J Allergy Clin Immunol 2000;105:193-204.
38. Amrani Y, Tliba O, Panettieri RA Jr. Biology of airway smooth muscle cells. In: Adkinson NF Jr, Bochner BS, Busse WW, Holgate ST, Lemanske RF Jr, Simons FER, editors. Middleton's allergy principles & practice. 7th ed Philadelphia: Elsevier; 2009. p. 399-411.
39. Lemanske RF Jr, Jackson DJ, Gangnon RE, Evans MD, Li Z, Shult PA, et al. Rhinovirus illnesses during infancy predict subsequent childhood wheezing. J Allergy Clin Immunol 2005;116:571-7.
40. Arbes SJ Jr, Gergen PJ, Vaughn B, Zeldin DC. Asthma cases attributable to atopy: results from the Third National Health and Nutrition Examination Survey. J Allergy Clin Immunol 2007;120:1139-45.
41. Brauer M, Hoek G, Smit HA, de Jongste JC, Gerritsen J, Postma DS, et al. Air pollution and development of asthma, allergy and infections in a birth cohort. Eur Respir J 2007;29:879-88.
42. Holgate ST. Pathogenesis of asthma. Clin Exp Allergy 2008;38:872-97.
43. Peden DB. The epidemiology and genetics of asthma risk associated with air pollution. J Allergy Clin Immunol 2005;115:213-9.
44. Wang C, Salam MT, Islam T, Wenten M, Gauderman WJ, Gilliland FD. Effects of in utero and childhood tobacco smoke exposure and beta2-adrenergic receptor genotype on childhood asthma and wheezing. Pediatrics 2008;122:e107-14.
45. Bergeron C, Boulet LP, Page N, Laviolette M, Zimmermann N, Rothenberg ME, et al. Influence of cigarette smoke on the arginine pathway in asthmatic airways: increased expression of arginase I. J Allergy Clin Immunol 2007;119:391-7.
46. Lazarus SC, Chinchilli VM, Rollings NJ, Boushey HA, Cherniack R, Craig TJ, et al. Smoking affects response to inhaled corticosteroids or leukotriene receptor antagonists in asthma. Am J Respir Crit Care Med 2007;175:783-90.
47. Dijkstra A, Vonk JM, Jongepier H, Koppelman GH, Schouten JP, ten Hacken NH, et al. Lung function decline in asthma: association with inhaled corticosteroids, smoking and sex. Thorax 2006;61:105-10.
48. Douwes J, Cheng S, Travier N, Cohet C, Niesink A, McKenzie J, et al. Farm exposure in utero may protect against asthma, hay fever and eczema. Eur Respir J 2008;32:603-11.
49. Greer FR, Sicherer SH, Burks AW. Effects of early nutritional interventions on the development of atopic disease in infants and children: the role of maternal dietary restriction, breastfeeding, timing of introduction of complementary foods, and hydrolyzed formulas. Pediatrics 2008;121:183-91.
50. Kusel MM, de Klerk N, Holt PG, Sly PD. Antibiotic use in the first year of life and risk of atopic disease in early childhood. Clin Exp Allergy 2008;38:1921-8.
51. Lowe AJ, Carlin JB, Bennett CM, Hosking CS, Abramson MJ, Hill DJ, et al. Do boys do the atopic march while girls dawdle? J Allergy Clin Immunol 2008;121:1190-5.
52. Shore SA. Obesity and asthma: possible mechanisms. J Allergy Clin Immunol 2008;121:1087-93.
53. Carroll KN, Wu P, Gebretsadik T, Griffin MR, Dupont WD, Mitchel EF, et al. The severity-dependent relationship of infant bronchiolitis on the risk and morbidity of early childhood asthma. J Allergy Clin Immunol 2009;123:1055-61.
54. Krishnan V, Diette GB, Rand CS, Bilderback AL, Merriman B, Hansel NN, et al. Mortality in patients hospitalized for asthma exacerbations in the United States. Am J Respir Crit Care Med 2006;174:633-8.
55. O'Byrne PM, Pedersen S, Lamm CJ, Tan WC, Busse WW. Severe exacerbations and decline in lung function in asthma. Am J Respir Crit Care Med 2009;179:19-24.
56. Bai TR, Vonk JM, Postma DS, Boezen HM. Severe exacerbations predict excess lung function decline in asthma. Eur Respir J 2007;30:452-6.
57. Ober C, Hoffjan S. Asthma genetics 2006: the long and winding road to gene discovery. Genes Immun 2006;7:95-100.
58. Yang IA, Holloway JW. Asthma: advancing gene-environment studies. Clin Exp Allergy 2007;37:1264-6.
59. von Mutius E. Genes and the environment: two readings of their interaction. J Allergy Clin Immunol 2008;122:99-100.
60. Wechsler ME, Israel E. How pharmacogenomics will play a role in the management of asthma. Am J Respir Crit Care Med 2005;172:12-8.
61. Platts-Mills TA. Allergen avoidance in the treatment of asthma: problems with the meta-analyses. J Allergy Clin Immunol 2008;122:694-6.
62. Celedon JC, Milton DK, Ramsey CD, Litonjua AA, Ryan L, Platts-Mills TA, et al. Exposure to dust mite allergen and endotoxin in early life and asthma and atopy in childhood. J Allergy Clin Immunol 2007;120:144-9.
63. Gruchalla RS, Pongracic J, Plaut M, Evans R III, Visness CM, Walter M, et al. Inner City Asthma Study: relationships among sensitivity, allergen exposure, and asthma morbidity. J Allergy Clin Immunol 2005;115:478-85.
64. Bush RK, Prochnau JJ. *Alternaria*-induced asthma. J Allergy Clin Immunol 2004;113:227-34.
65. Bufford JD, Reardon CL, Li Z, Roberg KA, Dasilva D, Eggleston PA, et al. Effects of dog ownership in early childhood on immune development and atopic diseases. Clin Exp Allergy 2008;38:1635-43.
66. Ownby DR, Johnson CC, Peterson EL. Exposure to dogs and cats in the first year of life and risk of allergic sensitization at 6 to 7 years of age. JAMA 2002;288:963-72.
67. Moller C, Dreborg S, Ferdousi HA, Halken S, Host A, Jacobsen L, et al. Pollen immunotherapy reduces the development of asthma in children with seasonal rhinoconjunctivitis (the PAT-study). J Allergy Clin Immunol 2002;109:251-6.
68. Kelly JT, Busse WW. Host immune responses to rhinovirus: mechanisms in asthma. J Allergy Clin Immunol 2008;122:671-82.
69. Wu P, Dupont WD, Griffin MR, Carroll KN, Mitchel EF, Gebretsadik T, et al. Evidence of a causal role of winter virus infection during infancy in early childhood asthma. Am J Respir Crit Care Med 2008;178:1123-9.
70. Newcomb DC, Peebles RS Jr. Bugs and asthma: a different disease? Proc Am Thorac Soc 2009;6:266-71.
71. Johnston NW, Johnston SL, Norman GR, Dai J, Sears MR. The September epidemic of asthma hospitalization: school children as disease vectors. J Allergy Clin Immunol 2006;117:557-62.
72. Green RM, Custovic A, Sanderson G, Hunter J, Johnston SL, Woodcock A. Synergism between allergens and viruses and risk of hospital admission with asthma: case-control study. BMJ 2002;324:763.

