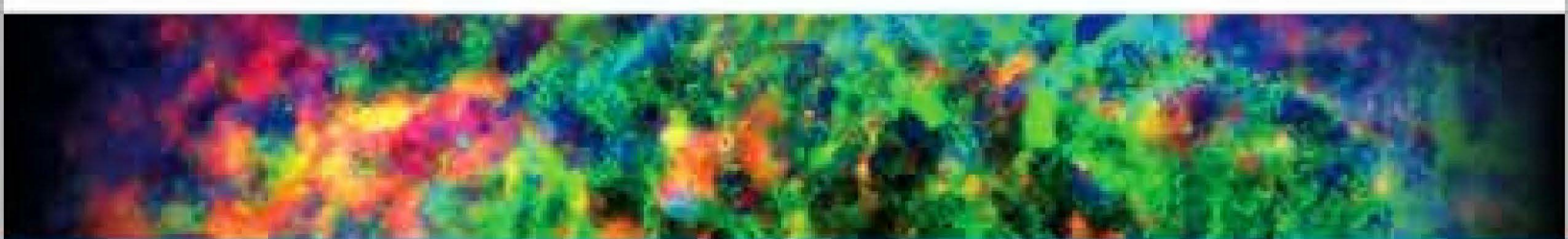
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Cellular and Molecular IMMUNOLOGY

7th EDITION

Abul K. Abbas
Andrew H. Lichtman
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Cellular and Molecular Immunology

Cellular and Molecular Immunology

SEVENTH EDITION

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Dedication

To

Ann, Jonathan, Rehana

Sheila, Eben, Ariella, Amos, Ezra

Honorine, Sohini

PREFACE

This seventh edition of *Cellular and Molecular Immunology* has been significantly rewritten and revised as part of our continuing effort to make the textbook current and, at the same time, preserve the easily understandable style that readers have enjoyed in past editions. We have added new information while striving to emphasize important concepts without increasing the length of the book. We have also changed many sections, when necessary, for increased clarity, accuracy, and completeness.

Among the major changes is a reorganization of the chapters in order to consolidate topics and present information in a more accessible manner. The chapter reorganization includes: a new chapter that discusses immune responses in mucosal tissues and other specialized sites; a new chapter on leukocyte migration, which brings together concepts that were previously discussed in multiple chapters; another new chapter that consolidates the discussions of immune receptors and signaling, which were also previously in several chapters; incorporation of discussions of cytokines into the relevant chapters rather than one chapter cataloguing all cytokines; and moving the discussion of autoimmunity into the chapter on tolerance, so the establishment and failure of immunologic tolerance is discussed as one cohesive theme. In addition, the entire book has been updated to include many recent advances in immunology. Some of the topics that have been significantly revised are the inflammasome, the biology of T_H17 cells, and the development and functions of follicular helper T cells. It is remarkable and fascinating to us that new principles continue to emerge from analysis of the complex systems that underlie immune responses. Perhaps one of the most satisfying developments for students of human disease is that basic principles of immunology are now laying the foundation for rational development of new immunologic therapies. Throughout the book, we have tried to emphasize these new therapeutics and the fundamental principles on which they are based.

Another major change in the seventh edition is a new illustration program, in which every figure in the book has been revised. The style of the new figures is based on the strengths of our popular illustrations in past editions, but incorporates many new features such as three dimensionality and new labeling conventions intended to enhance clarity and aesthetics. A large number of new illustrations have been added. We have also continued to improve the clarity of tables, and kept design features such as the use of bold italic text to highlight "take-home messages," to make the book easy and pleasant to read. The lists of selected readings continue to emphasize recent review articles that provide in-depth coverage of particular topics for the interested reader. We have divided the lists into sections based on themes to help readers find the most useful articles for their needs. A new table listing cytokines, their receptors, and their major cellular sources and functions has been added (Appendix II).

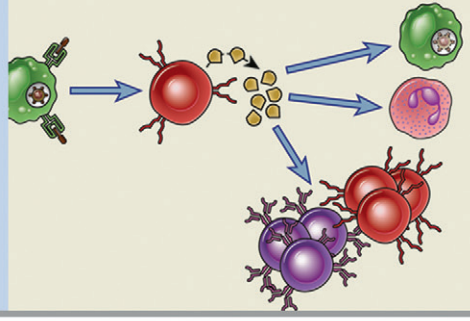
Many individuals have made valuable contributions to this edition. Drs. Richard Blumberg, Lisa Coussens, Jason Cyster, Francis Luscinskas, and Scott Plevy reviewed various sections, and all were generous with advice and comments. We thank Drs. Thorsten Mempel, Uli von Andrian, and Jason Cyster for help with cover illustrations for this and previous editions. Our illustrators, David and Alexandra Baker of DNA Illustrations,

remain full partners in the book and provide invaluable suggestions for clarity and accuracy. Several members of the Elsevier staff played critical roles. Our editor, Bill Schmitt, has been a source of support and encouragement. Our managing editor, Rebecca Gruliow, shepherded the book through its preparation and into production. Lou Forgione is responsible for the design, and Sarah Wunderly took charge of the production stage. Finally, our students were the original inspiration for the first edition of this book, and we remain continually grateful to them, because from them we learn how to think about the science of immunology, and how to communicate knowledge in the clearest and most meaningful way.

Abul K. Abbas

Andrew H. Lichtman

Shiv Pillai



CHAPTER

1

Properties and Overview of Immune Responses

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TYPES OF ADAPTIVE IMMUNE RESPONSES, 3

CARDINAL FEATURES OF ADAPTIVE IMMUNE RESPONSES, 6

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The term *immunity* is derived from the Latin word *immunitas,* which referred to the protection from legal prosecution offered to Roman senators during their tenures in office. Historically, immunity meant protection from disease and, more specifically, infectious disease. The cells and molecules responsible for immunity constitute the **immune system**, and their collective and coordinated response to the introduction of foreign substances is called the **immune response**.

The physiologic function of the immune system is defense against infectious microbes. However, even noninfectious foreign substances can elicit immune responses. Furthermore, mechanisms that normally protect individuals from infection and eliminate foreign substances are also capable of causing tissue injury and disease in some situations. Therefore, a more inclusive definition of the immune response is a reaction to components of microbes as well as to macromolecules, such as proteins and polysaccharides, and small chemicals that are recognized as foreign, regardless of the physiologic or pathologic consequence of such a reaction. Under some situations, even self molecules can elicit immune responses (so-called autoimmune responses). Immunology is the study of immune responses in this broader sense and of the cellular and molecular events that occur after an organism encounters microbes and other foreign macromolecules.

Historians often credit Thucydides, in the fifth century BC in Athens, as having first mentioned immunity to an infection that he called plague (but that was probably not the bubonic plague we recognize today). The concept of protective immunity may have existed long before, as suggested by the ancient Chinese custom of making children resistant to smallpox by having them inhale powders made from the skin lesions of patients recovering from the disease. Immunology, in its modern form, is an experimental science, in which explanations of immunologic phenomena are based on experimental observations and the conclusions drawn from them. The evolution of immunology as an experimental discipline has depended on our ability to manipulate the function of the immune system under controlled conditions. Historically, the first clear example of this manipulation, and one that remains among the most dramatic ever recorded, was Edward Jenner's successful vaccination against smallpox. Jenner, an English physician, noticed that milkmaids who had recovered from cowpox never contracted the more serious smallpox. On the basis of this observation, he injected the material from a cowpox pustule into the arm of an 8-year-old boy. When this boy was later intentionally inoculated with smallpox, the disease did not develop. Jenner's landmark treatise on **vaccination** (Latin *vaccinus,* of or from cows) was published in 1798. It led to the widespread acceptance of this method for inducing immunity to infectious diseases, and vaccination remains the most effective method for preventing infections (Table 1-1). An eloquent testament to the importance of immunology was the announcement by the World Health Organization in 1980 that smallpox was the first disease that had been eradicated worldwide by a program of vaccination.

TABLE 1–1 Effectiveness of Vaccines for Some Common Infectious Diseases

Disease	Maximum Number of Cases (year)	Number of Cases in 2009	Percentage Change
Diphtheria	206,939 (1921)	0	–99.99
Measles	894,134 (1941)	61	–99.99
Mumps	152,209 (1968)	982	–99.35
Pertussis	265,269 (1934)	13,506	–94.72
Polio (paralytic)	21,269 (1952)	0	–100.0
Rubella	57,686 (1969)	4	–99.99
Tetanus	1,560 (1923)	14	–99.10
Haemophilus influenzae type B	~20,000 (1984)	25	–99.88
Hepatitis B	26,611 (1985)	3,020	–87.66

This table illustrates the striking decrease in the incidence of selected infectious diseases for which effective vaccines have been developed.
Data from Orenstein WA, AR Hinman, KJ Bart, and SC Hadler. Immunization. In Mandell GL, JE Bennett, and R Dolin (eds). Principles and Practices of Infectious Diseases, 4th ed. Churchill Livingstone, New York, 1995, and Morbidity and Mortality Weekly Report 58:1458-1469, 2010.

Since the 1960s, there has been a remarkable transformation in our understanding of the immune system and its functions. Advances in cell culture techniques (including monoclonal antibody production), immunochemistry, recombinant DNA methodology, and x-ray crystallography and the creation of genetically altered animals (especially transgenic and knockout mice) have changed immunology from a largely descriptive science into one in which diverse immune phenomena can be explained in structural and biochemical terms. In this chapter, we outline the general features of immune responses and introduce the concepts that form the cornerstones of modern immunology and that recur throughout this book.

INNATE AND ADAPTIVE IMMUNITY

Defense against microbes is mediated by the early reactions of innate immunity and the later responses of adaptive immunity (Fig. 1-1 and Table 1-2). **Innate immunity** (also called natural or native immunity) provides the early line of defense against microbes. It consists of cellular and biochemical defense mechanisms that are in place even before infection and are poised to respond rapidly to infections. These mechanisms react to microbes and to the products of injured cells, and they respond in essentially the same way to repeated infections. The principal components of innate immunity are (1) physical and chemical barriers, such as epithelia and antimicrobial chemicals produced at epithelial surfaces; (2) phagocytic cells (neutrophils, macrophages), dendritic cells, and natural killer (NK) cells; (3) blood proteins, including members of the complement system and other mediators of inflammation; and (4) proteins called cytokines that regulate and coordinate many of the activities of the cells of innate immunity. The mechanisms of innate immunity are specific for structures that are common to groups of related microbes and may not distinguish fine differences between microbes.

In contrast to innate immunity, there are other immune responses that are stimulated by exposure to infectious agents and increase in magnitude and defensive capabilities with each successive exposure to a particular microbe. Because this form of immunity develops as a response to infection and adapts to the infection, it is called **adaptive immunity**. The defining characteristics of adaptive immunity are exquisite specificity for distinct molecules and an ability to "remember" and respond more vigorously to repeated exposures to the same microbe. The adaptive immune system is able to recognize and react to a large number of microbial and nonmicrobial substances. In addition, it has an extraordinary capacity to distinguish between different, even closely related, microbes and molecules, and for this reason it is also called **specific immunity**. It is also sometimes called acquired immunity, to emphasize that potent protective responses are "acquired" by experience. The main components of adaptive immunity are cells called **lymphocytes** and their secreted products, such as antibodies. Foreign substances that induce specific immune responses or are recognized by lymphocytes or antibodies are called **antigens**.

Mechanisms for defending the host against microbes are present in some form in all multicellular organisms. These mechanisms constitute innate immunity. The more specialized defense mechanisms that constitute adaptive immunity are found in vertebrates only. Two functionally similar but molecularly distinct adaptive immune systems developed at different times in evolution. About 500 million years ago, jawless fish, such as lampreys and hagfish, developed a unique immune system containing diverse lymphocyte-like cells that may function like lymphocytes in more advanced species and even responded to immunization. The antigen receptors on these cells were variable leucine-rich receptors that were capable of recognizing many antigens but were distinct from the antibodies and T cell receptors that appeared later in evolution. Most of the components of the adaptive immune system, including lymphocytes with highly

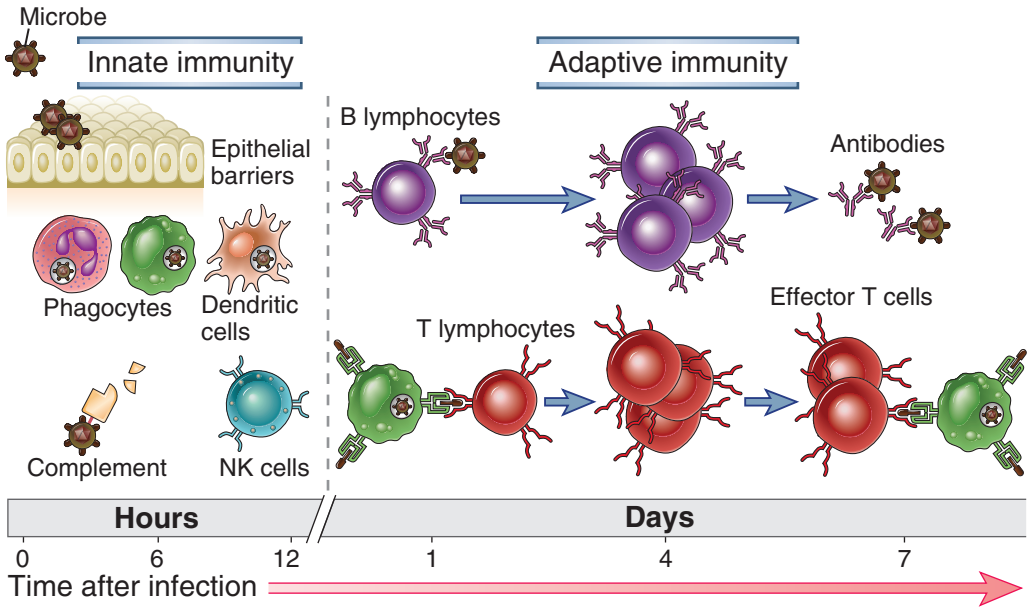


FIGURE 1–1 Innate and adaptive immunity. The mechanisms of innate immunity provide the initial defense against infections. Adaptive immune responses develop later and consist of activation of lymphocytes. The kinetics of the innate and adaptive immune responses are approximations and may vary in different infections.

TABLE 1–2 Features of Innate and Adaptive Immunity

	Innate	Adaptive
Characteristics		
Specificity	For molecules shared by groups of related microbes and molecules produced by damaged host cells	For microbial and nonmicrobial antigens
Diversity	Limited; germline encoded	Very large; receptors are produced by somatic recombination of gene segments
Memory	None	Yes
Nonreactivity to self	Yes	Yes
Components		
Cellular and chemical barriers	Skin, mucosal epithelia; antimicrobial molecules	Lymphocytes in epithelia; antibodies secreted at epithelial surfaces
Blood proteins	Complement, others	Antibodies
Cells	Phagocytes (macrophages, neutrophils), natural killer cells	Lymphocytes

diverse antigen receptors, antibodies, and specialized lymphoid tissues, evolved coordinately within a short time in jawed vertebrates (e.g., sharks), about 360 million years ago. The immune system has also become increasingly specialized with evolution.

Innate and adaptive immune responses are components of an integrated system of host defense in which numerous cells and molecules function cooperatively. The mechanisms of innate immunity provide effective initial defense against infections. However, many pathogenic microbes have evolved to resist innate immunity, and their elimination requires the more powerful mechanisms of adaptive immunity. There are many connections between the innate and adaptive immune systems. The innate immune response to microbes stimulates adaptive immune responses and influences the nature of the adaptive responses. Conversely, adaptive immune responses often work by enhancing the protective mechanisms of innate immunity, making them capable of effectively combating pathogenic microbes.

TYPES OF ADAPTIVE IMMUNE RESPONSES

There are two types of adaptive immune responses, called humoral immunity and cell-mediated immunity, that are

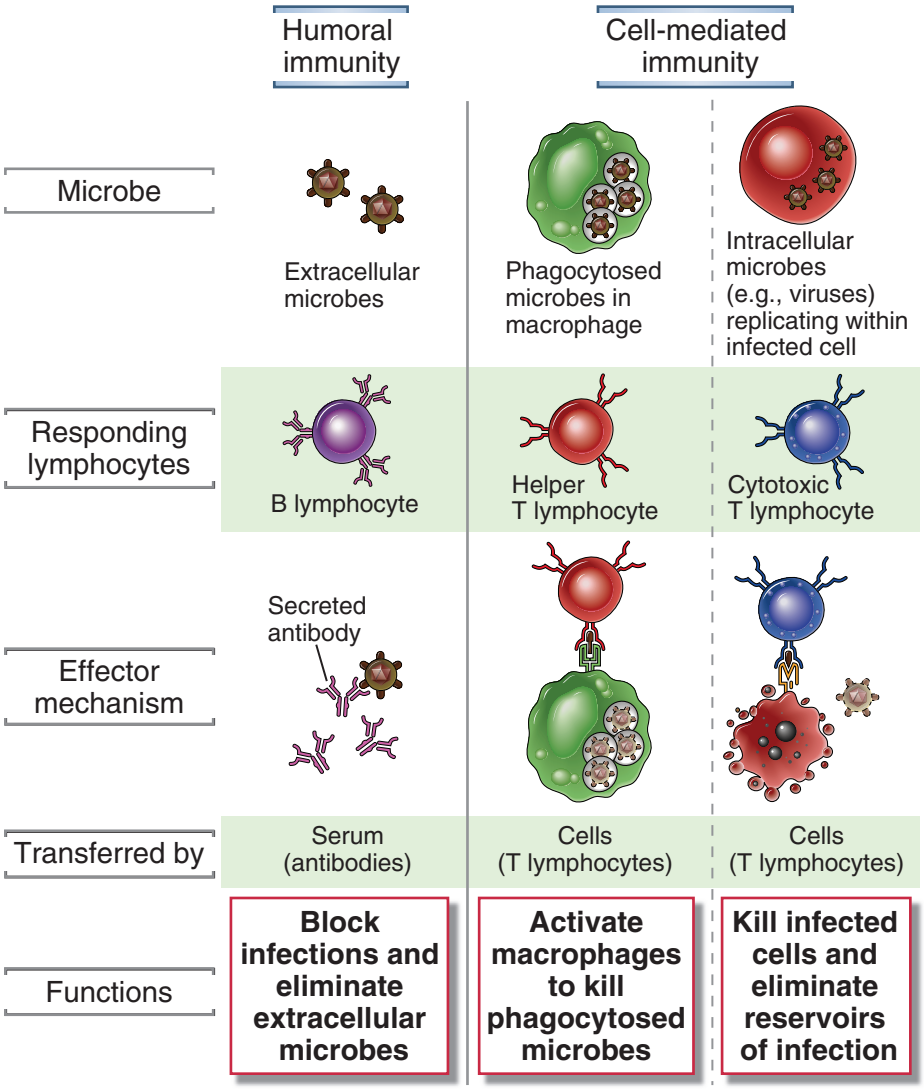


FIGURE 1–2 Types of adaptive immunity. In humoral immunity, B lymphocytes secrete antibodies that prevent infections by and eliminate extracellular microbes. In cell-mediated immunity, helper T lymphocytes activate macrophages to kill phagocytosed microbes or cytotoxic T lymphocytes directly destroy infected cells.

mediated by different components of the immune system and function to eliminate different types of microbes (Fig. 1-2). **Humoral immunity** is mediated by molecules in the blood and mucosal secretions, called antibodies, which are produced by cells called B lymphocytes (also called B cells). Antibodies recognize microbial antigens, neutralize the infectivity of the microbes, and target microbes for elimination by various effector mechanisms. Humoral immunity is the principal defense mechanism against extracellular microbes and their toxins because secreted antibodies can bind to these microbes and toxins and assist in their elimination. Antibodies themselves are specialized and may activate different effector mechanisms. For example, different types of antibodies promote the ingestion of microbes by host cells (phagocytosis), bind to and trigger the release of inflammatory mediators from cells, and are actively transported into the lumens of mucosal organs and through the placenta to provide defense against ingested and inhaled microbes and against infections of the newborn, respectively. **Cell-mediated immunity**, also called cellular immunity, is mediated by T lymphocytes (also called T cells). Intracellular microbes, such as viruses and some bacteria, survive and proliferate inside phagocytes and other host cells, where they are inaccessible to circulating antibodies. Defense against such infections is a function of cell-mediated immunity, which promotes the destruction of microbes residing in phagocytes or the killing of infected cells to eliminate reservoirs of infection.

Protective immunity against a microbe is usually induced by the host's response to the microbe (Fig. 1-3). The form of immunity that is induced by exposure to a foreign antigen is called **active immunity** because the immunized individual plays an active role in responding to the antigen. Individuals and lymphocytes that have not encountered a particular antigen are said to be naive, implying that they are immunologically inexperienced. Individuals who have responded to a microbial antigen and are protected from subsequent exposures to that microbe are said to be immune.

Immunity can also be conferred on an individual by transferring serum or lymphocytes from a specifically immunized individual, a process known as adoptive transfer in experimental situations (see Fig. 1-3). The

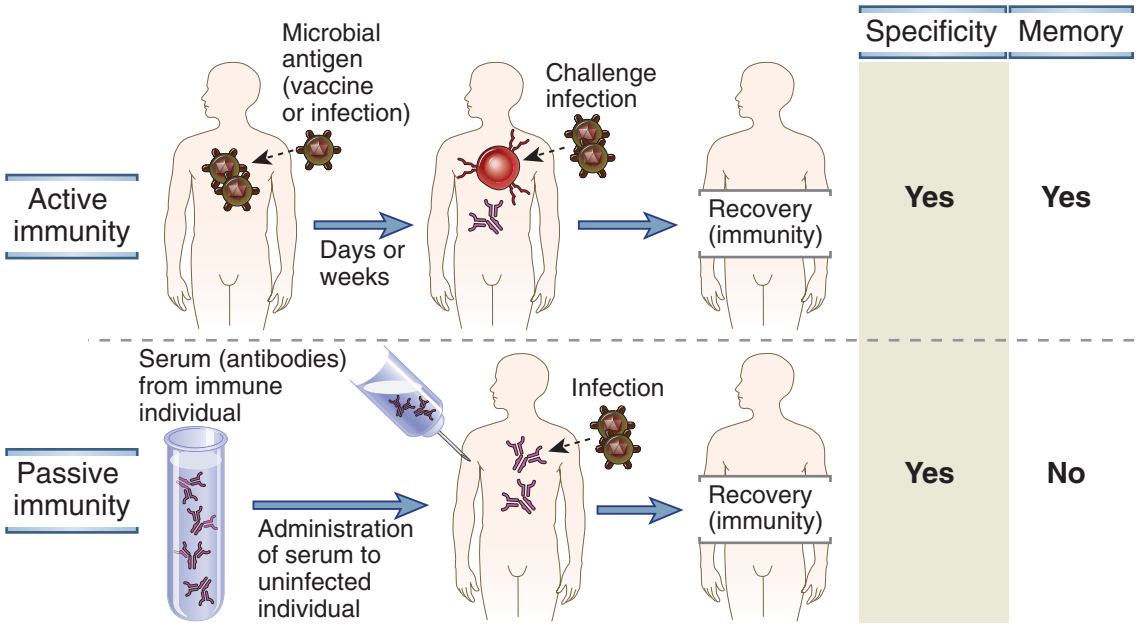


FIGURE 1–3 Active and passive immunity. Active immunity is conferred by a host response to a microbe or microbial antigen, whereas passive immunity is conferred by adoptive transfer of antibodies or T lymphocytes specific for the microbe. Both forms of immunity provide resistance to infection and are specific for microbial antigens, but only active immune responses generate immunologic memory. Cell transfers can be done only between genetically identical donor and recipient (e.g., inbred mice) to avoid rejection of the transferred cells.

recipient of such a transfer becomes immune to the particular antigen without ever having been exposed to or having responded to that antigen. Therefore, this form of immunity is called **passive immunity**. Passive immunization is a useful method for conferring resistance rapidly, without having to wait for an active immune response to develop. A physiologically important example of passive immunity is the transfer of maternal antibodies to the fetus, which enables newborns to combat infections before they develop the ability to produce antibodies themselves. Passive immunization against toxins by the administration of antibodies from immunized animals is a lifesaving treatment for potentially lethal infections, such as tetanus, and snake bites. The technique of adoptive transfer has also made it possible to define the various cells and molecules that are responsible for mediating specific immunity. In fact, humoral immunity was originally defined as the type of immunity that could be transferred to unimmunized, or naive, individuals by antibody-containing cell-free portions of the blood (i.e., plasma or serum) obtained from previously immunized individuals. Similarly, cell-mediated immunity was defined as the form of immunity that can be transferred to naive animals with cells (T lymphocytes) from immunized animals but not with plasma or serum.

The first experimental demonstration of humoral immunity was provided by Emil von Behring and Shibasaburo Kitasato in 1890. They showed that if serum from animals that had recovered from diphtheria infection was transferred to naive animals, the recipients became specifically resistant to diphtheria infection. The active components of the serum were called antitoxins because they neutralized the pathologic effects of the diphtheria toxin. This result led to the treatment of otherwise lethal diphtheria infection by the administration of antitoxin, an achievement that was recognized by the award of the first Nobel Prize in Physiology or Medicine to von Behring. In the early 1900s, Paul Ehrlich postulated that immune cells use receptors, which he called side chains, to recognize microbial toxins and subsequently secrete these receptors to combat microbes. He also coined the term **antibodies** (*antikörper* in German) for the serum proteins that bound toxins, and substances that stimulated the production of antibodies were called **antigens**. The modern definition of antigens includes substances that bind to specific lymphocyte receptors, whether or not they stimulate immune responses. According to strict definitions, substances that stimulate immune responses are called **immunogens**. The properties of antibodies and antigens are described in Chapter 5. Ehrlich's concepts are a remarkably prescient model for the function of B cells in humoral immunity. This early emphasis on antibodies led to the general acceptance of the humoral theory of immunity, according to which host defense against infections is mediated by substances present in body fluids (once called humors).

The cellular theory of immunity, which stated that host cells are the principal mediators of immunity, was championed initially by Elie Metchnikoff. His demonstration of phagocytes surrounding a thorn stuck into a translucent starfish larva, published in 1883, was perhaps the first experimental evidence that cells respond to foreign invaders. Ehrlich and Metchnikoff shared the Nobel Prize in 1908, in recognition of their contributions to establishing these fundamental principles of immunity. Sir Almroth Wright's observation in the early 1900s that factors in immune serum enhanced the phagocytosis of bacteria by coating the bacteria, a process known as

opsonization, lent support to the belief that antibodies prepared microbes for ingestion by phagocytes. These early "cellularists" were unable to prove that specific immunity to microbes could be mediated by cells. The cellular theory of immunity became firmly established in the 1950s, when it was shown that resistance to an intracellular bacterium, *Listeria monocytogenes*, could be adoptively transferred with cells but not with serum. We now know that the specificity of cell-mediated immunity is due to lymphocytes, which often function in concert with other cells, such as phagocytes, to eliminate microbes.

In the clinical setting, immunity to a previously encountered microbe is measured indirectly, either by assaying for the presence of products of immune responses (such as serum antibodies specific for microbial antigens) or by administering substances purified from the microbe and measuring reactions to these substances. A reaction to a microbial antigen is detectable only in individuals who have previously encountered the antigen; these individuals are said to be "sensitized" to the antigen, and the reaction is an indication of "sensitivity." Although the reaction to the purified antigen has no protective function, it implies that the sensitized individual is capable of mounting a protective immune response to the microbe.

CARDINAL FEATURES OF ADAPTIVE IMMUNE RESPONSES

All humoral and cell-mediated immune responses to foreign antigens have a number of fundamental properties that reflect the properties of the lymphocytes that mediate these responses (Table 1-3).

- ***Specificity and diversity.*** Immune responses are specific for distinct antigens and, in fact, for different portions of a single complex protein, polysaccharide, or other macromolecule (Fig. 1-4). The parts of such antigens that are specifically recognized by individual lymphocytes are called **determinants** or **epitopes**. This fine specificity exists because individual lymphocytes express membrane receptors that are able to distinguish subtle differences in structure between distinct epitopes. Clones of lymphocytes with different specificities are present in unimmunized individuals and are able to recognize and respond to foreign antigens. This concept is the basic tenet of the clonal selection hypothesis, which is discussed in more detail later in this chapter.

  The total number of antigenic specificities of the lymphocytes in an individual, called the **lymphocyte repertoire**, is extremely large. It is estimated that the immune system of an individual can discriminate 10^7 to 10^9 distinct antigenic determinants. This ability of the lymphocyte repertoire to recognize a very large number of antigens is the result of variability in the structures of the antigen-binding sites of lymphocyte receptors for antigens, called **diversity**. In other words, there are many different clones of lymphocytes that differ in the structures of their antigen receptors and therefore in their specificity for antigens, contributing to a total repertoire that is extremely diverse. The variation of antigen receptors among different clones of T and B cells is the reason that these receptors are said to be "clonally distributed." The molecular mechanisms that generate such diverse antigen receptors are discussed in Chapter 8.
- ***Memory.*** Exposure of the immune system to a foreign antigen enhances its ability to respond again to that antigen. Responses to second and subsequent exposures to the same antigen, called secondary immune responses, are usually more rapid, larger, and often qualitatively different from the first, or primary, immune response to that antigen (see Fig. 1-4). Immunologic memory occurs because each exposure to an antigen generates long-lived memory cells specific for the antigen, which are more numerous than the naive T cells specific for the antigen that exist before antigen exposure. In addition, these memory cells have special characteristics that make them more efficient at responding to and eliminating the antigen than are naive lymphocytes that have not previously been exposed to the antigen. For instance, memory B lymphocytes produce antibodies that bind antigens with higher affinities than do antibodies produced in primary immune responses, and memory T cells react much more rapidly and vigorously to antigen challenge than do naive T cells.
- ***Clonal expansion.*** Lymphocytes specific for an antigen undergo considerable proliferation after exposure to that antigen. The term *clonal expansion* refers to an increase in the number of cells that express identical receptors for the antigen and thus belong to a clone. This increase in antigen-specific cells enables the adaptive immune response to keep pace with rapidly dividing infectious pathogens.
- ***Specialization.*** As we have already noted, the immune system responds in distinct and special ways to

TABLE 1–3 Cardinal Features of Adaptive Immune Responses

Feature	Functional Significance
Specificity	Ensures that the immune response to a microbe (or nonmicrobial antigen) is targeted to that microbe (or antigen)
Diversity	Enables immune system to respond to a large variety of antigens
Memory	Increases ability to combat repeat infections by the same microbe
Clonal expansion	Increases number of antigen-specific lymphocytes to keep pace with microbes
Specialization	Generates responses that are optimal for defense against different types of microbes
Contraction and homeostasis	Allows immune system to recover from one response so that it can effectively respond to newly encountered antigens
Nonreactivity to self	Prevents injury to host during responses to foreign antigens

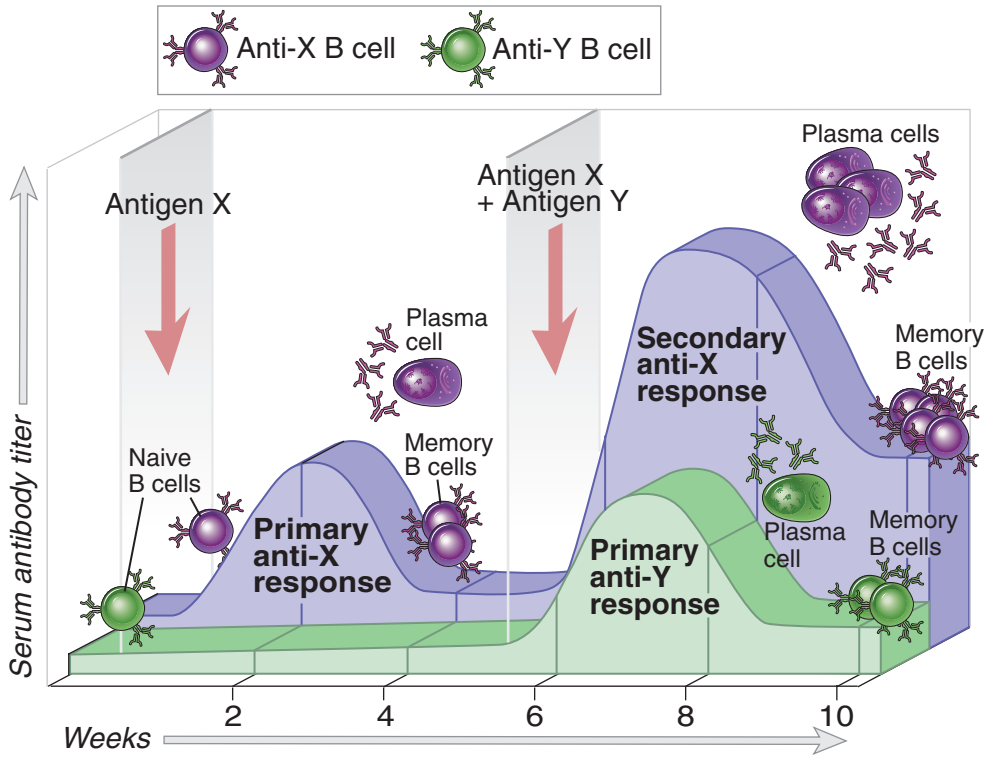


FIGURE 1–4 Specificity, memory, and contraction of adaptive immune responses. Antigens X and Y induce the production of different antibodies (specificity). The secondary response to antigen X is more rapid and larger than the primary response (memory). Antibody levels decline with time after each immunization (contraction, the process that maintains homeostasis). The same features are seen in cell-mediated immune responses.

different microbes, maximizing the effectiveness of antimicrobial defense mechanisms. Thus, humoral immunity and cell-mediated immunity are elicited by different classes of microbes or by the same microbe at different stages of infection (extracellular and intracellular), and each type of immune response protects the host against that class of microbe. Even within humoral or cell-mediated immune responses, the nature of the antibodies or T lymphocytes that are generated may vary from one class of microbe to another. We will return to the mechanisms and functional significance of such specialization in later chapters.