73. Rakes GP, Arruda E, Ingram JM, Hoover GE, Zambrano JC, Hayden FG, et al. Rhinovirus and respiratory syncytial virus in wheezing children requiring emergency care. IgE and eosinophil analyses. Am J Respir Crit Care Med 1999; 159:785-90.
74. Liu AH, Leung DY. Renaissance of the hygiene hypothesis. J Allergy Clin Immunol 2006;117:1063-6.
75. von Mutius E. Of attraction and rejection—asthma and the microbial world. N Engl J Med 2007;357:1545-7.
76. Braun-Fahrlander C, Riedler J, Herz U, Eder W, Waser M, Grize L, et al. Environmental exposure to endotoxin and its relation to asthma in school-age children. N Engl J Med 2002;347:869-77.
77. Bisgaard H, Hermansen MN, Buchvald F, Loland L, Halkjaer LB, Bonnelykke K, et al. Childhood asthma after bacterial colonization of the airway in neonates. N Engl J Med 2007;357:1487-95.
78. Prescott SL, Bjorksten B. Probiotics for the prevention or treatment of allergic diseases. J Allergy Clin Immunol 2007;120:255-62.
79. Weiler JM, Bonini S, Coifman R, Craig T, Delgado L, Capao-Filipe M, et al. American Academy of Allergy, Asthma & Immunology Work Group Report: exercise-induced asthma. J Allergy Clin Immunol 2007;119:1349-58.
80. Fitch KD, Sue-Chu M, Anderson SD, Boulet LP, Hancox RJ, McKenzie DC, et al. Asthma and the elite athlete: summary of the International Olympic Committee's consensus conference, Lausanne, Switzerland, January 22-24, 2008. J Allergy Clin Immunol 2008;122:254-60.
81. Watson MA, King CS, Holley AB, Greenburg DL, Mikita JA. Clinical and lung-function variables associated with vocal cord dysfunction. Respir Care 2009;54: 467-73.
82. Hallstrand TS, Moody MW, Aitken ML, Henderson WR Jr. Airway immunopathology of asthma with exercise-induced bronchoconstriction. J Allergy Clin Immunol 2005;116:586-93.
83. Szczeklik A, Stevenson DD. Aspirin-induced asthma: Advances in pathogenesis, diagnosis, and management. J Allergy Clin Immunol 2003;111:913-21.
84. Fischer AR, Rosenberg MA, Lilly CM, Callery JC, Rubin P, Cohn J, et al. Direct evidence for role of the mast cell in the nasal response to aspirin in aspirin-sensitive asthma. J Allergy Clin Immunol 1994;94:1046-56.
85. Lee JY, Simon RA, Stevenson DD. Selection of aspirin dosages for aspirin desensitization treatment in patients with aspirin-exacerbated respiratory disease. J Allergy Clin Immunol 2007;119:157-64.
86. Stevenson DD. Aspirin desensitization in patients with AERD. Clin Rev Allergy Immunol 2003;24:159-68.
87. Coughlan JL, Gibson PG, Henry RL. Medical treatment for reflux oesophagitis does not consistently improve asthma control: a systematic review. Thorax 2001;56:198-204.
88. Kiljander TO, Harding SM, Field SK, Stein MR, Nelson HS, Ekelund J, et al. Effects of esomeprazole 40 mg twice daily on asthma: a randomized placebo-controlled trial. Am J Respir Crit Care Med 2006;173:1091-7.
89. Mastronarde JG, Anthonisen NR, Castro M, Holbrook JT, Leone FT, Teague WG, et al. Efficacy of esomeprazole for treatment of poorly controlled asthma. N Engl J Med 2009;360:1487-99.
90. Wright RJ, Cohen S, Carey V, Weiss ST, Gold DR. Parental stress as a predictor of wheezing in infancy: a prospective birth-cohort study. Am J Respir Crit Care Med 2002;165:358-65.
91. Liu LY, Coe CL, Swenson CA, Kelly EA, Kita H, Busse WW. School examinations enhance airway inflammation to antigen challenge. Am J Respir Crit Care Med 2002;165:1062-7.
92. Chen E, Hanson MD, Paterson LQ, Griffin MJ, Walker HA, Miller GE. Socioeconomic status and inflammatory processes in childhood asthma: the role of psychological stress. J Allergy Clin Immunol 2006;117:1014-20.
93. Hasler G, Gergen PJ, Kleinbaum DG, Ajdacic V, Gamma A, Eich D, et al. Asthma and panic in young adults: a 20-year prospective community study. Am J Respir Crit Care Med 2005;171:1224-30.
94. Holloway JW, Yang IA, Holgate ST. Interpatient variability in rates of asthma progression: can genetics provide an answer? J Allergy Clin Immunol 2008;121:573-9.
95. Martinez FD. Asthma treatment and asthma prevention: a tale of 2 parallel pathways. J Allergy Clin Immunol 2007;119:30-3.
96. Knuffman JE, Sorkness CA, Lemanske RF Jr, Mauger DT, Boehmer SJ, Martinez FD, et al. Phenotypic predictors of long-term response to inhaled corticosteroid and leukotriene modifier therapies in pediatric asthma. J Allergy Clin Immunol 2009;123:411-6.
97. Szefler SJ, Phillips BR, Martinez FD, Chinchilli VM, Lemanske RF Jr, Strunk RC, et al. Characterization of within-subject responses to fluticasone and montelukast in childhood asthma. J Allergy Clin Immunol 2005;115:233-42.
98. Guilbert TW, Morgan WJ, Zeiger RS, Mauger DT, Boehmer SJ, Szefler SJ, et al. Long-term inhaled corticosteroids in preschool children at high risk for asthma. N Engl J Med 2006;354:1985-97.
99. Bisgaard H, Hermansen MN, Loland L, Halkjaer LB, Buchvald F. Intermittent inhaled corticosteroids in infants with episodic wheezing. N Engl J Med 2006;354: 1998-2005.
100. Bacharier LB, Phillips BR, Zeiger RS, Szefler SJ, Martinez FD, Lemanske RF Jr, et al. Episodic use of an inhaled corticosteroid or leukotriene receptor antagonist in preschool children with moderate-to-severe intermittent wheezing. J Allergy Clin Immunol 2008;122:1127-35.
101. Ducharme FM, Lemire C, Noya FJD, Davis GM, Alos N, Leblond H, et al. Preemptive use of high-dose fluticasone for virus-induced wheezing in young children. N Engl J Med 2009;360:339-53.
102. Panickar J, Lakhanpaul M, Lambert PC, Kenia P, Stephenson T, Smyth A, et al. Oral prednisolone for preschool children with acute virus-induced wheezing. N Engl J Med 2009;360:329-38.
103. Bush A. Practice imperfect—treatment for wheezing in preschoolers. N Engl J Med 2009;360:409-10.
104. Jartti T, Lehtinen P, Vanto T, Vuorinen T, Hiekkanen H, Hartiala J, et al. Atopic characteristics of wheezing children and responses to prednisolone. Pediatr Pulmonol 2007;42:1125-33.
105. Jaeschke R, O'Byrne PM, Mejza F, Nair P, Lesniak W, Brozek J, et al. The safety of long-acting beta-agonists among patients with asthma using inhaled corticosteroids: systematic review and metaanalysis. Am J Respir Crit Care Med 2008;178: 1009-16.
106. Martinez FD. Safety of long-acting beta-agonists—an urgent need to clear the air. N Engl J Med 2005;353:2637-9.
107. Kramer JM. Balancing the benefits and risks of inhaled long-acting beta-agonists—the influence of values. N Engl J Med 2009;360:1592-5.
108. Drazen JM, O'Byrne PM. Risks of long-acting beta-agonists in achieving asthma control. N Engl J Med 2009;360:1671-2.