- ***Contraction and homeostasis.*** All normal immune responses wane with time after antigen stimulation, thus returning the immune system to its resting basal state, a state that is called **homeostasis** (see Fig. 1-4). This contraction of immune responses occurs largely because responses that are triggered by antigens function to eliminate the antigens, thus eliminating an essential stimulus for lymphocyte survival and activation. Lymphocytes, other than memory cells, that are deprived of these stimuli die by apoptosis.
- ***Nonreactivity to self.*** One of the most remarkable properties of every normal individual's immune system is its ability to recognize, respond to, and eliminate many foreign (non-self) antigens while not reacting harmfully to that individual's own (self) antigenic substances. Immunologic unresponsiveness is also called **tolerance**. Tolerance to self antigens, or self-tolerance, is maintained by several mechanisms. These include eliminating lymphocytes that express receptors specific for some self antigens, inactivating self-reactive lymphocytes, or suppressing these cells by the actions of other (regulatory) cells. Abnormalities in the induction or maintenance of self-tolerance lead to immune responses against self (autologous) antigens, which may result in disorders called **autoimmune diseases**. The mechanisms of self-tolerance and its failure are discussed in Chapter 14.

These features of adaptive immunity are necessary if the immune system is to perform its normal function of host defense (see Table 1-3). Specificity and memory enable the immune system to mount heightened responses to persistent or recurring exposure to the same antigen and thus to combat infections that are prolonged or occur repeatedly. Diversity is essential if the immune system is to defend individuals against the many potential pathogens in the environment. Specialization enables the host to "custom design" responses to best combat different types of microbes. Contraction of the response allows the system to return to a state of rest after it eliminates each foreign antigen and to be prepared to respond to other antigens. Self-tolerance is vital for preventing harmful reactions against one's own cells and tissues while maintaining a diverse repertoire of lymphocytes specific for foreign antigens.

Immune responses are regulated by a system of positive feedback loops that amplify the reaction and by control

mechanisms that prevent inappropriate or pathologic reactions. When lymphocytes are activated, they trigger mechanisms that further increase the magnitude of the response. This positive feedback is important to enable the small number of lymphocytes that are specific for any microbe to generate the response needed to eradicate that infection. Many control mechanisms become active in immune responses to prevent excessive activation of lymphocytes, which may cause collateral damage to normal tissues, and to avoid responses against self antigens. In fact, a balance between activating and inhibitory signals is characteristic of all immune responses. We will mention specific examples of these fundamental features of the immune system throughout the book.

CELLULAR COMPONENTS OF THE ADAPTIVE IMMUNE SYSTEM

The principal cells of the immune system are lymphocytes, antigen-presenting cells, and effector cells. Lymphocytes are the cells that specifically recognize and respond to foreign antigens and are therefore the mediators of humoral and cellular immunity. There are distinct subpopulations of lymphocytes that differ in how they recognize antigens and in their functions (Fig. 1-5). **B lymphocytes** are the only cells capable of producing antibodies. They recognize extracellular (including cell surface) antigens and differentiate into antibody-secreting plasma cells, thus functioning as the mediators of humoral immunity. **T lymphocytes**, the cells of cell-mediated immunity, recognize the antigens of intracellular microbes and either help phagocytes to destroy these microbes or directly kill the infected cells. T cells do not produce antibody molecules. Their antigen receptors are membrane molecules distinct from but structurally related to antibodies (see Chapter 7). T lymphocytes have a restricted specificity for antigens; they recognize peptides derived from foreign proteins that are bound to host proteins called major histocompatibility complex (MHC) molecules, which are expressed on the surfaces of other cells. As a result, these T cells recognize and respond to cell surface–associated but not soluble antigens (see Chapter 6). T lymphocytes consist of functionally distinct populations, the best defined of which are **helper T cells** and **cytotoxic** (or **cytolytic**) **T lymphocytes** (CTLs). In response to antigenic stimulation, helper T cells secrete proteins called **cytokines**, which are responsible for many of the cellular responses of innate and adaptive immunity and thus function as the "messenger molecules" of the immune system. The cytokines secreted by helper T lymphocytes stimulate the proliferation and differentiation of the T cells themselves and activate other cells, including B cells, macrophages, and other leukocytes. CTLs kill cells that produce foreign antigens, such as cells infected by viruses and other intracellular microbes. Some T lymphocytes, which are called **regulatory T cells**, function mainly to inhibit immune responses. A third class of lymphocytes, **natural killer (NK) cells**, is involved in innate immunity against viruses and other intracellular microbes. A small population of T lymphocytes that express a cell surface protein found on NK cells are called **NKT cells;** their specificities and role in host defense are not well understood. We will return to a more detailed discussion of the properties of lymphocytes in Chapter 2 and in later chapters. Different classes of lymphocytes can be distinguished by the expression of surface proteins that are named CD molecules and numbered (see Chapter 2).

The initiation and development of adaptive immune responses require that antigens be captured and displayed to specific lymphocytes. The cells that serve this role are called **antigen-presenting cells** (APCs). The most specialized APCs are dendritic cells, which capture microbial antigens that enter from the external environment, transport these antigens to lymphoid organs, and present the antigens to naive T lymphocytes to initiate immune responses. Other cell types function as APCs at different stages of cell-mediated and humoral immune responses. We will describe the functions of APCs in Chapter 6.

The activation of lymphocytes by antigen leads to the generation of numerous mechanisms that function to eliminate the antigen. Antigen elimination often requires the participation of cells that are called **effector cells** because they mediate the final effect of the immune response, which is to get rid of the microbes. Activated T lymphocytes, mononuclear phagocytes, and other leukocytes function as effector cells in different immune responses.

Lymphocytes and APCs are concentrated in anatomically discrete lymphoid organs, where they interact with one another to initiate immune responses. Lymphocytes are also present in the blood; from the blood, they can recirculate through lymphoid tissues and home to peripheral tissue sites of antigen exposure to eliminate the antigen (see Chapter 3).

The cells of innate immunity interact with one another and with other host cells during the initiation and effector stages of innate and adaptive immune responses. Many of these interactions are mediated by secreted proteins called **cytokines**. We will describe the properties and functions of individual cytokines when we discuss immune responses in which these proteins play important roles. We summarize some of the general features and functional categories of cytokines below.

CYTOKINES, SOLUBLE MEDIATORS OF THE IMMUNE SYSTEM

Cytokines, a large and heterogeneous group of secreted proteins produced by many different cell types, mediate and regulate all aspects of innate and adaptive immunity. The human genome contains about 180 genes that may encode proteins with the structural characteristics of cytokines. The nomenclature of cytokines is somewhat haphazard, with many cytokines arbitrarily named on the basis of one of the biologic activities they were discovered to have (e.g., tumor necrosis factor, interferons) and others called **interleukins**, with a number suffix, because cytokines were thought to be made by and to act on leukocytes.

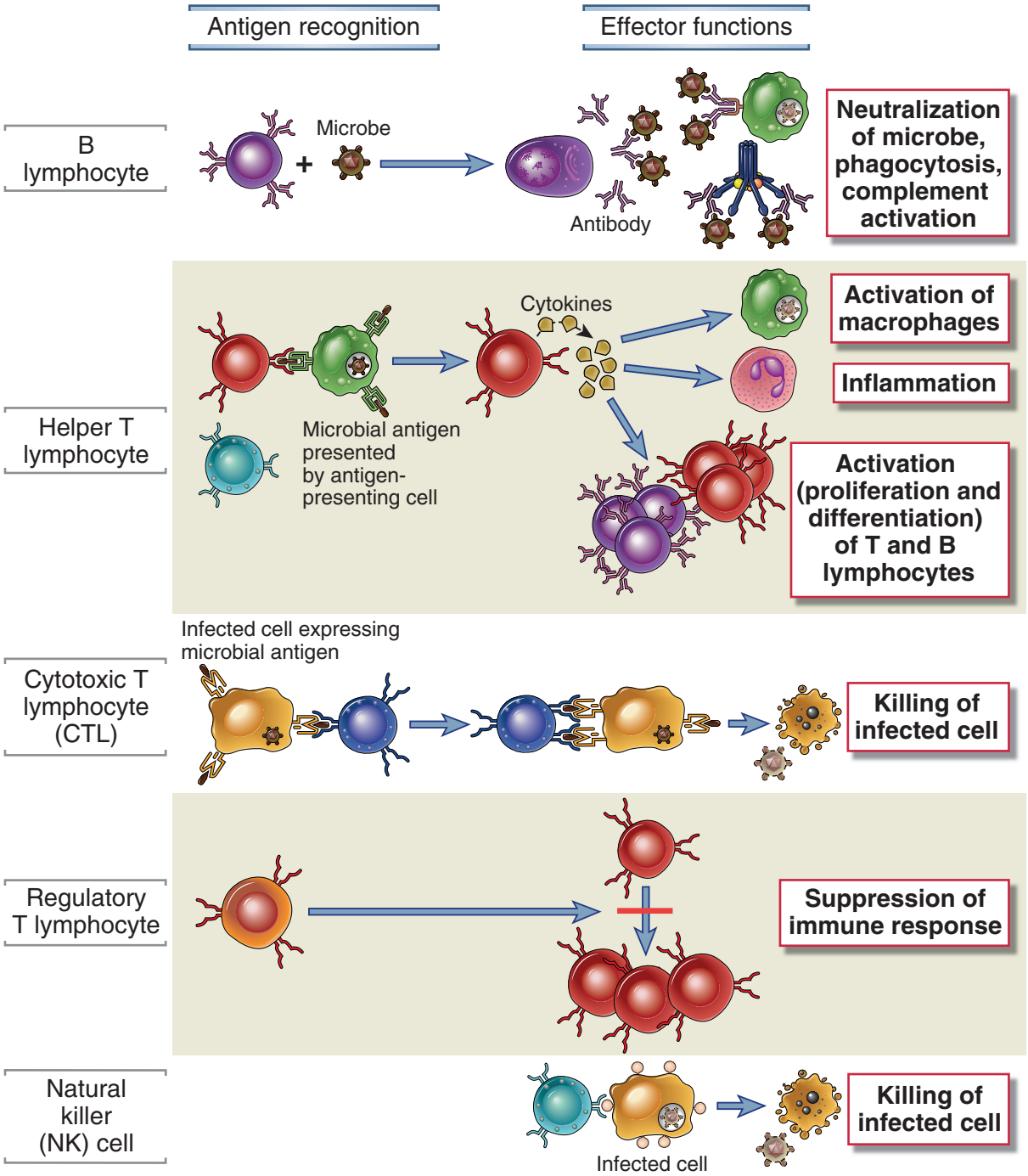


FIGURE 1–5 Classes of lymphocytes. B lymphocytes recognize soluble antigens and develop into antibody-secreting cells. Helper T lymphocytes recognize antigens on the surfaces of antigen-presenting cells and secrete cytokines, which stimulate different mechanisms of immunity and inflammation. Cytotoxic T lymphocytes recognize antigens on infected cells and kill these cells. Regulatory T cells suppress and prevent immune response (e.g., to self antigens). NK cells use receptors with more limited diversity than T or B cell antigen receptors to recognize and kill their targets, such as infected cells.

Cytokines are not usually stored as preformed molecules, and their synthesis is initiated by new gene transcription as a result of cellular activation. Such transcriptional activation is transient, and the messenger RNAs encoding most cytokines are unstable and often rapidly degraded, so cytokine synthesis is also transient. The production of some cytokines may additionally be regulated by RNA processing and by post-translational mechanisms, such as proteolytic release of an active product from an inactive precursor. Once synthesized, cytokines are rapidly secreted, resulting in a burst of release when needed.

Cytokines share many other general properties. One cytokine can act on diverse cell types and have multiple biologic effects, a property that is referred to as pleiotropism. Conversely, multiple cytokines may have the same

action, and are said to be redundant. One cytokine can stimulate or inhibit the production of others, and cytokines may antagonize one another or produce additive or synergistic effects.

Most cytokines act close to where they are produced, either on the same cell that secretes the cytokine (**autocrine** action) or on a nearby cell (**paracrine** action). T cells often secrete cytokines at the site of contact with APCs, the so-called immune synapse (see Chapter 9). This may be one reason that cytokines often act on cells that are in contact with the cytokine producers. When produced in large amounts, cytokines may enter the circulation and act at a distance from the site of production (**endocrine** action). Tumor necrosis factor (TNF) is an example of a cytokine that has important local and distant (systemic) effects.

Some cytokines are mediators and regulators of innate immunity. They are produced by innate immune cells such as dendritic cells, macrophages, and mast cells, and they drive the process of inflammation or contribute to defense against viral infections. Other cytokines, especially those produced by helper T cell subsets, contribute to host defense mediated by the adaptive immune system and also regulate immune responses. Members of this category of cytokines are also responsible for the activation and differentiation of T cells and B cells. Some cytokines are growth factors for hematopoiesis and regulate the generation of different types of immune cells from precursors in the bone marrow.

In general, the cytokines of innate and adaptive immunity are produced by different cell populations, act on different target cells, and have other distinct properties. However, these distinctions are not absolute because the same cytokine may be produced during innate and adaptive immune reactions, and different cytokines produced during such reactions may have overlapping actions.

OVERVIEW OF IMMUNE RESPONSES TO MICROBES

Now that we have introduced the major components of the immune system and their properties, it is useful to summarize the principles of immune responses to different types of microbes. Such a summary will be a foundation for the topics that are discussed throughout the book. The immune system has to combat many and diverse microbes. As we shall see shortly, immune responses to all infectious pathogens share some common features, and responses to different classes of these microbes may also have unique features. How these adaptive immune reactions are initiated, orchestrated, and controlled are the fundamental questions of immunology. We start with a discussion of the innate immune response.

The Early Innate Immune Response to Microbes

The innate immune system blocks the entry of microbes and eliminates or limits the growth of many microbes that are able to colonize tissues. The main sites of interaction between individuals and their environment—the skin and gastrointestinal and respiratory tracts—are lined by continuous epithelia, which serve as barriers to prevent the entry of microbes from the external environment. If microbes successfully breach the epithelial barriers, they encounter the cells of innate immunity. The cellular innate immune response to microbes consists of two main types of reactions—inflammation and antiviral defense. **Inflammation** is the process of recruitment of leukocytes and plasma proteins from the blood, their accumulation in tissues, and their activation to destroy the microbes. Many of these reactions involve cytokines, which are produced by dendritic cells, macrophages, and other types of cells during innate immune reactions. The major leukocytes that are recruited in inflammation are the phagocytes, neutrophils (which have short life spans in tissues), and monocytes (which develop into tissue macrophages). These phagocytes express on their surfaces receptors that bind and ingest microbes and other receptors that recognize different microbial molecules and activate the cells. On engagement of these receptors, the phagocytes produce reactive oxygen and nitrogen species and lysosomal enzymes, which destroy the microbes that have been ingested. Resident macrophages in the tissues serve much the same functions. **Antiviral defense** consists of a cytokine-mediated reaction in which cells acquire resistance to viral infection and killing of virus-infected cells by NK cells.

Microbes that are able to withstand these defense reactions in the tissues may enter the blood, where they are recognized by the circulating proteins of innate immunity. Among the most important plasma proteins of innate immunity are the components of the alternative pathway of the complement system. When this pathway is activated by microbial surfaces, proteolytic cleavage products are generated that mediate inflammatory responses, coat the microbes for enhanced phagocytosis, and directly lyse microbes. (As we shall discuss later, complement can also be activated by antibodies—called the classical pathway, for historical reasons—with the same functional consequences.) Many of the circulating proteins enter sites of infection during inflammatory reactions and thus help combat microbes in extravascular tissues.

The reactions of innate immunity are effective at controlling and even eradicating infections. However, a hallmark of many pathogenic microbes is that they have evolved to resist innate immunity. Defense against these pathogens requires the more powerful and specialized mechanisms of adaptive immunity, which prevents them from invading and replicating in the cells and tissues of the host.

The Adaptive Immune Response

The adaptive immune system uses three main strategies to combat most microbes.

- Secreted ***antibodies*** bind to extracellular microbes, block their ability to infect host cells, and promote

their ingestion and subsequent destruction by phagocytes.

- ***Phagocytes*** ingest microbes and kill them, and helper T cells enhance the microbicidal abilities of the phagocytes.
- ***Cytotoxic T lymphocytes (CTLs)*** destroy cells infected by microbes that are inaccessible to antibodies and phagocytic destruction.

The goal of the adaptive response is to activate one or more of these defense mechanisms against diverse microbes that may be in different anatomic locations, such as intestinal lumens, the circulation, or inside cells.

All adaptive immune responses develop in steps, each of which corresponds to particular reactions of lymphocytes (Fig. 1-6). We start this overview of adaptive immunity with the first step, which is the recognition of antigens.

The Capture and Display of Microbial Antigens

Because the number of naive lymphocytes specific for any antigen is very small (on the order of 1 in 10^5 or 10^6 lymphocytes) and the quantity of the available antigen may also be small, special mechanisms are needed to capture microbes, to concentrate their antigens in the correct location, and to deliver the antigens to specific lymphocytes. Dendritic cells are the APCs that display microbial peptides to naive $CD4^+$ and $CD8^+$ T lymphocytes and initiate adaptive immune responses to protein antigens. Dendritic cells located in epithelia and connective tissues capture microbes, digest their proteins into peptides, and express on their surface peptides bound to MHC molecules, the specialized peptide display molecules of the adaptive immune system. Dendritic cells carry their antigenic cargo to draining lymph nodes and take up residence in the same regions of the nodes through which naive T lymphocytes continuously recirculate. Thus, the chance of a lymphocyte with receptors for an antigen finding that antigen is greatly increased by concentrating the antigen in recognizable form in the correct anatomic location. Dendritic cells also display the peptides of microbes that enter other lymphoid tissues, such as the spleen.

Intact microbes or microbial antigens that enter lymph nodes and spleen are recognized in unprocessed (native) form by specific B lymphocytes. There are also specialized APCs that display antigens to B lymphocytes.

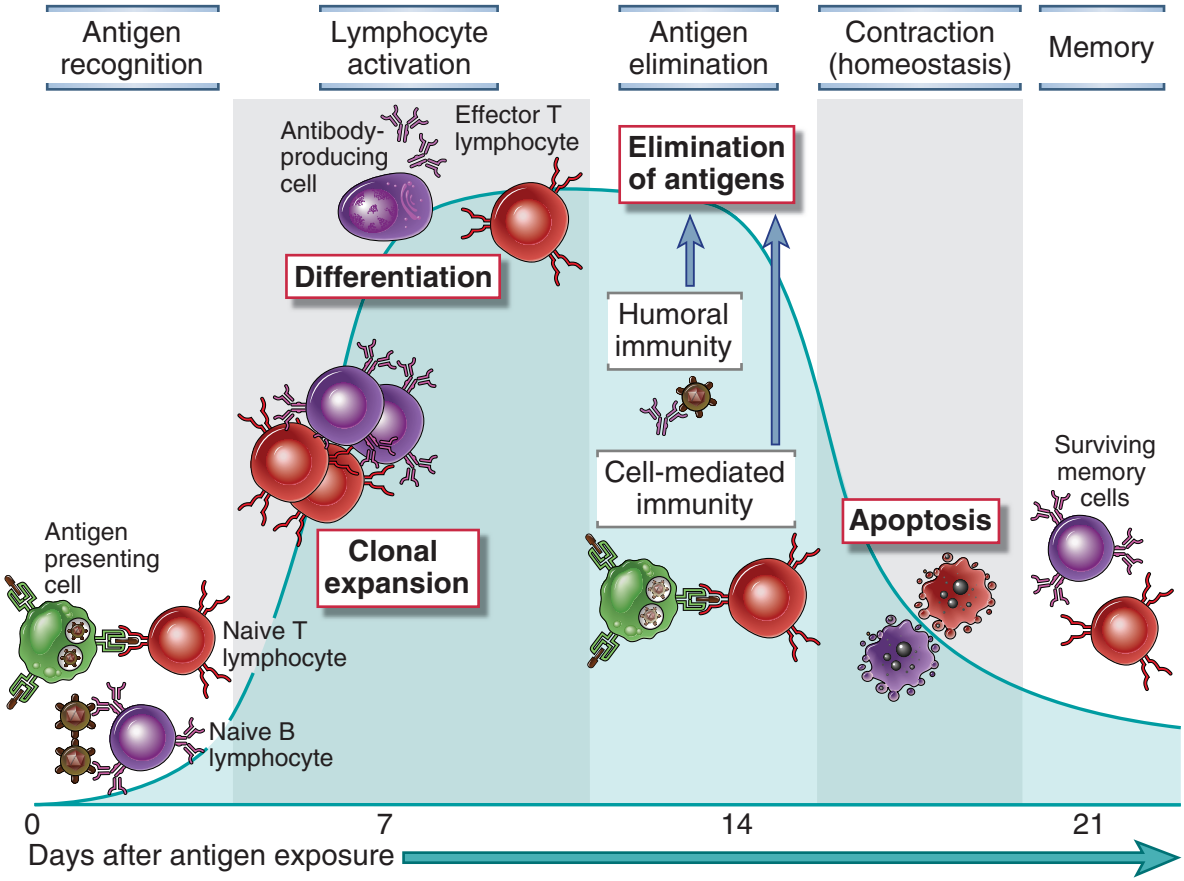


FIGURE 1–6 Phases of adaptive immune responses. Adaptive immune responses consist of distinct phases, the first three being the recognition of antigen, the activation of lymphocytes, and the elimination of antigen (the effector phase). The response contracts (declines) as antigen-stimulated lymphocytes die by apoptosis, restoring homeostasis, and the antigen-specific cells that survive are responsible for memory. The duration of each phase may vary in different immune responses. The y-axis represents an arbitrary measure of the magnitude of the response. These principles apply to humoral immunity (mediated by B lymphocytes) and cell-mediated immunity (mediated by T lymphocytes).

Antigen Recognition by Lymphocytes

Lymphocytes specific for a large number of antigens exist before exposure to the antigen, and when an antigen enters, it selects the specific cells and activates them (Fig. 1-7). This fundamental concept is called the **clonal selection hypothesis**. It was first suggested by Niels Jerne in 1955, and most clearly enunciated by Macfarlane Burnet in 1957, as a hypothesis to explain how the immune system could respond to a large number and variety of antigens. According to this hypothesis, antigen-specific clones of lymphocytes develop before and independent of exposure to antigen. A "clone" refers to a lymphocyte of one specificity and its progeny. A characteristic of the immune system is that a very large number of clones is generated during the maturation of lymphocytes, thus maximizing the potential for recognizing diverse microbes.

The activation of naive T lymphocytes requires recognition of peptide-MHC complexes presented on dendritic cells. The nature of the antigen that activates T cells (i.e., peptides bound to MHC molecules) ensures that these lymphocytes can interact only with other cells (because MHC molecules are cell surface proteins) and not with free antigen. This feature is necessary because all the functions of T lymphocytes are dependent on their physical interactions with other cells. To respond, the T cells need to recognize not only antigens but also other molecules, called costimulators, which are induced on the APCs by microbes. Antigen recognition provides specificity to the immune response, and the need for costimulation ensures that T cells respond to microbes (the inducers of costimulatory molecules) and not to harmless substances.

B lymphocytes use their antigen receptors (membrane-bound antibody molecules) to recognize antigens of many different chemical types.

Engagement of antigen receptors and other signals trigger lymphocyte proliferation and differentiation. The reactions and functions of T and B lymphocytes differ in important ways and are best considered separately.

Cell-Mediated Immunity: Activation of T Lymphocytes and Elimination of Intracellular Microbes

Activated $CD4^+$ helper T lymphocytes proliferate and differentiate into effector cells whose functions are mediated largely by secreted cytokines. One of the earliest responses of $CD4^+$ helper T cells is secretion of the cytokine interleukin-2 (IL-2). IL-2 is a growth factor that acts on the antigen-activated lymphocytes and stimulates their proliferation (clonal expansion). Some of the progeny differentiate into effector cells that can secrete different sets of cytokines and thus perform different functions. Many of these effector cells leave the lymphoid organs where they were generated and migrate to sites of infection and accompanying inflammation. When these differentiated effectors again encounter cell-associated microbes, they are activated to perform the functions that are responsible for elimination of the

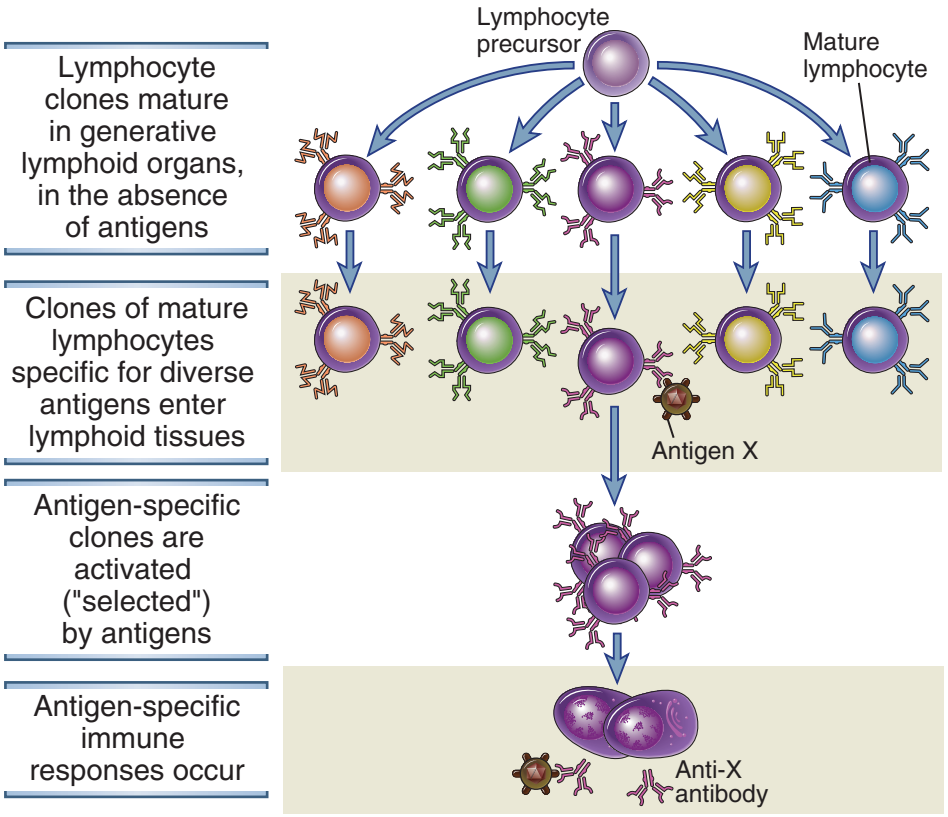


FIGURE 1–7 The clonal selection hypothesis. Each antigen (X or Y) selects a preexisting clone of specific lymphocytes and stimulates the proliferation and differentiation of that clone. The diagram shows only B lymphocytes giving rise to antibody-secreting effector cells, but the same principle applies to T lymphocytes.

microbes. Some effector T cells of the $CD4^+$ helper cell lineage secrete cytokines that recruit leukocytes and stimulate production of microbicidal substances in phagocytes. Thus, these helper T cells help the phagocytes kill the infectious pathogens. Other $CD4^+$ effector T cells secrete cytokines that stimulate the production of a special class of antibody called immunoglobulin E (IgE) and activate leukocytes called eosinophils, which are able to kill parasites that may be too large to be phagocytosed. As we discuss next, some $CD4^+$ helper T cells stay in the lymphoid organs and stimulate B cell responses.

Activated $CD8^+$ lymphocytes proliferate and differentiate into CTLs that kill cells harboring microbes in the cytoplasm. These microbes may be viruses that infect many cell types or bacteria that are ingested by macrophages but escape from phagocytic vesicles into the cytoplasm (where they are inaccessible to the killing machinery of phagocytes, which is largely confined to vesicles). By destroying the infected cells, CTLs eliminate the reservoirs of infection.

Humoral Immunity: Activation of B Lymphocytes and Elimination of Extracellular Microbes

On activation, B lymphocytes proliferate and differentiate into cells that secrete different classes of antibodies with distinct functions. The response of B cells to protein antigens requires activating signals ("help") from $CD4^+$ T cells (which is the historical reason for calling these T cells "helper" cells). B cells can respond to many nonprotein antigens without the participation of other cells.

Some of the progeny of the expanded B cell clones differentiate into antibody-secreting plasma cells. Each plasma cell secretes antibodies that have the same antigen-binding site as the cell surface antibodies (B cell receptors) that first recognized the antigen. Polysaccharides and lipids stimulate secretion mainly of the antibody class called IgM. Protein antigens induce the production of antibodies of functionally different classes (IgG, IgA, IgE) from a single clone of B cells. The production of these different antibodies, all with the same specificity, is called class switching and requires the action of helper T cells; it provides plasticity in the antibody response, enabling it to serve many functions. Helper T cells also stimulate the production of antibodies with increased affinity for the antigen. This process, called affinity maturation, improves the quality of the humoral immune response.

The humoral immune response combats microbes in many ways. Antibodies bind to microbes and prevent them from infecting cells, thus "neutralizing" the microbes and blocking their ability to infect host cells or to colonize tissues. In fact, antibodies are the only mechanisms of adaptive immunity that prevent an infection from becoming established; this is why eliciting the production of potent antibodies is a key goal of vaccination. IgG antibodies coat microbes and target them for phagocytosis because phagocytes (neutrophils and macrophages) express receptors for the tails of IgG. IgG and IgM activate the complement system, by the classical pathway, and complement products promote phagocytosis and destruction of microbes. Some antibodies serve special roles at particular anatomic sites. IgA is secreted from mucosal epithelia and neutralizes microbes in the lumens of the respiratory and gastrointestinal tracts (and other mucosal tissues). Maternal IgG is actively transported across the placenta and protects the newborn until the baby's immune system becomes mature. Most antibodies have half-lives of a few days, but some IgG antibodies have half-lives of about 3 weeks. Some antibody-secreting plasma cells migrate to the bone marrow and live for years, continuing to produce low levels of antibodies. The antibodies that are secreted by these long-lived plasma cells provide immediate protection if the microbe returns to infect the individual. More effective protection is provided by memory cells that are activated by the microbe and rapidly differentiate to generate large numbers of plasma cells.

Immunologic Memory

An effective immune response eliminates the microbes that initiated the response. This is followed by a contraction phase, in which the expanded lymphocyte clones die and homeostasis is restored.

The initial activation of lymphocytes generates long-lived memory cells, which may survive for years after the infection. Memory cells are more effective in combating microbes than are naive lymphocytes because, as mentioned earlier, memory cells represent an expanded pool of antigen-specific lymphocytes (more numerous than naive cells specific for the antigen), and memory cells respond faster and more effectively against the antigen than do naive cells. This is why generating memory responses is another important goal of vaccination. We will discuss the properties of memory lymphocytes in later chapters.

In the remainder of the book, we describe in detail the recognition, activation, regulation, and effector phases of innate and adaptive immune responses. The principles introduced in this chapter recur throughout the book.

SUMMARY

- Protective immunity against microbes is mediated by the early reactions of innate immunity and the later responses of adaptive immunity. Innate immune responses are stimulated by molecular structures shared by groups of microbes and by molecules expressed by damaged host cells. Adaptive immunity is specific for different microbial and nonmicrobial antigens and is increased by repeated exposures to antigen (immunologic memory).
- Humoral immunity is mediated by B lymphocytes and their secreted products, antibodies, and functions in defense against extracellular microbes. Cell-mediated immunity is mediated by T lymphocytes and their products, such as cytokines, and is important for defense against intracellular microbes.
- Immunity may be acquired by a response to antigen (active immunity) or conferred by transfer

of antibodies or cells from an immunized individual (passive immunity).

- The immune system possesses several properties that are of fundamental importance for its normal functions. These include specificity for different antigens, a diverse repertoire capable of recognizing a wide variety of antigens, memory of antigen exposure, the capacity for rapid expansion of clones of antigen-specific lymphocytes in response to the antigen, specialized responses to different microbes, maintenance of homeostasis, and the ability to discriminate between foreign antigens and self antigens.
- Lymphocytes are the only cells capable of specifically recognizing antigens and are thus the principal cells of adaptive immunity. The two major subpopulations of lymphocytes are B cells and T cells, and they differ in their antigen receptors and functions. Specialized antigen-presenting cells capture microbial antigens and display these antigens for recognition by lymphocytes. The elimination of antigens often requires the participation of various effector cells.
- The adaptive immune response is initiated by the recognition of foreign antigens by specific lymphocytes. Lymphocytes respond by proliferating and by differentiating into effector cells, whose function is to eliminate the antigen, and into memory cells, which show enhanced responses on subsequent encounters with the antigen. The activation of lymphocytes requires antigen and additional signals that may be provided by microbes or by innate immune responses to microbes.
- $CD4^+$ helper T lymphocytes help macrophages to eliminate ingested microbes and help B cells to produce antibodies. $CD8^+$ CTLs kill cells harboring intracellular pathogens, thus eliminating reservoirs of infection. Antibodies, the products of B lymphocytes, neutralize the infectivity of microbes and promote the elimination of microbes by phagocytes and by activation of the complement system.