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.

Disclosure of potential conflict of interest: M. S. Dykewicz is an advisor for Alcon, Boehringer Ingelheim, and Ista; is on the speakers' bureau for Merck and Sanofi-Aventis; is on the Board of Directors for the American Board of Allergy and Immunology; is on the Board of Directors and is a member of the Rhinitis Committee for the American Academy of Allergy, Asthma & Immunology; and is Vice Chair and on the Committee on Rhinitis and Sinusitis for the American College of Allergy, Asthma & Immunology. D. L. Hamilos has received research support from Merck and the Flight Attendant Medical Research Institute and has served an as expert witness on the topics of anaphylaxis and occupational asthma.

Received for publication November 2, 2009; revised December 30, 2009; accepted for publication December 30, 2009.

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.

0091-6749/$36.00

doi:10.1016/j.jaci.2009.12.989

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.

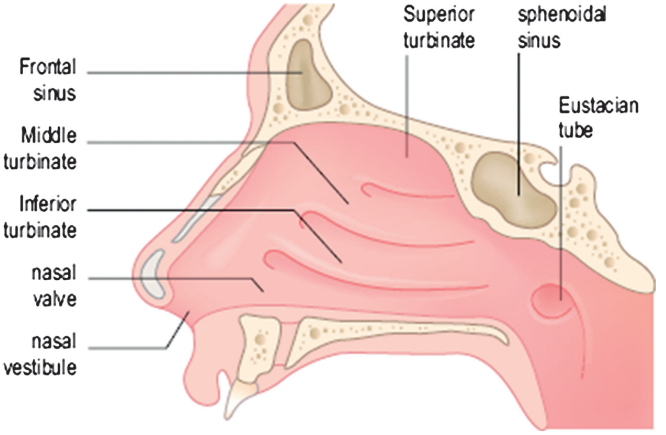

FIG 1. Nasal anatomy. Reprinted with permission from Dykewicz MS. Rhinitis and sinusitis. In: Rich RR, Fleischer TA, Shearer WT, Schroeder HW Jr, Frew AJ, Weyand CM, editors. *Clinical Immunology*. 3rd ed. London: Mosby Elsevier; 2008. p. 626-39.

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

Reprinted with permission from Wallace et al.[1]
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

Reprinted with permission from Wallace et al.[1]

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

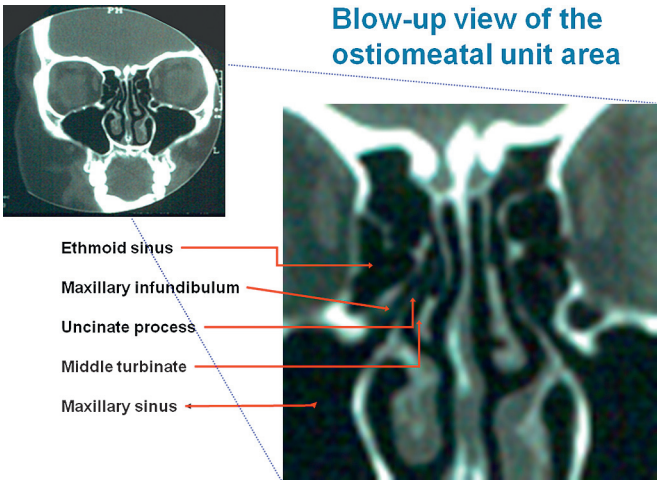

FIG 2. The ostiomeatal unit is well visualized on this blow-up of a normal sinus CT scan. The major structures illustrated include the maxillary infundibulum, the ethmoid infundibulum, the uncinate process, and the middle turbinate. The ostiomeatal unit is a 3-dimensional structure made up of these individual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

REFERENCES

1. Wallace DV, Dykewicz MS, Bernstein DI, Bernstein IL, Blessing-Moore J, Cox L, et al. The Joint Force on Practice Parameters, representing the AAAAI, ACAAI, JCAAI. The diagnosis and management of rhinitis: An updated practice parameter. J Allergy Clin Immunol 2008;122(suppl):S1-84.
2. Bousquet J, Khaltaev N, Cruz AA, Denburg J, Fokkens WJ, Togias A, et al. Allergic Rhinitis and Its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). Allergy 2008;63(suppl 86):8-160.
3. Slavin RG, Spector SL, Bernstein IL, Kaliner MA, Kennedy DW, Virant FS, et al. The diagnosis and management of sinusitis: a practice parameter update. J Allergy Clin Immunol 2005;116(suppl):S13-47.
4. Meltzer EO, Hamilos DL, Hadley JA, Lanza DC, Marple BF, Nicklas RA, et al. Rhinosinusitis: establishing definitions for clinical research and patient care. J Allergy Clin Immunol 2004;114:155-212.
5. Settipane RA, Charnock DR. Epidemiology of rhinitis: allergic and nonallergic. Clin Allergy Immunol 2007;19:23-34.
6. US Census Bureau. Available at: http://www.census.gov/index.html. Accessed October 24, 2009.
7. Rosenwasser L. New insights into the pathophysiology of allergic rhinitis. Allergy Asthma Proc 2007;28:10-5.
8. Hammad H, Lambrecht BN. Dendritic cells and epithelial cells: linking innate and adaptive immunity in asthma. Nat Rev Immunol 2008;8:193-204.
9. Trompette A, Divanovic S, Visintin A, Blanchard C, Hegde RS, Madan R, et al. Allergenicity resulting from functional mimicry of a Toll-like receptor complex protein. Nature 2009;457:585-8.
10. Heppt W, Dinh QT, Cryer A, Zweng M, Noga O, Peiser C, et al. Phenotypic alteration of neuropeptide-containing nerve fibres in seasonal intermittent allergic rhinitis. Clin Exp Allergy 2004;34:1105-10.

11. Wachs M, Proud D, Lichtenstein LM, Kagey-Sobotka A, Norman PS, Naclerio RM. Observations on the pathogenesis of nasal priming. J Allergy Clin Immunol 1989;84:492-501.
12. Bousquet J, Knani J, Hejjaoui A, Ferrando R, Cour P, Dhivert H, et al. Heterogeneity of atopy. I. Clinical and immunologic characteristics of patients allergic to cypress pollen. Allergy 1993;48:183-8.
13. Braunstahl GJ, Kleinjan A, Overbeek SE, Prins JB, Hoogsteden HC, Fokkens WJ. Segmental bronchial provocation induces nasal inflammation in allergic rhinitis patients. Am J Respir Crit Care Med 2000;161:2051-7.
14. Corren J, Adinoff AD, Irvin CG. Changes in bronchial responsiveness following nasal provocation with allergen. J Allergy Clin Immunol 1992;89:611-8.
15. Foresi A, Pelucchi A, Gherson G, Mastropasqua B, Chiapparino A, Testi R. Once daily intranasal fluticasone propionate (200 micrograms) reduces nasal symptoms and inflammation but also attenuates the increase in bronchial responsiveness during the pollen season in allergic rhinitis. J Allergy Clin Immunol 1996;98:274-82.
16. Ellis A, Keith P. Nonallergic rhinitis with eosinophilia syndrome. Curr Allergy Asthma Rep 2006;6:215-20.
17. Schiavino D, Nucera E, Milani A, Della Corte AM, D'Ambrosio C, Pagliari G, et al. Nasal lavage cytometry in the diagnosis of nonallergic rhinitis with eosinophilia syndrome (NARES). Allergy Asthma Proc 1997;18:363-6.
18. Rupp GH, Friedman RA. Eosinophilic nonallergic rhinitis in children. Pediatrics 1982;70:437-9.
19. Moneret-Vautrin DA, Hsieh V, Wayoff M, Guyot JL, Mouton C, Maria Y. Nonallergic rhinitis with eosinophilia syndrome a precursor of the triad: nasal polyposis, intrinsic asthma, and intolerance to aspirin. Ann Allergy 1990;64:513-8.
20. Kramer MF, de la Chaux R, Fintelmann R, Rasp G. NARES: a risk factor for obstructive sleep apnea? Am J Otolaryngol 2004;25:173-7.
21. Ellegard EK. Pregnancy rhinitis. Immunol Allergy Clin North Am 2006;26: 119-35.
22. Knipping S, Holzhausen HJ, Goetze G, Riederer A, Bloching MB. Rhinitis medicamentosa: electron microscopic changes of human nasal mucosa. Otolaryngol Head Neck Surg 2007;136:57-61.
23. Raphael GD, Hauptschein-Raphael M, Kaliner M. Gustatory rhinitis: a syndrome of food-induced rhinorrhea. J Allergy Clin Immunol 1989;83:110-5.
24. Zohar Y, Talmi YP, Strauss M, Finkelstein Y, Shvilli Y. Ozena revisited. J Otolaryngol 1990;19:345-9.
25. Moore EJ, Kern EB. Atrophic rhinitis: a review of 242 cases. Am J Rhinol 2001; 15:355-61.
26. Rowe-Jones JM, Shembekar M, Trendell-Smith N, Mackay IS. Polypoidal rhinosinusitis in cystic fibrosis: a clinical and histopathological study. Clin Otolaryngol 1997;22:167-71.
27. Joint Task Force on Practice Parameters; American Academy of Allergy, Asthma and Immunology; American College of Allergy, Asthma and Immunology; Joint Council of Allergy, Asthma and Immunology. Allergen immunotherapy: a practice parameter second update. J Allergy Clin Immunol 2007;120(suppl): S25-85.
28. Niggemann B, Jacobsen L, Dreborg S, Ferdousi HA, Halken S, Host A, et al. Five-year follow-up on the PAT study: specific immunotherapy and long-term prevention of asthma in children. Allergy 2006;61:855-9.
29. Skoner D, Rachelefsky G, Meltzer E, Chervinsky P, Morris R, Seltzer J, et al. Detection of growth suppression in children during treatment with intranasal belcomethasone dipropionate. Pediatrics 2000;105:E23.
30. Agertoft L, Pedersen S. Short-term lower leg growth rate in children with rhinitis treated with intranasal mometasone furoate and budesonide. J Allergy Clin Immunol 1999;104:948-52.
31. Schenkel E, Skoner D, Bronsky E, Miller S, Pearlman D, Rooklin A, et al. Absence of growth retardation in children with perennial allergic rhinitis following 1 year treatment with mometasone furoate aqueous nasal spray. Pediatrics 2000; 101:E22.
32. Gilbert C, Mazzotta P, Loebstein R, Koren G. Fetal safety of drugs used in the treatment of allergic rhinitis: a critical review. Drug Saf 2005;28:707-19.
33. Schatz M, Zeiger RS, Harden K, Hoffman CC, Chilingar L, Petitti D. The safety of asthma and allergy medications during pregnancy. J Allergy Clin Immunol 1997; 100:301-6.
34. Werler MM, Sheehan JE, Mitchell AA. Maternal medication use and risks of gastroschisis and small intestinal atresia. Am J Epidemiol 2002;155:26-31.
35. Baroody FM. Mucociliary transport in chronic rhinosinusitis. Clin Allergy Immunol 2007;20:103-19.
36. Tieu DD, Kern RC, Schleimer RP. Alterations in epithelial barrier function and host defense responses in chronic rhinosinusitis. J Allergy Clin Immunol 2009; 124:37-42.