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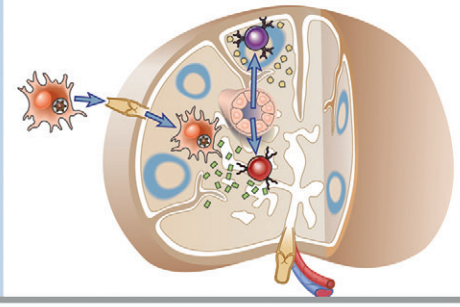
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CHAPTER

2

Cells and Tissues of the Immune System

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The cells of the innate and adaptive immune system are normally present as circulating cells in the blood and lymph, as anatomically defined collections in lymphoid organs, and as scattered cells in virtually all tissues. The anatomic organization of these cells and their ability to circulate and exchange among blood, lymph, and tissues are of critical importance for the generation of immune responses. The immune system faces numerous challenges to generate effective protective responses against infectious pathogens. First, the system must be able to respond rapidly to small numbers of many different microbes that may be introduced at any site in the body. Second, in the adaptive immune response, very few naive lymphocytes specifically recognize and respond to any one antigen. Third, the effector mechanisms of the adaptive immune system (antibodies and effector T cells) may have to locate and destroy microbes at sites that are distant from the site where the immune response was induced. The ability of the immune system to meet these challenges and to perform its protective functions optimally is dependent on several properties of its cells and tissues. The major cells and tissues of the immune system and their important roles are the following:

- Macrophages are phagocytes that are constitutively present in tissues and respond rapidly to microbes that enter these tissues.
- Neutrophils, an abundant type of phagocyte, and monocytes, the precursors of tissue macrophages, are always present in the blood and can be quickly delivered anywhere in the body.
- Specialized tissues, called peripheral lymphoid organs, function to concentrate microbial antigens that are introduced through the common portals of entry (skin and gastrointestinal and respiratory tracts). The capture of antigen and its transport to lymphoid organs are the first steps in adaptive immune responses. Antigens that are transported to lymphoid organs are displayed by antigen-presenting cells (APCs) for recognition by specific lymphocytes.
- Almost all tissues contain dendritic cells, which are APCs that are specialized to capture microbial antigens, to transport them to lymphoid tissues, and to present them for recognition by lymphocytes.
- Naive lymphocytes (lymphocytes that have not previously encountered antigens) migrate through these peripheral lymphoid organs, where they recognize antigens and initiate adaptive immune responses. The anatomy of lymphoid organs promotes cell-cell interactions that are required for antigen recognition by lymphocytes and for the activation of naive lymphocytes, resulting in the generation of effector and memory lymphocytes.
- Effector and memory lymphocytes circulate in the blood, home to peripheral sites of antigen entry, and are efficiently retained at these sites. This ensures that immunity is systemic (i.e., that protective mechanisms can act anywhere in the body).

Immune responses develop through a series of steps, in each of which the special properties of immune cells and tissues play critical roles. This chapter describes the cells, tissues, and organs that compose the immune system. In Chapter 3, we describe the traffic patterns of lymphocytes throughout the body and the mechanisms of migration of lymphocytes and other leukocytes.

TABLE 2–1 Normal Blood Cell Counts

	Mean Number per Microliter	Normal Range
White blood cells (leukocytes)	7400	4500-11,000
Neutrophils	4400	1800-7700
Eosinophils	200	0-450
Basophils	40	0-200
Lymphocytes	2500	1000-4800
Monocytes	300	0-800

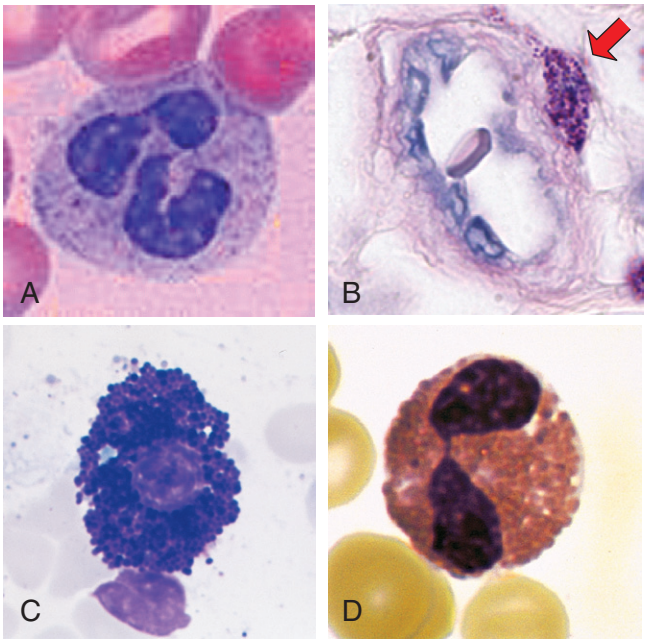


FIGURE 2–1 Morphology of neutrophils, mast cells, basophils, and eosinophils. A, The light micrograph of a Wright-Giemsa–stained blood neutrophil shows the multilobed nucleus, because of which these cells are also called polymorphonuclear leukocytes, and the faint cytoplasmic granules. **B,** The light micrograph of a Wright-Giemsa–stained section of skin shows a mast cell *(arrow)* adjacent to a small blood vessel, identifiable by the red blood cell in the lumen. The cytoplasmic granules in the mast cell, which are stained purple, are filled with histamine and other mediators that act on adjacent blood vessels to promote increased blood flow and delivery of plasma proteins and leukocytes into the tissue. *(Courtesy of Dr. George Murphy, Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts.)* **C,** The light micrograph of a Wright-Giemsa–stained blood basophil shows the characteristic blue-staining cytoplasmic granules. *(Courtesy of Dr. Jonathan Hecht, Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts.)* **D,** The light micrograph of a Wright-Giemsa–stained blood eosinophil shows the characteristic segmented nucleus and red staining of the cytoplasmic granules.

CELLS OF THE IMMUNE SYSTEM

The cells that serve specialized roles in innate and adaptive immune responses are phagocytes, dendritic cells, antigen-specific lymphocytes, and various other leukocytes that function to eliminate antigens. The cells of the immune system were introduced briefly in Chapter 1. Here we describe the morphology and functional characteristics of phagocytes, other leukocytes, APCs, and lymphocytes and how these cells are organized in lymphoid tissues. The numbers of some of these cell types in the blood are listed in Table 2-1. Although most of these cells are found in the blood, their responses to microbes are usually localized to tissues and are generally not reflected in changes in the total numbers of circulating leukocytes.

Phagocytes

Phagocytes, including neutrophils and macrophages, are cells whose primary function is to identify, ingest, and destroy microbes. The functional responses of phagocytes in host defense consist of sequential steps: recruitment of the cells to the sites of infection, recognition of and activation by microbes, ingestion of the microbes by the process of phagocytosis, and destruction of ingested microbes. In addition, through direct contact and by secreting proteins, phagocytes communicate with other cells in ways that promote or regulate immune responses. The effector functions of phagocytes are important in innate immunity, discussed in Chapter 4, and also in the effector phase of some adaptive immune responses, as we will discuss in Chapter 10. As a prelude to more detailed discussions of the role of phagocytes in immune responses in later chapters, we will now describe their morphologic features and briefly introduce the functional responses of neutrophils and macrophages.

Neutrophils

Neutrophils, also called polymorphonuclear leukocytes, are the most abundant population of circulating white blood cells and mediate the earliest phases of inflammatory reactions. Neutrophils circulate as spherical cells about 12 to 15 μm in diameter with numerous membranous projections. The nucleus of a neutrophil is segmented into three to five connected lobules, hence the synonym *polymorphonuclear leukocyte* (Fig. 2-1A). The cytoplasm contains granules of two types. The majority, called specific granules, are filled with enzymes such as lysozyme, collagenase, and elastase. These granules do not stain strongly with either basic or acidic dyes (hematoxylin and eosin, respectively), which distinguishes neutrophil granules from those of two other types of circulating granulocytes, called basophils and eosinophils. The remainder of the granules of neutrophils, called azurophilic granules, are lysosomes containing enzymes and other microbicidal substances, including defensins and cathelicidins, which we will discuss in Chapter 4. Neutrophils are produced in the bone marrow and arise from a common lineage with mononuclear phagocytes. Production of neutrophils is stimulated by granulocyte colony-stimulating factor (G-CSF). An adult human produces more than 1×10^{11} neutrophils per day, each of which circulates in the blood for only about 6 hours. Neutrophils may migrate to sites of infection within a few hours after the entry of microbes. If a circulating neutrophil is not recruited into a site of inflammation within this period, it undergoes apoptosis and is usually phagocytosed by resident macrophages in the

liver or spleen. After entering tissues, neutrophils function for a few hours and then die.

Mononuclear Phagocytes

The mononuclear phagocyte system consists of cells whose primary function is phagocytosis and that play central roles in innate and adaptive immunity. The cells of the mononuclear phagocyte system originate from a common precursor in the bone marrow, circulate in the blood, and mature and become activated in various tissues (Fig. 2-2). The cell type in this lineage that enters the peripheral blood from the marrow is incompletely differentiated and is called the monocyte. Monocytes are 10 to 15 μm in diameter, and they have bean-shaped nuclei and finely granular cytoplasm containing lysosomes, phagocytic vacuoles, and cytoskeletal filaments (Fig. 2-3). Monocytes are heterogeneous and consist of at least two subsets, which are distinguishable by cell surface proteins and kinetics of migration into tissues. One population is called inflammatory because it is rapidly recruited from the blood into sites of tissue inflammation. The other type may be the source of tissue resident macrophages and some dendritic cells.

Once they enter tissues, these monocytes mature and become macrophages. Macrophages in different tissues have been given special names to designate specific locations. For instance, in the central nervous system, they are called microglial cells; when lining the vascular sinusoids of the liver, they are called Kupffer cells; in pulmonary airways, they are called alveolar macrophages; and multinucleate phagocytes in bone are called osteoclasts.

Macrophages perform several important functions in innate and adaptive immunity.

- A major function of macrophages in host defense is to ingest and kill microbes. The mechanisms of killing, which are discussed in Chapter 4, include the enzymatic generation of reactive oxygen and nitrogen species that are toxic to microbes, and proteolytic digestion.

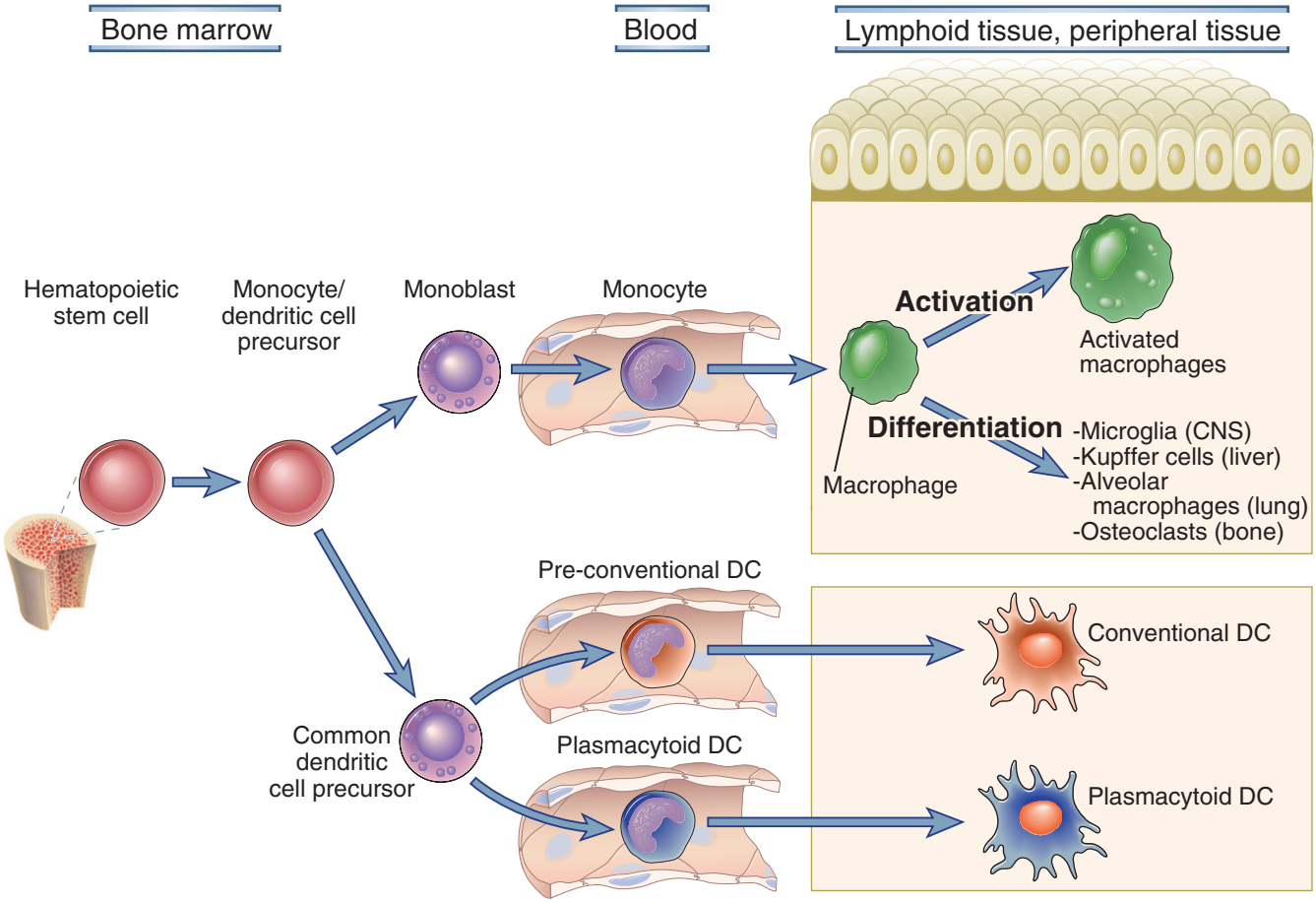


FIGURE 2–2 Maturation of mononuclear phagocytes and dendritic cells. Both dendritic cells and monocytes arise from a common precursor cell of the myeloid lineage in the bone marrow, and differentiation into monocytes or dendritic cells is driven by the cytokines monocyte colony-stimulating factor and Flt3 ligand, respectively (not shown). Dendritic cells further differentiate into subsets, the two major being conventional dendritic cells and plasmacytoid dendritic cells. Some dendritic cells may arise from monocytes in inflamed tissues. When blood monocytes are recruited into tissues, they become macrophages. Long-lived resident macrophages are present in all tissues of the body. At least two populations of blood monocytes exist (not shown), which are precursors, respectively, of macrophages that accumulate in response to infections and macrophages that are constitutively present in normal tissues. Macrophages in tissues become activated to perform antimicrobial and tissue repair functions in response to infections and tissue injury. Macrophages differentiate into specialized forms in particular tissues. CNS, central nervous system; DC, dendritic cell.

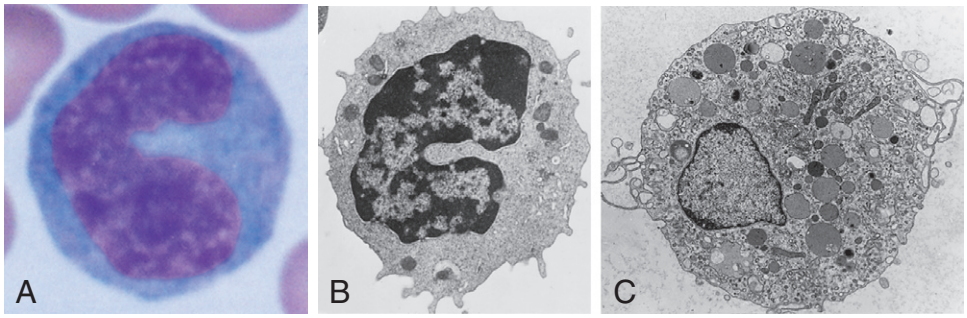


FIGURE 2–3 Morphology of mononuclear phagocytes. A, Light micrograph of a monocyte in a peripheral blood smear. **B,** Electron micrograph of a peripheral blood monocyte. *(Courtesy of Dr. Noel Weidner, Department of Pathology, University of California, San Diego.)* **C,** Electron micrograph of an activated tissue macrophage showing numerous phagocytic vacuoles and cytoplasmic organelles. *(From Fawcett DW. Bloom and Fawcett: A Textbook of Histology, 12th ed. Chapman & Hall, New York, 1994. With kind permission of Springer Science and Business Media.)*

- In addition to ingesting microbes, macrophages also ingest dead host cells as part of the cleaning up process after infection or sterile tissue injury. For example, they phagocytose dead neutrophils, which rapidly accumulate in sites of infection or tissue death caused by trauma or interrupted blood supply. Macrophages also recognize and engulf apoptotic cells before the dead cells can release their contents and induce inflammatory responses. Throughout the body and throughout the life of an individual, unwanted cells die by apoptosis, as part of many physiologic processes, such as development, growth, and renewal of healthy tissues, and the dead cells must be cleaned up by macrophages.
- Activated macrophages secrete proteins, called **cytokines**, that bind to signaling receptors on other cells and thereby instruct those cells to respond in ways that contribute to host defense. For example, some cytokines act on endothelial cells lining blood vessels to enhance the recruitment of more monocytes from the blood into sites of infections, thereby amplifying the protective response against the microbes. There are many different cytokines that are involved in every aspect of immune responses. The general properties and different classes of cytokines were discussed in Chapter 1.
- Macrophages serve as APCs that display antigens to and activate T lymphocytes. This function is important in the effector phase of T cell–mediated immune responses (see Chapter 10).
- Another important function of macrophages is to promote repair of damaged tissues by stimulating new blood vessel growth (angiogenesis) and synthesis of collagen-rich extracellular matrix (fibrosis). This function is mediated by certain cytokines secreted by the macrophages that act on various tissue cells.

Macrophages are activated to perform their functions by recognizing many different kinds of microbial molecules as well as host molecules produced in response to infections. These various activating molecules bind to specific signaling receptors located on the surface of or inside the macrophage. An example of these receptors is the Toll-like receptors, which are of central importance in innate immunity and will be discussed in detail in Chapter 4. Macrophages are also activated when receptors on their plasma membrane bind opsonins on the surface of microbes. Opsonins are substances that coat particles for phagocytosis. Examples of these opsonin receptors are complement receptors and antibody Fc receptors, discussed in Chapter 12. In adaptive immunity, macrophages are activated by secreted cytokines and membrane proteins made by T lymphocytes, discussed in Chapter 10.

Macrophages can acquire distinct functional capabilities, depending on the types of activating stimuli. The clearest example of this is the response of macrophages to different cytokines made by subsets of T cells. Some of these cytokines activate macrophages to become efficient at killing microbes, called classical activation. Other cytokines activate macrophages to promote tissue remodeling and repair, called alternative activation. The details of these different forms of activation, and the cytokines involved, are discussed in Chapter 10. Macrophages may also assume different morphologic forms after activation by external stimuli, such as microbes. Some develop abundant cytoplasm and are called epithelioid cells because of their resemblance to epithelial cells of the skin. Activated macrophages can fuse to form multinucleate giant cells.

Macrophage-like cells are phylogenetically the oldest mediators of innate immunity. *Drosophila* responds to infection by surrounding microbes with "hemocytes," which are similar to macrophages, and these cells phagocytose the microbes and wall off the infection by inducing coagulation of the surrounding hemolymph. Similar phagocyte-like cells have been identified even in plants.

Macrophages typically respond to microbes nearly as rapidly as neutrophils do, but macrophages survive much longer at sites of inflammation. Unlike neutrophils, macrophages are not terminally differentiated and can undergo cell division at an inflammatory site. Therefore, macrophages are the dominant effector cells of the later stages of the innate immune response, several days after infection.

Mast Cells, Basophils, Eosinophils

Mast cells, basophils, and eosinophils are three additional cells that play roles in innate and adaptive immune

responses. All three cell types share the common feature of having cytoplasmic granules filled with various inflammatory and antimicrobial mediators. Another common feature of these cells is their involvement in immune responses that protect against helminths and immune responses that cause allergic diseases. We will describe the major features of these cells in this section and discuss their functions in more detail in Chapter 19.

Mast Cells

Mast cells are bone marrow–derived cells that are present in the skin and mucosal epithelium and contain abundant cytoplasmic granules filled with cytokines histamine, and other mediators. Stem cell factor (also called c-Kit ligand) is a cytokine that is essential for mast cell development. Normally, mature mast cells are not found in the circulation but are constitutively present in healthy tissues, usually adjacent to small blood vessels and nerves. Human mast cells vary in shape and have round nuclei, and the cytoplasm contains membrane-bound granules (see Fig. 2-1B). The granules contain acidic proteoglycans that bind basic dyes. Mast cells express plasma membrane receptors for IgE and IgG antibodies and are usually coated with these antibodies. When these antibodies on the mast cell surface also bind antigen, signaling events are induced that lead to release of the cytoplasmic granule contents into the extracellular space. The released contents of the granules, including cytokines and histamine, promote changes in the blood vessels that cause inflammation. Mast cells also express other activating receptors that recognize complement proteins, neuropeptides, and microbial products. Mast cells provide defense against helminths but are also responsible for symptoms of allergic diseases (see Chapter 19).

Basophils

Basophils are blood granulocytes with many structural and functional similarities to mast cells. Like other granulocytes, basophils are derived from bone marrow progenitors (a lineage different from that of mast cells), mature in the bone marrow, and circulate in the blood. Basophils constitute less than 1% of blood leukocytes (see Table 2-1). Although they are normally not present in tissues, basophils may be recruited to some inflammatory sites. Basophils contain granules that bind basic dyes (see Fig. 2-1C), and they are capable of synthesizing many of the same mediators as mast cells. Like mast cells, basophils express IgG and IgE receptors, bind IgE, and can be triggered by antigen binding to the IgE. Because basophil numbers are low in tissues, their importance in host defense and allergic reactions is uncertain.

Eosinophils

Eosinophils are blood granulocytes that express cytoplasmic granules containing enzymes that are harmful to the cell walls of parasites but can also damage host tissues. The granules of eosinophils contain basic proteins that bind acidic dyes such as eosin (see Fig. 2-1D). Like neutrophils and basophils, eosinophils are bone marrow derived. GM-CSF, IL-3, and IL-5 promote eosinophil maturation from myeloid precursors. Some eosinophils are normally present in peripheral tissues, especially in mucosal linings of the respiratory, gastrointestinal, and genitourinary tracts, and their numbers can increase by recruitment from the blood in the setting of inflammation.

Antigen-Presenting Cells

Antigen-presenting cells (APCs) are cell populations that are specialized to capture microbial and other antigens, display them to lymphocytes, and provide signals that stimulate the proliferation and differentiation of the lymphocytes. By convention, APC usually refers to a cell that displays antigens to T lymphocytes. The major type of APC that is involved in initiating T cell responses is the dendritic cell. Macrophages and B cells present antigens to T lymphocytes in different types of immune responses, and a specialized cell type called the follicular dendritic cell displays antigens to B lymphocytes during particular phases of humoral immune responses. APCs link responses of the innate immune system to responses of the adaptive immune system, and therefore they may be considered components of both systems. In addition to the introduction presented here, APC function will be described in more detail in Chapter 6.

Dendritic Cells

Dendritic cells are the most important APCs for activating naive T cells, and they play major roles in innate responses to infections and in linking innate and adaptive immune responses. They have long membranous projections and phagocytic capabilities and are widely distributed in lymphoid tissues, mucosal epithelium, and organ parenchyma (Fig. 2-4). Dendritic cells are part of the myeloid lineage of hematopoietic cells and arise from a precursor that can also differentiate into monocytes but not granulocytes (see Fig. 2-2). Maturation of dendritic cells is dependent on a cytokine called Flt3 ligand, which binds to the Flt3 tyrosine kinase receptor on the precursor cells. Similar to macrophages, dendritic cells express receptors that recognize molecules typically made by microbes and not mammalian cells, and they respond to the microbes by secreting cytokines. The majority of dendritic cells are called conventional dendritic cells. In response to activation by microbes, conventional dendritic cells in skin, mucosa, and organ parenchyma become mobile, migrate to lymph nodes, and display microbial antigens to T lymphocytes. Thus, these cells function in both innate and adaptive immune responses and are a link between these two components of host defense. One subpopulation of dendritic cells, called plasmacytoid dendritic cells, are early cellular responders to viral infection. They recognize nucleic acids of intracellular viruses and produce soluble proteins called type I interferons, which have potent antiviral activities. We will discuss the role of dendritic cells as mediators of innate immunity and as APCs in Chapters 4 and 6, respectively.

Antigen-Presenting Cells for Effector T Lymphocytes

In addition to dendritic cells, macrophages and B lymphocytes perform important antigen-presenting

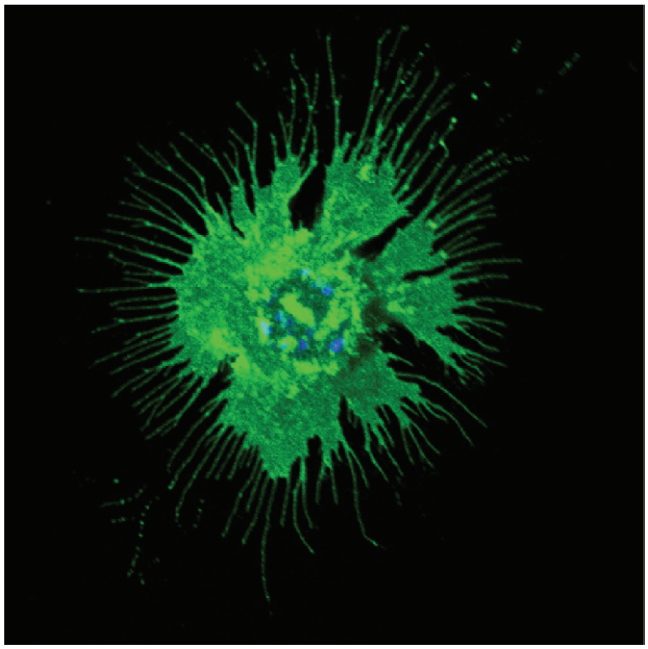


FIGURE 2–4 A dendritic cell. The fluorescence photomicrograph shows a bone marrow–derived dendritic cell in which class II MHC molecules appear green, highlighting the fine cytoplasmic processes characteristic of dendritic cells, and the nucleus appears blue. Class II MHC molecules are highly expressed in dendritic cells and are important for their function (see Chapter 6). *(Courtesy of Scott Loughhead and Uli Van Andrian, Harvard Medical School, Boston, Massachusetts.)*

functions in $CD4^+$ helper T cell–mediated immune responses. Macrophages present antigen to helper T lymphocytes at the sites of infection, which leads to helper T cell activation and production of molecules that further activate the macrophages. This process is important for the eradication of microbes that are ingested by the phagocytes but resist killing; in these cases, helper T cells greatly enhance the microbicidal activities of the macrophages. B cells present antigens to helper T cells in lymph nodes and spleen, which is a key step in the cooperation of helper T cells with B cells in humoral immune responses to protein antigens. These functions of macrophages and B cells will be discussed in Chapters 10 and 11. Cytotoxic T lymphocytes (CTLs) are effector $CD8^+$ T cells that can recognize antigens on any type of nucleated cell and become activated to kill the cell. Therefore, all nucleated cells are potentially APCs for CTLs.

Follicular Dendritic Cells

Follicular dendritic cells (FDCs) are cells with membranous projections that are found intermingled in specialized collections of activated B cells, called germinal centers, in the lymphoid follicles of the lymph nodes, spleen, and mucosal lymphoid tissues. FDCs are not derived from precursors in the bone marrow and are unrelated to the dendritic cells that present antigens to T lymphocytes. FDCs trap antigens complexed to antibodies or complement products and display these antigens on their surfaces for recognition by B lymphocytes. This is important for the selection of activated B lymphocytes whose antigen receptors bind the displayed antigens with high affinity (see Chapter 11).

Lymphocytes

Lymphocytes, the unique cells of adaptive immunity, are the only cells in the body that express clonally distributed antigen receptors, each with a fine specificity for a different antigenic determinant. Each clone of lymphocytes consists of the progeny of one cell and expresses antigen receptors with a single specificity. This is why the total population of antigen receptors in the adaptive immune system is said to be clonally distributed. As we shall discuss here and in later chapters, there are millions of lymphocyte clones in the body, enabling the organism to recognize and respond to millions of foreign antigens.

The role of lymphocytes as the cells that mediate adaptive immunity was established during decades of research by several lines of evidence. One of the earliest clues about the importance of lymphocytes in adaptive immunity came from the discovery that humans with congenital and acquired immune deficiency states had reduced numbers of lymphocytes in the peripheral circulation and in lymphoid tissues. Furthermore, physicians noted that depletion of lymphocytes with drugs or irradiation impaired immune protection against infection. Experiments done mainly with mice showed that protective immunity to microbes can be adoptively transferred from immunized to naive animals only by lymphocytes or their secreted products. In vitro experiments established that stimulation of lymphocytes with antigens leads to responses that show many of the characteristics of immune responses induced under more physiologic conditions in vivo. Following the identification of lymphocytes as the mediators of humoral and cellular immunity, many discoveries were made at a rapid pace about different types of lymphocytes, their origins in the bone marrow and thymus, and the consequences of the absence of each type of lymphocyte. These discoveries relied on many tools, including genetically modified mice and reagents that selectively deplete one or another type of lymphocyte. Among the most important of these discoveries was that clonally distributed, highly diverse and specific receptors for antigens are produced by lymphocytes but not by any other types of cells. During the past two decades, there has been an enormous expansion of information about lymphocyte genes, proteins, and functions. We probably now know more about lymphocytes than about any other cells in all of biology.

One of the most interesting questions about lymphocytes has been how the enormously diverse repertoire of antigen receptors, and therefore specificities, is generated from a small number of genes for these receptors in the germline. It is now known that the genes encoding the antigen receptors of lymphocytes are formed by recombination of DNA segments during the maturation of these cells. There is a random aspect to these somatic recombination events that results in the generation of millions of different receptor genes and a highly diverse repertoire of antigen specificities among different clones of lymphocytes (see Chapter 8).

TABLE 2–2 Lymphocyte Classes

Class	Functions	Antigen Receptor and Specificity	Selected Phenotype Markers	Percentage of Total Lymphocytes (Human)		
				Blood	Lymph Node	Spleen
αβ T lymphocytes						
$CD4^+$ helper T lymphocytes	B cell differentiation (humoral immunity) Macrophage activation (cell-mediated immunity) Stimulation of inflammation	αβ heterodimers Diverse specificities for peptide–class II MHC complexes	$CD3^+$, $CD4^+$, $CD8^-$	50-60*	50-60	50-60
$CD8^+$ cytotoxic T lymphocytes	Killing of cells infected with viruses or intracellular bacteria; rejection of allografts	αβ heterodimers Diverse specificities for peptide–class I MHC complexes	$CD3^+$, $CD4^+$, $CD8^-$	20-25	15-20	10-15
Regulatory T cells	Suppress function of other T cells (regulation of immune responses, maintenance of self-tolerance)	αβ heterodimers Unresolved	$CD3^+$, $CD4^+$, $CD25^+$ (most common, but other phenotypes as well)	Rare	10	10
γδ T lymphocytes	Helper and cytotoxic functions (innate immunity)	γδ heterodimers Limited specificities for peptide and nonpeptide antigens	$CD3^+$, $CD4^+$, and CD8 variable			
B lymphocytes	Antibody production (humoral immunity)	Surface antibody Diverse specificities for all types of molecules	Fc receptors; class II MHC; CD19, CD21	10-15	20-25	40-45
Natural killer cells	Cytotoxic killing of virus-infected or damaged cells (innate immunity)	Various activating and inhibitory receptors Limited specificities for MHC or MHC-like molecules	CD16 (Fc receptor for IgG)	10	Rare	10
NKT cells	Suppress or activate innate and adaptive immune responses	αβ heterodimers Limited specificity for glycolipid-CD1 complexes	CD16 (Fc receptor for IgG); CD3	10	Rare	10

*In most cases, the ratio of $CD4^+CD8^-$ to $CD8^+CD4^-$ is about 2:1.
IgG, immunoglobulin G; MHC, major histocompatibility complex.

The total number of lymphocytes in a healthy adult is about 5×10^{11}. Of these, ~2% are in the blood, ~10% in the bone marrow, ~15% in the mucosal lymphoid tissues of the gastrointestinal and respiratory tracts, and ~65% in lymphoid organs (mainly the lymph nodes and spleen). We first describe the properties of these cells and then their organization in various lymphoid tissues.