37. Stringer SP, Mancuso AA, Avino AJ. Effect of a topical vasoconstrictor on computed tomography of paranasal sinus disease. Laryngoscope 1993;103:6-9.
38. Lanza DC, Kennedy DW. Adult rhinosinusitis defined. Otolaryngol Head Neck Surg 1997;117(suppl):S1-7.
39. Gwaltney JM Jr. Acute community-acquired sinusitis. Clin Infect Dis 1996;23: 1209-23.
40. Rosenfeld RM, Andes D, Bhattacharyya N, Cheung D, Eisenberg S, Ganiats TG, et al. Clinical practice guideline: adult sinusitis. Otolaryngol Head Neck Surg 2007;137(suppl):S1-31.
41. Benninger MS, Ferguson BJ, Hadley JA, Hamilos DL, Jacobs M, Kennedy DW, et al. Adult chronic rhinosinusitis: definitions, diagnosis, epidemiology, and pathophysiology. Otolaryngol Head Neck Surg 2003;129(suppl):S1-32.
42. Bhattacharyya N. The economic burden and symptom manifestations of chronic rhinosinusitis. Am J Rhinol 2003;17:27.
43. Bhattacharyya T, Piccirillo J. Wippold FJ 2nd. Relationship between patient-based descriptions of sinusitis and paranasal sinus computed tomographic findings. Arch Otolaryngol Head Neck Surg 1997;123:1189.
44. Hopkins C, Browne JP, Slack R, Lund V, Brown P. The Lund-Mackay staging system for chronic rhinosinusitis: How is it used and what does it predict? Otolaryngol Head Neck Surg 2007;137:555-61.
45. Jones NS. Midfacial segment pain: implications for rhinitis and sinusitis. Curr Allergy Asthma Rep 2004;4:187.
46. Rosbe KW, Jones KR. Usefulness of patient symptoms and nasal endoscopy in the diagnosis of chronic sinusitis. Am J Rhinol 1998;12:167.
47. Kim JH. Available at: http://www.bcm.edu/oto/grand/121792.html. Accessed October 26, 2009.
48. Malekzadeh S, Hamburger MD, Whelan PJ, Biedlingmaier JF, Baraniuk JN. Density of middle turbinate subepithelial mucous glands in patients with chronic rhinosinusitis. Otolaryngol Head Neck Surg 2002;127:190-5.
49. Watelet JB, Claeys C, Perez-Novo C, Gevaert P, Van Cauwenberge P, Bachert C. Transforming growth factor beta1 in nasal remodeling: differences between chronic rhinosinusitis and nasal polyposis. Am J Rhinol 2004;18:267-72.
50. Hamilos DL. Chronic rhinosinusitis patterns of illness. Clin Allergy Immunol 2007;20:1.
51. Drake-Lee AB, McLaughlan P. Clinical symptoms, free histamine and IgE in patients with nasal polyposis. Int Arch Allergy Appl Immunol 1982;69:268.
52. Hamilos DL. Nasal polyps as immunoreactive tissue. Allergy Asthma Proc 1996; 17:293.
53. Schubert MS, Goetz DW. Evaluation and treatment of allergic fungal sinusitis. I. Demographics and diagnosis. J Allergy Clin Immunol 1998;102:387.
54. Marple BF, Mabry RL. Comprehensive management of allergic fungal sinusitis. Am J Rhinol 1998;12:263-8.
55. Corey JP. Allergic fungal sinusitis. Otolaryngol Clin North Am 1992;25:225-30.
56. Ponikau JU, Sherris DA, Kern EB, Homburger HA, Frigas E, Gaffey TA, et al. The diagnosis and incidence of allergic fungal sinusitis. Mayo Clin Proc 1999; 74:877.
57. Shin SH, Ponikau JU, Sherris DA, Congdon D, Frigas E, Homburger HA, et al. Chronic rhinosinusitis: an enhanced immune response to ubiquitous airborne fungi. J Allergy Clin Immunol 2004;114:1369.
58. Ponikau JU, Sherris DA, Kephart GM, Adolphson C, Kita H. The role of ubiquitous airborne fungi in chronic rhinosinusitis. Curr Allergy Asthma Rep 2005;5: 472-6.
59. Van Zele T, Gevaert P, Watelet JB, Claeys G, Haoltappels G, Claeys C, et al. *Staphylococcus aureus* colonization and IgE antibody formation to enterotoxins is increased in nasal polyposis. J Allergy Clin Immunol 2004;114: 981-3.
60. Bernstein JM, Kansal R. Superantigen hypothesis for the early development of chronic hyperplastic sinusitis with massive nasal polyposis. Curr Opin Otolaryngol Head Neck Surg 2005;13:39.
61. Ramadan HH. Chronic rhinosinusitis and bacterial biofilms. Curr Opin Otolaryngol Head Neck Surg 2006;14:183.
62. Harvey RJ, Lund VJ. Biofilms and chronic rhinosinusitis: systematic review of evidence, current concepts and directions for research. Rhinology 2007;45:3.
63. Lee JT, Kennedy DW, Palmer JN, Feldman M, Chiu AG. The incidence of concurrent osteitis in patients with chronic rhinosinusitis: a clinicopathological study. Am J Rhinol 2006;20:278-82.
64. Hickner JM, Bartlett JG, Besser RE, Gonzales R, Hoffman JR, Sande MA, et al. Principles of appropriate antibiotic use for acute rhinosinusitis in adults: background. Ann Intern Med 2001;134:498-505.
65. Anon J, Jacobs M, Poole M, Ambrose P, Benninger M, Hadley J, et al. Antimicrobial treatment guidelines for acute bacterial rhinosinusitis. Otolaryngol Head Neck Surg 2004;130:1-45.
66. Fokkens W, Lund V, Bachert C, Clement P, Hellings P, Holmstrom W, et al. EAACI position paper on rhinosinusitis and nasal polyps executive summary. Allergy 2005;60:583-601.

67. Kennedy DW, Kuhn FA, Hamilos DL, Zinreich SJ, Butler D, Warsi G, et al. Treatment of chronic rhinosinusitis with high-dose oral terbinafine: a double blind, placebo-controlled study. Laryngoscope 2005;115:1793-9.
68. Ponikau JU, Sherris DA, Weaver A, Kita H. Treatment of chronic rhinosinusitis with intranasal amphotericin B: a randomized, placebo-controlled, double-blind pilot trial. J Allergy Clin Immunol 2005;115:125.
69. Ebbens FA, Scadding GK, Badia L, Hellings PW, Jorissen M, Mullol J, et al. Amphotericin B nasal lavages: not a solution for patients with chronic rhinosinusitis. J Allergy Clin Immunol 2006;118:1149.
70. Benitez P, Alobid I, de Haro J, Brenguer J, Bernal-Spreckelsen M, Pujols L, et al. A short course of oral prednisone followed by intranasal budesonide is an effective treatment of severe nasal polyps. Laryngoscope 2006;116:770-5.
71. Small CB, Hernandez J, Reyes A, Schenkel E, Damiano A, Staudinger H, et al. Efficacy and safety of mometasone furoate nasal spray in nasal polyposis. J Allergy Clin Immunol 2005;116:1275-81.
72. Stammberger H, Posawetz W. Functional endoscopic sinus surgery. Concept, indications and results of the Messerklinger technique. Eur Arch Otorhinolaryngol 1990;247:63-76.
73. Chambers DW, Davis WE, Cook PR, Nishioka GJ, Rudman DT. Long-term outcome analysis of functional endoscopic sinus surgery: correlation of symptoms with endoscopic examination findings and potential prognostic variables. Laryngoscope 1997;107:504-10.
74. Senior BA, Kennedy DW, Tanabodee J, Kroger H, Hassab M, Lanza D. Long-term results of functional endoscopic sinus surgery. Laryngoscope 1998;108:151-7.
75. Jakobsen J, Svendstrup F. Functional endoscopic sinus surgery in chronic sinusitis—a series of 237 consecutively operated patients. Acta Otolaryngol Suppl 2000;543:158-61.
76. Berrettini S, Carabelli A, Sellari-Franceschini S, Bruschini L, Abruzesse A, Quartieri F, et al. Perennial allergic rhinitis and chronic sinusitis: correlation with rhinologic risk factors. Allergy 1999;54:242-8.
77. Hamilos DL, Leung DY, Wood R, Cunningham L, Bean DK, Yasruel Z, et al. Evidence for distinct cytokine expression in allergic versus nonallergic chronic sinusitis. J Allergy Clin Immunol 1995;96:537-44.
78. al Ghamdi K, Ghaffar O, Small P, Frenkiel S, Hamid Q. IL-4 and IL-13 expression in chronic sinusitis: relationship with cellular infiltrate and effect of topical corticosteroid treatment. J Otolaryngol 1997;26:160-6.
79. Psaltis AJ, Bruhn MA, Ooi EH, Tan LW, Wormald PJ. Nasal mucosa expression of lactoferrin in patients with chronic rhinosinusitis. Laryngoscope 2007;117:2030-5.
80. Barbero GJ. Gastroesophageal reflux and upper airway disease. Otolaryngol Clin North Am 1996;29:27-38.
81. Schreiber S, Garten D, Sudhoff H. Pathophysiological mechanisms of extraesophageal reflux in otolaryngeal disorders. Eur Arch Otorhinolaryngol 2009;266:17-24.
82. Jang YJ, Kwon HJ, Park HW, Lee BJ. Detection of rhinovirus in turbinate epithelial cells of chronic sinusitis. Am J Rhinol 2006;20:634.
83. Lohrmann C, Uhl M, Warnatz K, Kotter E, Ghanem N, Langer M. Sinonasal computed tomography in patients with Wegener's granulomatosis. J Comput Assist Tomogr 2006;30:122-5.
84. Alobid I, Guilemany JM, Mullol J. Nasal manifestations of systemic illnesses. Curr Allergy Asthma Rep 2004;4:208.
85. Kay DJ, Har-El G. The role of endoscopic sinus surgery in chronic sinonasal sarcoidosis. Am J Rhinol 2001;15:249.
86. Lusk RP, McAlister B, el Fouley A. Anatomic variation in pediatric chronic sinusitis: a CT study. Otolaryngol Clin North Am 1996;29:75.
87. Bolger WE, Butzin CA, Parsons DS. Paranasal sinus bony anatomic variations and mucosal abnormalities: CT analysis for endoscopic sinus surgery. Laryngoscope 1991;101:56.
88. Jones NS, Strobl A, Holland I. A study of the CT findings in 100 patients with rhinosinusitis and 100 controls. Clin Otolaryngol Allied Sci 1997;22:47.
89. Danese M, Duvoisin B, Agrifoglio A, Cherpillod J, Krayenbuhl M. Influence of naso-sinusal anatomic variants on recurrent, persistent or chronic sinusitis. X-ray computed tomographic evaluation in 112 patients. J Radiol 1997;78:651-7.
90. Jani AL, Hamilos DL. Current thinking on the relationship between rhinosinusitis and asthma. J Asthma 2005;42:1.
91. Samter M, Beers RF Jr. Intolerance to aspirin. Clinical studies and consideration of its pathogenesis. Ann Intern Med 1968;68:975-83.
92. Berges-Gimeno MP, Simon RA, Stevenson DD. The natural history and clinical characteristics of aspirin-exacerbated respiratory disease. Ann Allergy Asthma Immunol 2002;89:474-8.