Subsets of Lymphocytes

Lymphocytes consist of distinct subsets that are different in their functions and protein products (Table 2-2). The major classes of lymphocytes were introduced in Chapter 1 (see Fig. 1-5). Morphologically, all lymphocytes are similar, and their appearance does not reflect their heterogeneity or their diverse functions. **B lymphocytes**, the cells that produce antibodies, were so called because in birds they were found to mature in an organ called the bursa of Fabricius. In mammals, no anatomic equivalent of the bursa exists, and the early stages of B cell maturation occur in the bone marrow. Thus, "B" lymphocytes refer to bursa-derived lymphocytes or bone marrow–derived lymphocytes. **T lymphocytes**, the mediators of cellular immunity, were named because their precursors, which arise in the bone marrow, migrate to and mature in the thymus; "T" lymphocytes refer to thymus-derived lymphocytes. B and T lymphocytes each consist of subsets with distinct phenotypic and functional characteristics. The major subsets of B cells are follicular B cells, marginal zone B cells, and B-1 B cells, each of which is found in distinct anatomic locations within lymphoid tissues. The two major T cell subsets are helper $CD4^+$ T lymphocytes and $CD8^+$ CTLs, which express an antigen receptor called the αβ receptor. $CD4^+$ regulatory T cells are a third unique subset of T cells expressing the αβ receptor. Another population of T cells, called γδ T cells, expresses a similar but structurally distinct type of antigen receptor. The different functions of these classes of B and T cells will be discussed in later chapters.

The major populations of B cells and T cells express highly diverse, clonally distributed sets of antigen receptors. Some numerically minor subsets of lymphocytes, including γδ T cells, marginal zone B cells, and B-1 B cells, are restricted in their use of DNA segments that contribute to their antigen receptor genes, and these lymphocyte subsets have very limited diversity.

In addition to B and T cells, there exist other populations of cells that are called lymphocytes on the basis of morphology and certain functional and molecular criteria but that are not readily categorized as T or B cells. **Natural killer (NK) cells**, which are described in Chapter 4, have similar effector functions as CTLs, but their receptors are distinct from B or T cell antigen receptors and are not encoded by somatically recombined genes. NKT cells are a numerically small population of T lymphocytes that are so named because they express a surface molecule typically found on NK cells. They express αβ antigen receptors that are encoded by somatically recombined genes, but like γδ T cells and B-1 B cells, they lack diversity. NKT cells, γδ T cells, and B-1 B cells may all be considered part of both adaptive and innate immune systems.

Membrane proteins are used as phenotypic markers to distinguish distinct populations of lymphocytes (see Table 2-2). For instance, most helper T cells express a surface protein called CD4, and most CTLs express a different surface protein called CD8. These and many other surface proteins are often called markers because they identify and discriminate between ("mark") different cell populations. These markers not only delineate the different classes of lymphocytes but also have many functions in the cell types in which they are expressed. The most common way to determine if a surface phenotypic marker is expressed on a cell is to test if antibodies specific for the marker bind to the cell. In this context, the antibodies are used by investigators or clinicians as analytical tools. There are available thousands of different pure antibody preparations, called monoclonal antibodies, each specific for a different molecule and labeled with probes that can be readily detected on cell surfaces by use of appropriate instruments. (Monoclonal antibodies are described in Chapter 5, and methods to detect labeled antibodies bound to cells are discussed in Appendix IV.) The cluster of differentiation (CD) system is a widely adopted uniform method for naming cell surface molecules that are characteristic of a particular cell lineage or differentiation stage, have a defined structure, and are recognized by a group ("cluster") of monoclonal antibodies. Thus, all structurally well defined cell surface molecules are given a CD number designation (e.g., CD1, CD2). A current list of CD markers for leukocytes that are mentioned in the book is provided in Appendix III.

Development of Lymphocytes

After birth, lymphocytes, like all blood cells, arise from stem cells in the bone marrow. The origin of lymphocytes from bone marrow progenitors was first demonstrated by experiments with radiation-induced bone marrow chimeras. Lymphocytes and their precursors are radiosensitive and are killed by high doses of γ-irradiation. If a mouse of one inbred strain is irradiated and then injected with bone marrow cells or small numbers of hematopoietic stem cells of another strain that can be distinguished from the host, all the lymphocytes that develop subsequently are derived from the bone marrow cells or hematopoietic stem cells of the donor. Such approaches have proved useful for examining the maturation of lymphocytes and other blood cells.

All lymphocytes go through complex maturation stages during which they express antigen receptors and acquire the functional and phenotypic characteristics of mature cells. The anatomic sites where the major steps

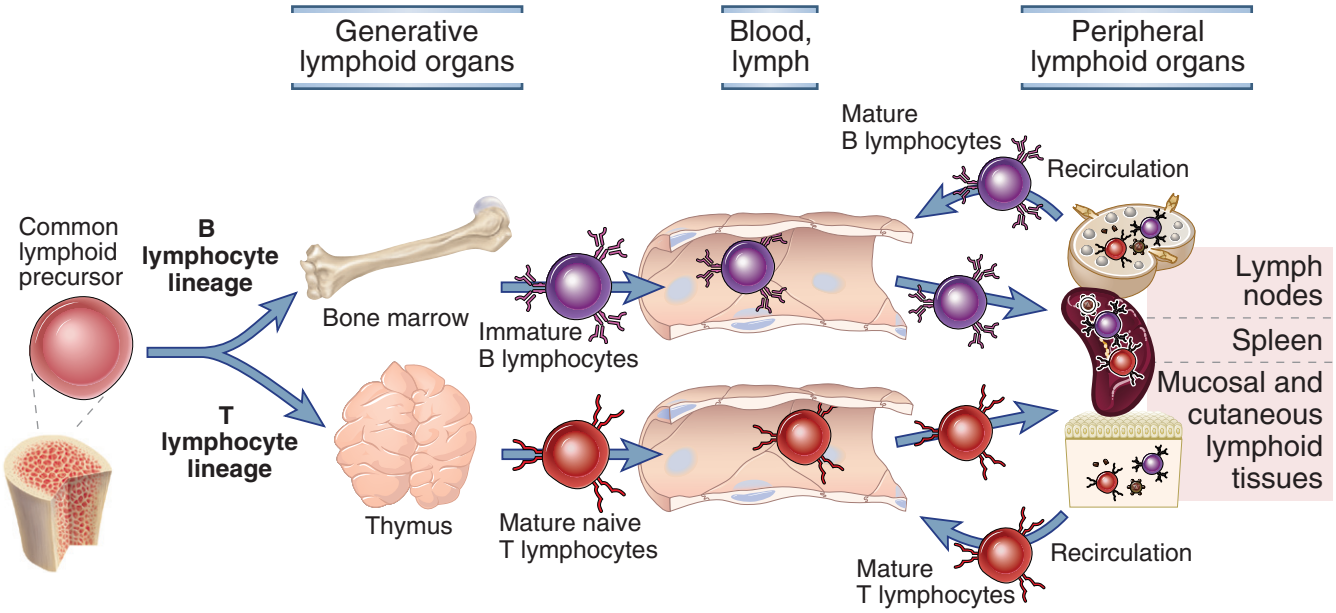


FIGURE 2–5 Maturation of lymphocytes. Lymphocytes develop from bone marrow stem cells and mature in the generative lymphoid organs (bone marrow and thymus for B and T cells, respectively) and then circulate through the blood to secondary lymphoid organs (lymph nodes, spleen, regional lymphoid tissues such as mucosa-associated lymphoid tissues). Fully mature T cells leave the thymus, but immature B cells leave the bone marrow and complete their maturation in secondary lymphoid organs. Naive lymphocytes may respond to foreign antigens in these secondary lymphoid tissues or return by lymphatic drainage to the blood and recirculate through other secondary lymphoid organs.

in lymphocyte development occur are called the generative lymphoid organs. These include the bone marrow, where precursors of all lymphocytes arise and B cells mature, and the thymus, where T cells mature (Fig. 2-5). We will discuss the processes of B and T lymphocyte maturation in much more detail in Chapter 8. These mature B and T cells are called **naive lymphocytes**. After activation by antigen, lymphocytes go through sequential changes in phenotype and functional capacity.

Populations of Lymphocytes Distinguished by History of Antigen Exposure

In adaptive immune responses, naive lymphocytes that emerge from the bone marrow or thymus migrate into peripheral lymphoid organs, where they are activated by antigens to proliferate and differentiate into effector and memory cells, some of which then migrate into tissues (Fig. 2-6). The activation of lymphocytes follows a series of sequential steps beginning with the synthesis of new proteins, such as cytokine receptors and cytokines, which are required for many of the subsequent changes. The naive cells then undergo proliferation, resulting in increased size of the antigen-specific clones, a process that is called **clonal expansion**. In some infections, the numbers of microbe-specific T cells may increase more than 50,000-fold, and the numbers of specific B cells may increase up to 5000-fold. This rapid clonal expansion of microbe-specific lymphocytes is needed to keep pace with the ability of microbes to rapidly replicate and expand in numbers. Concurrently with clonal expansion, antigen-stimulated lymphocytes differentiate into **effector cells** whose function is to eliminate the antigen. Some of the progeny of antigen-stimulated B and T lymphocytes differentiate into long-lived **memory cells**, whose function is to mediate rapid and enhanced (i.e., secondary) responses to subsequent exposures to antigens. Distinct populations of lymphocytes (naive, effector, and memory) are always present in various sites throughout the body, and these populations can be distinguished by several functional and phenotypic criteria (Table 2-3).

The details of lymphocyte activation and differentiation as well as the functions of each of these populations will be addressed later in this book. Here we summarize the phenotypic characteristics of each population.

Naive Lymphocytes

Naive lymphocytes are mature T or B cells that reside in the peripheral lymphoid organs and circulation and have never encountered foreign antigen. (The term *naive* refers to the idea that these cells are immunologically

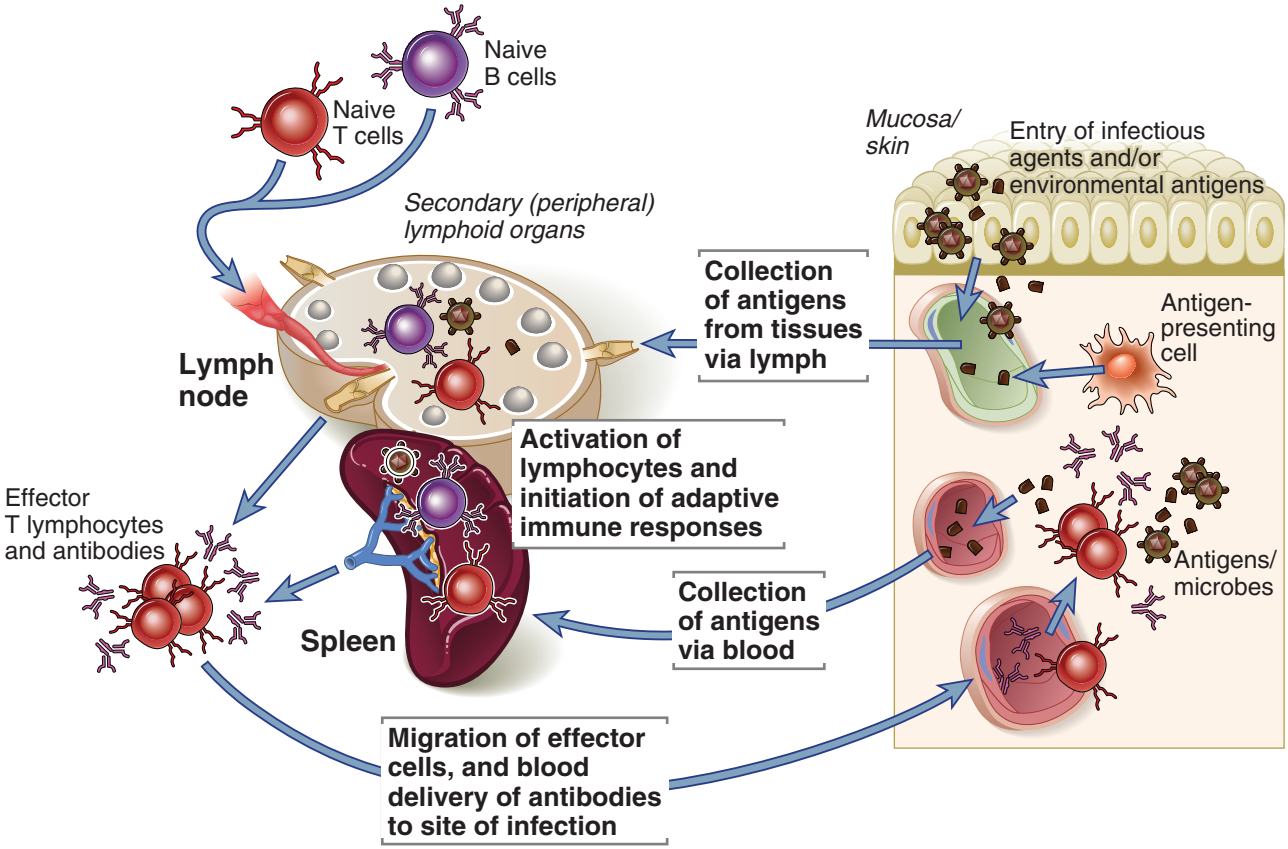


FIGURE 2–6 The anatomy of lymphocyte activation. Naive T cells emerging from the thymus and immature B cells emerging from the bone marrow migrate into secondary lymphoid organs, including lymph nodes and spleen. In these locations, B cells complete their maturation; naive B and T cells activated by antigens differentiate into effector and memory lymphocytes. Some effector and memory lymphocytes migrate into peripheral tissue sites of infection. Antibodies secreted by effector B cells in lymph node, spleen, and bone marrow (not shown) enter the blood and are delivered to sites of infection.

TABLE 2–3 Characteristics of Naive, Effector, and Memory Lymphocytes

	Naive Cell	Activated or Effector Lymphocytes	Memory Lymphocytes
T Lymphocytes			
Migration	Preferentially to peripheral lymph nodes	Preferentially to inflamed tissues	Preferentially to inflamed tissues, mucosal tissues
Frequency of cells responsive to particular antigen	Very low	High	Low
Effector functions	None	Cytokine secretion; cytotoxic activity	None
Cell cycling	No	Yes	+/–
Surface protein expression			
IL-2R (CD25)	Low	High	Low
L-selectin (CD62L)	High	Low	Variable
IL-7R (CD127)	Moderately high	Low	High
Adhesion molecules: integrins, CD44	Low	High	High
Chemokine receptor: CCR7	High	Low	Variable
Major CD45 isoform (humans only)	CD45RA	CD45RO	CD45RO; variable
Morphology	Small; scant cytoplasm	Large; more cytoplasm	Small
B Lymphocytes			
Membrane immunoglobulin (Ig) isotype	IgM and IgD	Frequently IgG, IgA, IgE	Frequently IgG, IgA, IgE
Affinity of Ig produced	Relatively low	Increases during immune response	Relatively high
Effector function	None	Antibody secretion	None
Morphology	Small; scant cytoplasm	Large; more cytoplasm; plasma cell	Small
Surface protien expression			
Chemokine receptor: CXCR5	High	Low	?
CD27	Low	High	High

inexperienced because they have not encountered antigen.) Naive lymphocytes typically die after 1 to 3 months if they do not recognize antigens. Naive and memory lymphocytes, discussed later, are both called resting lymphocytes because they are not actively dividing, nor are they performing effector functions. Naive (and memory) B and T lymphocytes cannot be readily distinguished morphologically and are both often called small lymphocytes when observed in blood smears or by flow cytometry (a technique described in Appendix IV). A small lymphocyte is 8 to 10 μm in diameter and has a large nucleus with dense heterochromatin and a thin rim of cytoplasm that contains a few mitochondria, ribosomes, and lysosomes but no visible specialized organelles (Fig. 2-7). Before antigenic stimulation, naive lymphocytes are in a state of rest, or in the G_0 stage of the cell cycle. In response to stimulation, they enter the G_1 stage of the cell cycle before going on to divide. Activated lymphocytes are larger (10 to 12 μm in diameter), have more cytoplasm and organelles and increased amounts of cytoplasmic RNA, and are called large lymphocytes or lymphoblasts (see Fig. 2-7).

The survival of naive lymphocytes depends on two types of signals, some of which are generated by antigen receptors and others by cytokines. It is postulated that the antigen receptor of naive B cells generates survival signals even in the absence of antigen, and naive T lymphocytes recognize various self antigens "weakly," enough to generate survival signals but without triggering the stronger signals that are needed to initiate clonal expansion and differentiation into effector cells. The need for antigen receptor expression to maintain the pool of naive lymphocytes in peripheral lymphoid organs has been demonstrated by studies with mice in which the genes that encode the antigen receptors of B cells or T cells were deleted after the lymphocytes matured. (The method used, called the Cre/lox recombinase technique, is described in Appendix IV.) In these studies, naive lymphocytes that lost their antigen receptors died within 2 or 3 weeks.