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.

Disclosure of potential conflict of interest: S. H. Sicherer is a consultant for the Food Allergy Initiative and a medical advisor for the Food Allergy & Anaphylaxis Network and has received research support from the National Institutes of Health/National Institute of Allergy and Infectious Diseases. H. A. Sampson is a consultant for and holds shares in Allertein Pharmaceuticals, LLC; is a consultant and scientific advisor for the Food Allergy Initiative; and has received research support from the Food Allergy Initiative and the National Institutes of Health/National Institute of Allergy and Infectious Diseases.

Received for publication July 20, 2009; revised August 18, 2009; accepted for publication August 21, 2009.

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.

0091-6749/$36.00

doi:10.1016/j.jaci.2009.08.028

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.

REFERENCES

1. Johansson SG, Bieber T, Dahl R, Friedmann PS, Lanier BQ, Lockey RF, et al. Revised nomenclature for allergy for global use: report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. J Allergy Clin Immunol 2004;113:832-6.
2. Rona RJ, Keil T, Summers C, Gislason D, Zuidmeer L, Sodergren E, et al. The prevalence of food allergy: a meta-analysis. J Allergy Clin Immunol 2007;120:638-46.
3. Zuidmeer L, Goldhahn K, Rona RJ, Gislason D, Madsen C, Summers C, et al. The prevalence of plant food allergies: a systematic review. J Allergy Clin Immunol 2008;121:1210-8.
4. Food allergy. a practice parameter. Ann Allergy Asthma Immunol 2006;96(suppl 2):S1-68.
5. Sicherer SH, Sampson HA. Peanut allergy: emerging concepts and approaches for an apparent epidemic. J Allergy Clin Immunol 2007;120:491-503.
6. Branum AM, Lukacs SL. Food allergy among U.S. children: trends in prevalence and hospitalizations. NCHS Data Brief 2008;10:1-8.
7. Ross MP, Ferguson M, Street D, Klontz K, Schroeder T, Luccioli S. Analysis of food-allergic and anaphylactic events in the National Electronic Injury Surveillance System. J Allergy Clin Immunol 2008;121:166-71.
8. Decker WW, Campbell RL, Manivannan V, Luke A, St Sauver JL, Weaver A, et al. The etiology and incidence of anaphylaxis in Rochester, Minnesota: a report from the Rochester Epidemiology Project. J Allergy Clin Immunol 2008;122:1161-5.
9. Bock SA, Munoz-Furlong A, Sampson HA. Further fatalities caused by anaphylactic reactions to food, 2001-2006. J Allergy Clin Immunol 2007;119:1016-8.
10. Sampson HA. Update on food allergy. J Allergy Clin Immunol 2004;113:805-19.
11. Savage JH, Matsui EC, Skripak JM, Wood RA. The natural history of egg allergy. J Allergy Clin Immunol 2007;120:1413-7.
12. Skripak JM, Matsui EC, Mudd K, Wood RA. The natural history of IgE-mediated cow's milk allergy. J Allergy Clin Immunol 2007;120:1172-7.
13. Lack G. Epidemiologic risks for food allergy. J Allergy Clin Immunol 2008;121:1331-6.
14. Du TG, Katz Y, Sasieni P, Mesher D, Maleki SJ, Fisher HR, et al. Early consumption of peanuts in infancy is associated with a low prevalence of peanut allergy. J Allergy Clin Immunol 2008;122:984-91.
15. Fox AT, Sasieni P, Du TG, Syed H, Lack G. Household peanut consumption as a risk factor for the development of peanut allergy. J Allergy Clin Immunol 2009;123:417-23.
16. Chehade M, Mayer L. Oral tolerance and its relation to food hypersensitivities. J Allergy Clin Immunol 2005;115:3-12.
17. Untersmayr E, Jensen-Jarolim E. The role of protein digestibility and antacids on food allergy outcomes. J Allergy Clin Immunol 2008;121:1301-8.
18. Groschwitz KR, Hogan SP. Intestinal barrier function: molecular regulation and disease pathogenesis. J Allergy Clin Immunol 2009;124:3-20.
19. Mowat AM. Anatomical basis of tolerance and immunity to intestinal antigens. Nat Rev Immunol 2003;3:331-41.
20. Strobel S, Mowat AM. Oral tolerance and allergic responses to food proteins. Curr Opin Allergy Clin Immunol 2006;6:207-13.
21. Husby S, Jensenius J, Svehag S. Passage of undegraded dietary antigen into the blood of healthy adults. Quantification, estimation of size distribution and relation of uptake to levels of specific antibodies. Scand J Immunol 1985;22:83-92.
22. Brunner M, Walzer M. Absorption of undigested proteins in human beings: the absorption of unaltered fish protein in adults. Arch Intern Med 1928;42:173-9.
23. Walzer M. Allergy of the abdominal organs. J Lab Clin Med 1941;26:1867-77.
24. Sudo N, Sawamura S, Tanaka K, Aiba Y, Kubo C, Koga Y. The requirement of intestinal bacterial flora for the development of an IgE production system fully susceptible to oral tolerance induction. J Immunol 1997;159:1739-45.
25. Bashir ME, Louie S, Shi HN, Nagler-Anderson C. Toll-like receptor 4 signaling by intestinal microbes influences susceptibility to food allergy. J Immunol 2004;172:6978-87.
26. Prescott SL, Bjorksten B. Probiotics for the prevention or treatment of allergic diseases. J Allergy Clin Immunol 2007;120:255-62.
27. Blazquez AB, Berin MC. Gastrointestinal dendritic cells promote Th2 skewing via OX40L. J Immunol 2008;180:4441-50.
28. Yang PC, Xing Z, Berin CM, Soderholm JD, Feng BS, Wu L, et al. TIM-4 expressed by mucosal dendritic cells plays a critical role in food antigen-specific Th2 differentiation and intestinal allergy. Gastroenterology 2007;133:1522-33.
29. Turcanu V, Maleki SJ, Lack G. Characterization of lymphocyte responses to peanuts in normal children, peanut-allergic children, and allergic children who acquired tolerance to peanuts. J Clin Invest 2003;111:1065-72.
30. Thottingal TB, Stefura BP, Simons FE, Bannon GA, Burks W, HayGlass KT. Human subjects without peanut allergy demonstrate T cell-dependent, TH2-biased, peanut-specific cytokine and chemokine responses independent of TH1 expression. J Allergy Clin Immunol 2006;118:905-14.
31. Fernandez-Rivas M, Bolhaar S, Gonzalez-Mancebo E, Asero R, van Leeuwen A, Bohle B, et al. Apple allergy across Europe: how allergen sensitization profiles determine the clinical expression of allergies to plant foods. J Allergy Clin Immunol 2006;118:481-8.
32. Navuluri L, Parvataneni S, Hassan H, Birmingham NP, Kelly C, Gangur V. Allergic and anaphylactic response to sesame seeds in mice: identification of Ses i 3 and basic subunit of 11s globulins as allergens. Int Arch Allergy Immunol 2006;140:270-6.
33. Strid J, Thomson M, Hourihane J, Kimber I, Strobel S. A novel model of sensitization and oral tolerance to peanut protein. Immunology 2004;113:293-303.
34. Benlounes N, Candalh C, Matarazzo P, Dupont C, Heyman M. The time-course of milk antigen-induced TNF-alpha secretion differs according to the clinical symptoms in children with cow's milk allergy. J Allergy Clin Immunol 1999;104:863-9.
35. Chung HL, Hwang JB, Park JJ, Kim SG. Expression of transforming growth factor beta1, transforming growth factor type I and II receptors, and TNF-alpha in the mucosa of the small intestine in infants with food protein-induced enterocolitis syndrome. J Allergy Clin Immunol 2002;109:150-4.
36. Husby S. Normal immune responses to ingested foods. J Pediatr Gastroenterol Nutr 2000;30(suppl):S13-9.
37. Stapel SO, Asero R, Ballmer-Weber BK, Knol EF, Strobel S, Vieths S, et al. Testing for IgG4 against foods is not recommended as a diagnostic tool: EAACI Task Force Report. Allergy 2008;63:793-6.
38. Sicherer SH, Sampson HA. 9. Food allergy. J Allergy Clin Immunol 2006;117(2 Suppl Mini-Primer):S470-5.
39. Radauer C, Breiteneder H. Evolutionary biology of plant food allergens. J Allergy Clin Immunol 2007;120:518-25.
40. Jenkins JA, Breiteneder H, Mills EN. Evolutionary distance from human homologs reflects allergenicity of animal food proteins. J Allergy Clin Immunol 2007;120:1399-405.
41. Maleki SJ, Viquez O, Jacks T, Dodo H, Champagne ET, Chung SY, et al. The major peanut allergen, Ara h 2, functions as a trypsin inhibitor, and roasting enhances this function. J Allergy Clin Immunol 2003;112:190-5.
42. Beyer K, Morrow E, Li XM, Bardina L, Bannon GA, Burks AW, et al. Effects of cooking methods on peanut allergenicity. J Allergy Clin Immunol 2001;107:1077-81.
43. Nowak-Wegrzyn A, Bloom KA, Sicherer SH, Shreffler WG, Noone S, Wanich N, et al. Tolerance to extensively heated milk in children with cow's milk allergy. J Allergy Clin Immunol 2008;122:342-7.
44. Lemon-Mule H, Sampson HA, Sicherer SH, Shreffler WG, Noone S, Nowak-Wegrzyn A. Immunologic changes in children with egg allergy ingesting extensively heated egg. J Allergy Clin Immunol 2008;122:977-83.
45. Shreffler WG, Castro RR, Kucuk ZY, Charlop-Powers Z, Grishina G, Yoo S, et al. The major glycoprotein allergen from *Arachis hypogaea*, Ara h 1, is a ligand of dendritic cell-specific ICAM-grabbing nonintegrin and acts as a Th2 adjuvant in vitro. J Immunol 2006;177:3677-85.
46. Meyer S, van Liempt E, Imberty A, van Kooyk Y, Geyer H, Geyer R, et al. DC-SIGN mediates binding of dendritic cells to authentic pseudo-LewisY glycolipids

of *Schistosoma mansoni* cercariae, the first parasite-specific ligand of DC-SIGN. J Biol Chem 2005;280:37349-59.
47. Commins SP, Satinover SM, Hosen J, Mozena J, Borish L, Lewis BD, et al. Delayed anaphylaxis, angioedema, or urticaria after consumption of red meat in patients with IgE antibodies specific for galactose-alpha-1,3-galactose. J Allergy Clin Immunol 2009;123:426-33.
48. Sicherer SH, Sampson HA. Food allergy: recent advances in pathophysiology and treatment. Annu Rev Med 2009;60:261-77.
49. Sampson HA, Sicherer SH, Birnbaum AH. AGA. Technical review on the evaluation of food allergy in gastrointestinal disorders. Gastroenterology 2001;120: 1026-40.
50. Xanthakos SA, Schwimmer JB, Melin-Aldana H, Rothenberg ME, Witte DP, Cohen MB. Prevalence and outcome of allergic colitis in healthy infants with rectal bleeding: a prospective cohort study. J Pediatr Gastroenterol Nutr 2005; 41:16-22.
51. Arvola T, Ruuska T, Keranen J, Hyoty H, Salminen S, Isolauri E. Rectal bleeding in infancy: clinical, allergological, and microbiological examination. Pediatrics 2006;117:e760-8.
52. Knight AK, Shreffler WG, Sampson HA, Sicherer SH, Noone S, Mofidi S, et al. Skin prick test to egg white provides additional diagnostic utility to serum egg white-specific IgE antibody concentration in children. J Allergy Clin Immunol 2006;117:842-7.
53. Sporik R, Hill DJ, Hosking CS. Specificity of allergen skin testing in predicting positive open food challenges to milk, egg and peanut in children. Clin Exp Allergy 2000;30:1541-6.
54. Hill DJ, Heine RG, Hosking CS. The diagnostic value of skin prick testing in children with food allergy. Pediatr Allergy Immunol 2004;15:435-41.
55. Pucar F, Kagan R, Lim H, Clarke AE. Peanut challenge: a retrospective study of 140 patients. Clin Exp Allergy 2001;31:40-6.
56. Hamilton RG, Franklin AN Jr. In vitro assays for the diagnosis of IgE-mediated disorders. J Allergy Clin Immunol 2004;114:213-25.
57. Sampson HA. Utility of food-specific IgE concentrations in predicting symptomatic food allergy. J Allergy Clin Immunol 2001;107:891-6.
58. Boyano-Martinez T, Garcia-Ara C, Diaz-Pena JM, Martin-Esteban M. Prediction of tolerance on the basis of quantification of egg white- specific IgE antibodies in children with egg allergy. J Allergy Clin Immunol 2002;110:304-9.
59. Garcia-Ara C, Boyano-Martinez T, Diaz-Pena JM, Martin-Munoz F, Reche-Frutos M, Martin-Esteban M. Specific IgE levels in the diagnosis of immediate hypersensitivity to cows' milk protein in the infant. J Allergy Clin Immunol 2001;107:185-90.
60. Osterballe M, Bindslev-Jensen C. Threshold levels in food challenge and specific IgE in patients with egg allergy: is there a relationship? J Allergy Clin Immunol 2003;112:196-201.
61. Celik-Bilgili S, Mehl A, Verstege A, Staden U, Nocon M, Beyer K, et al. The predictive value of specific immunoglobulin E levels in serum for the outcome of oral food challenges. Clin Exp Allergy 2005;35:268-73.
62. Perry TT, Matsui EC, Kay Conover-Walker M, Wood RA. The relationship of allergen-specific IgE levels and oral food challenge outcome. J Allergy Clin Immunol 2004;114:144-9.
63. Komata T, Soderstrom L, Borres MP, Tachimoto H, Ebisawa M. The predictive relationship of food-specific serum IgE concentrations to challenge outcomes for egg and milk varies by patient age. J Allergy Clin Immunol 2007;119:1272-4.
64. Roberts G, Lack G. Diagnosing peanut allergy with skin prick and specific IgE testing. J Allergy Clin Immunol 2005;115:1291-6.
65. Shek LP, Soderstrom L, Ahlstedt S, Beyer K, Sampson HA. Determination of food specific IgE levels over time can predict the development of tolerance in cow's milk and hen's egg allergy. J Allergy Clin Immunol 2004;114:387-91.
66. Sampson HA, Ho DG. Relationship between food-specific IgE concentrations and the risk of positive food challenges in children and adolescents. J Allergy Clin Immunol 1997;100:444-51.
67. Sicherer SH. Clinical implications of cross-reactive food allergens. J Allergy Clin Immunol 2001;108:881-90.
68. Wang J, Godbold JH, Sampson HA. Correlation of serum allergy (IgE) tests performed by different assay systems. J Allergy Clin Immunol 2008;121:1219-24.
69. Cerecedo I, Zamora J, Shreffler WG, Lin J, Bardina L, Dieguez MC, et al. Mapping of the IgE and IgG4 sequential epitopes of milk allergens with a peptide microarray-based immunoassay. J Allergy Clin Immunol 2008;122:589-94.
70. Steckelbroeck S, Ballmer-Weber BK, Vieths S. Potential, pitfalls, and prospects of food allergy diagnostics with recombinant allergens or synthetic sequential epitopes. J Allergy Clin Immunol 2008;121:1323-30.
71. Mehl A, Rolinck-Werninghaus C, Staden U, Verstege A, Wahn U, Beyer K, et al. The atopy patch test in the diagnostic workup of suspected food-related symptoms in children. J Allergy Clin Immunol 2006;118:923-9.
72. Spergel JM, Brown-Whitehorn T, Beausoleil JL, Shuker M, Liacouras CA. Predictive values for skin prick test and atopy patch test for eosinophilic esophagitis. J Allergy Clin Immunol 2007;119:509-11.
73. Fogg MI, Brown-Whitehorn TA, Pawlowski NA, Spergel JM. Atopy patch test for the diagnosis of food protein-induced enterocolitis syndrome. Pediatr Allergy Immunol 2006;17:351-5.
74. Wanich N, Nowak-Wegrzyn A, Sampson HA, Shreffler WG. Allergen-specific basophil suppression associated with clinical tolerance in patients with milk allergy. J Allergy Clin Immunol 2009;123:789-94.
75. Furuta GT, Liacouras CA, Collins MH, Gupta SK, Justinich C, Putnam PE, et al. Eosinophilic esophagitis in children and adults: a systematic review and consensus recommendations for diagnosis and treatment. Gastroenterology 2007;133: 1342-63.
76. Bernstein IL, Li JT, Bernstein DI, Hamilton R, Spector SL, Tan R, et al. Allergy diagnostic testing: an updated practice parameter. Ann Allergy Asthma Immunol 2008;100(suppl 3):S1-148.
77. Flinterman AE, Knulst AC, Meijer Y, Bruijnzeel-Koomen CA, Pasmans SG. Acute allergic reactions in children with AEDS after prolonged cow's milk elimination diets. Allergy 2006;61:370-4.
78. Bindslev-Jensen C, Ballmer-Weber BK, Bengtsson U, Blanco C, Ebner C, Hourihane J, et al. Standardization of food challenges in patients with immediate reactions to foods—position paper from the European Academy of Allergology and Clinical Immunology. Allergy 2004;59:690-7.
79. Bock SA, Sampson HA, Atkins FM, Zeiger RS, Lehrer S, Sachs M, et al. Double-blind, placebo-controlled food challenge (DBPCFC) as an office procedure: a manual. J Allergy Clin Immunol 1988;82:986-97.
80. Nowak-Wegrzyn A, Assa'ad AH, Bahna SL, Bock SA, Sicherer SH, Teuber SS. Work Group report: oral food challenge testing. J Allergy Clin Immunol 2009; 123(suppl):S365-83.
81. Rothenberg ME. Eosinophilic gastrointestinal disorders (EGID). J Allergy Clin Immunol 2004;113:11-28.
82. Greer FR, Sicherer SH, Burks AW. Effects of early nutritional interventions on the development of atopic disease in infants and children: the role of maternal dietary restriction, breastfeeding, timing of introduction of complementary foods, and hydrolyzed formulas. Pediatrics 2008;121:183-91.
83. Host A, Halken S, Muraro A, Dreborg S, Niggemann B, Aalberse R, et al. Dietary prevention of allergic diseases in infants and small children. Pediatr Allergy Immunol 2008;19:1-4.
84. Nowak-Wegrzyn A, Sicherer SH. Immunotherapy for food and latex allergy. Clin Allergy Immunol 2008;21:429-46.
85. Li XM. Traditional Chinese herbal remedies for asthma and food allergy. J Allergy Clin Immunol 2007;120:25-31.
86. Nelson HS, Lahr J, Rule R, Bock A, Leung D. Treatment of anaphylactic sensitivity to peanuts by immunotherapy with injections of aqueous peanut extract. J Allergy Clin Immunol 1997;99:744-51.
87. Jones SM, Pons L, Roberts JL, Scurlock AM, Perry TT, Kulis M, et al. Clinical efficacy and immune regulation with peanut oral immunotherapy. J Allergy Clin Immunol 2009;124:292-300.
88. Skripak JM, Nash SD, Rowley H, Brereton NH, Oh S, Hamilton RG, et al. A randomized, double-blind, placebo-controlled study of milk oral immunotherapy for cow's milk allergy. J Allergy Clin Immunol 2008;122:1154-60.
89. Buchanan AD, Green TD, Jones SM, Scurlock AM, Christie L, Althage KA, et al. Egg oral immunotherapy in nonanaphylactic children with egg allergy. J Allergy Clin Immunol 2007;119:199-205.
90. Staden U, Rolinck-Werninghaus C, Brewe F, Wahn U, Niggemann B, Beyer K. Specific oral tolerance induction in food allergy in children: efficacy and clinical patterns of reaction. Allergy 2007;62:1261-9.
91. Hofmann AM, Scurlock AM, Jones SM, Palmer KP, Lokhnygina Y, Steele PH, et al. Safety of a peanut oral immunotherapy protocol in children with peanut allergy. J Allergy Clin Immunol 2009;124:286-91.