Cytokines are also essential for the survival of naive lymphocytes, and naive T and B cells constitutively express receptors for these cytokines. The most important of these cytokines are interleukin-7 (IL-7), which promotes survival and, perhaps, low-level cycling of naive T cells, and B cell–activating factor (BAFF) belonging to the TNF family, which is required for naive B cell survival.

In the steady state, the pool of naive lymphocytes is maintained at a fairly constant number because of a balance between spontaneous death of these cells and the generation of new cells in the generative lymphoid organs. Any loss of lymphocytes leads to a compensatory proliferation of the remaining ones and increased output from the generative organs. A demonstration of the ability of the lymphocyte population to "fill" the available space is the phenomenon of **homeostatic**

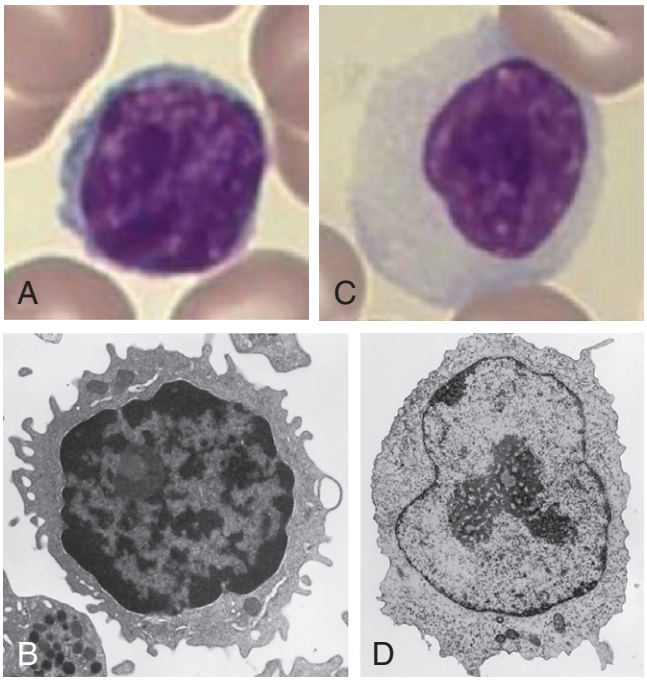


FIGURE 2–7 Morphology of lymphocytes. A, Light micrograph of a lymphocyte in a peripheral blood smear. *(Courtesy of Jean Shafer, Department of Pathology, University of California, San Diego. Copyright 1995-2008, Carden Jennings Publishing Co., Ltd.)* **B,** Electron micrograph of a small lymphocyte. *(Courtesy of Dr. Noel Weidner, Department of Pathology, University of California, San Diego.)* **C,** Light micrograph of a large lymphocyte (lymphoblast). *(Courtesy of Jean Shafer, Department of Pathology, University of California, San Diego. Copyright 1995-2008, Carden Jennings Publishing Co., Ltd.)* **D,** Electron micrograph of a large lymphocyte (lymphoblast). *(From Fawcett DW. Bloom and Fawcett: A Textbook of Histology, 12th ed. Chapman & Hall, New York, 1994. With kind permission of Springer Science and Business Media.)*

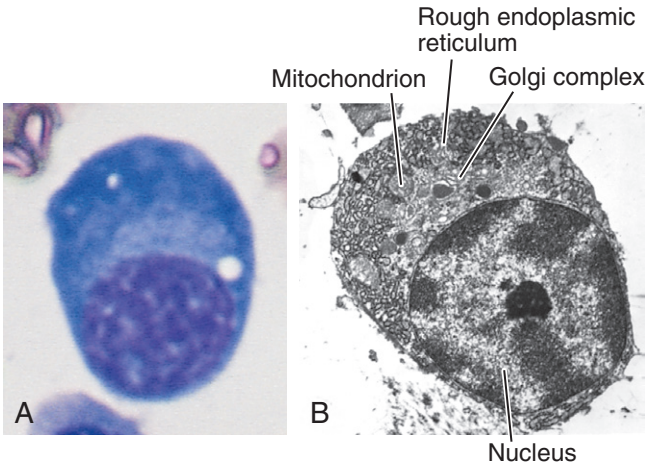


FIGURE 2–8 Morphology of plasma cells. A, Light micrograph of a plasma cell in tissue. **B,** Electron micrograph of a plasma cell. *(Courtesy of Dr. Noel Weidner, Department of Pathology, University of California, San Diego.)*

proliferation. If naive cells are transferred into a host that is deficient in lymphocytes (said to be lymphopenic) because of inherited defects or the effects of irradiation, the transferred lymphocytes begin to proliferate and increase in number until they reach roughly the numbers of lymphocytes in normal animals. Homeostatic proliferation appears to be driven by the same signals—weak recognition of some self antigens and cytokines, mainly IL-7—that are required for the maintenance of naive lymphocytes.

Effector Lymphocytes

After naive lymphocytes are activated, they become larger and proliferate and are called lymphoblasts. Some of these cells differentiate into effector lymphocytes that have the ability to produce molecules capable of eliminating foreign antigens; effector lymphocytes include helper T cells, CTLs, and antibody-secreting plasma cells. Helper T cells, which are usually $CD4^+$, express surface molecules such as CD40 ligand (CD154) and secrete cytokines that interact with macrophages and B lymphocytes, leading to their activation. CTLs have cytoplasmic granules filled with proteins that, when released, kill the cells that the CTLs recognize, which are usually virus-infected and tumor cells. Both $CD4^+$ and $CD8^+$ effector T cells usually express surface proteins indicative of recent activation, including CD25 (a component of the receptor for the T cell growth factor IL-2), and altered patterns of adhesion molecules (selectins and integrins, discussed in Chapter 3). The majority of differentiated effector T lymphocytes are short-lived and not self-renewing.

Many antibody-secreting B cells are morphologically identifiable as **plasma cells**. They have characteristic nuclei, abundant cytoplasm containing dense, rough endoplasmic reticulum that is the site where antibodies (and other secreted and membrane proteins) are synthesized, and distinct perinuclear Golgi complexes where antibody molecules are converted to their final forms and packaged for secretion (Fig. 2-8). It is estimated that half or more of the messenger RNA in plasma cells codes for antibody proteins. Plasma cells develop in lymphoid organs and at sites of immune responses and some of them migrate to the bone marrow, where they may live and secrete antibodies for long periods after the immune response is induced and even after the antigen is eliminated. Circulating antibody-secreting cells, called **plasmablasts**, are rare, and may be precursors of long-lived plasma cells in tissues.

Memory Lymphocytes

Memory cells may survive in a functionally quiescent or slowly cycling state for months or years without a need for stimulation by antigen and presumably after the antigen is eliminated. They can be identified by their expression of surface proteins that distinguish them from naive and recently activated effector lymphocytes, although it is still not clear which of these surface proteins are definitive markers of memory populations (see Table 2-3). Memory B lymphocytes express certain classes (isotypes) of membrane Ig, such as IgG, IgE, or IgA, as a result of isotype switching, whereas naive B cells express only IgM and IgD (see Chapters 5 and 11). In humans, CD27 expression is a good marker for memory B cells. Memory T cells, like naive but not effector T cells, express high levels of the IL-7 receptor (CD127). Memory T cells also express surface molecules that promote their

migration into sites of infection anywhere in the body (discussed later in the chapter). In humans, most naive T cells express a 200-kD isoform of a surface molecule called CD45 that contains a segment encoded by an exon designated A. This CD45 isoform can be recognized by antibodies specific for the A-encoded segment and is therefore called CD45RA (for "restricted A"). In contrast, most activated and memory T cells express a 180-kD isoform of CD45 in which the A exon RNA has been spliced out; this isoform is called CD45RO. However, this way of distinguishing naive from memory T cells is not perfect, and interconversion between $CD45RA^+$ and $CD45RO^+$ populations has been documented.

Memory cells appear to be heterogeneous, and there are subsets that differ, especially with respect to their location and migratory properties. More details about memory T and B cells will be discussed in Chapters 9 and 11, respectively.

The distinguishing features of naive, effector, and memory lymphocytes reflect different programs of gene expression that are regulated by transcription factors and by stable epigenetic changes, including DNA methylation and chromatin remodeling. Our understating of these molecular determinants of mature lymphocyte phenotype is still incomplete and evolving. For example, a transcription factor called Kruppel-like factor 2 (KLF-2) is required for maintenance of the naive T cell phenotype. The phenotypes of functionally different types of $CD4^+$ effector T cells, called T_H1, T_H2, and T_H17 cells, depend on transcription factors T-bet, GATA-3, and RORγT, respectively, as well as epigenetic changes in cytokine gene loci (see Chapter 9). Other transcription factors are required for maintaining the phenotypes of memory T and B cells.

ANATOMY AND FUNCTIONS OF LYMPHOID TISSUES

To optimize the cellular interactions necessary for antigen recognition and lymphocyte activation in adaptive immune responses, lymphocytes and APCs are localized and concentrated in anatomically defined tissues or organs, which are also the sites where foreign antigens are transported and concentrated. Such anatomic compartmentalization is not fixed because, as we will discuss in Chapter 3, many lymphocytes recirculate and constantly exchange between the circulation and the tissues.

Lymphoid tissues are classified as generative organs, also called primary or central lymphoid organs, where lymphocytes first express antigen receptors and attain phenotypic and functional maturity, and as peripheral organs, also called secondary lymphoid organs, where lymphocyte responses to foreign antigens are initiated and develop (see Fig. 2-5). Included in the generative lymphoid organs of adult mammals are the bone marrow and the thymus for B cells and T cells, respectively. B lymphocytes partially mature in the bone marrow, enter the circulation, and then populate peripheral lymphoid organs, including spleen and lymph nodes, where they complete their maturation. T lymphocytes mature completely in the thymus, then enter the circulation and populate peripheral lymphoid organs and tissues. Two important functions shared by the generative organs are to provide growth factors and other molecular signals needed for lymphocyte maturation and to present self antigens for recognition and selection of maturing lymphocytes (see Chapter 8). The peripheral lymphoid organs and tissues include the lymph nodes, spleen, cutaneous immune system, and mucosal immune system. In addition, poorly defined aggregates of lymphocytes are found in connective tissue and in virtually all organs except the central nervous system. All peripheral lymphoid organs also share common functions, including the delivery of antigens and responding naive lymphocytes to the same location so that adaptive immune responses can be initiated and the anatomic segregation of B and T lymphocytes except for specific times when they need to interact.

Bone Marrow

The bone marrow is the site of generation of most mature circulating blood cells, including red cells, granulocytes, and monocytes, and the site of early events in B cell maturation. The generation of all blood cells, called **hematopoiesis** (Fig. 2-9), occurs initially, during fetal development, in blood islands of the yolk sac and the para-aortic mesenchyme, then shifts to the liver between the third and fourth months of gestation, and gradually shifts again to the bone marrow. At birth, hematopoiesis takes place mainly in the bones throughout the skeleton, but it becomes restricted increasingly to the marrow of the flat bones so that by puberty, hematopoiesis occurs mostly in the sternum, vertebrae, iliac bones, and ribs. The red marrow that is found in these bones consists of a sponge-like reticular framework located between long trabeculae. The spaces in this framework contain a network of blood-filled sinusoids lined by endothelial cells attached to a discontinuous basement membrane. Outside the sinusoids are clusters of the precursors of blood cells in various stages of development as well as mature fat cells. The blood cell precursors mature and migrate through the sinusoidal basement membrane and between endothelial cells to enter the vascular circulation. When the bone marrow is injured or when an exceptional demand for production of new blood cells occurs, the liver and spleen often become sites of extramedullary hematopoiesis.

Red cells, granulocytes, monocytes, dendritic cells, platelets, B and T lymphocytes, and NK cells all originate from a common hematopoietic stem cell (HSC) in the bone marrow (see Fig. 2-9). HSCs are pluripotent, meaning that a single HSC can generate all different types of mature blood cells. HSCs are also self-renewing because each time they divide, at least one daughter cell maintains the properties of a stem cell while the other can differentiate along a particular lineage (called asymmetric division). HSCs can be identified by the presence of surface markers, including the proteins CD34 and c-Kit, and the absence of lineage-specific markers. HSCs are maintained within specialized microscopic anatomic niches in the marrow. In these locations, nonhematopoietic stromal cells provide contact-dependent signals and soluble factors required for continuous self-renewing

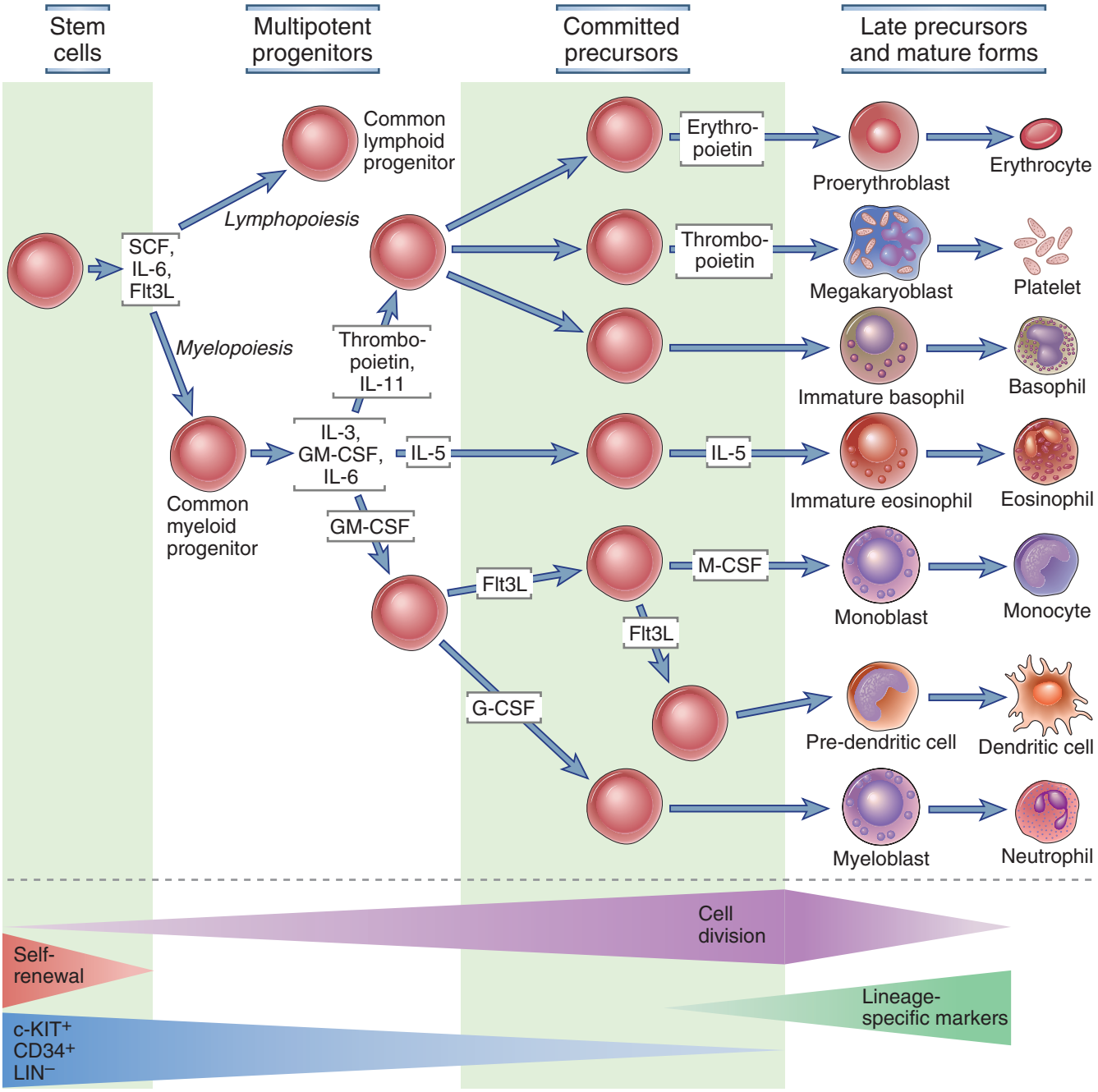


FIGURE 2–9 Hematopoiesis. The development of the different lineages of blood cells is depicted in this "hematopoietic tree." Also shown are the principal cytokines that drive the maturation of different lineages. The development of lymphocytes forming the common lymphoid precursor is described later in this chapter and in Figure 8-2, Chapter 8. SCF, stem cell factor; Flt3L, Flt3 ligand; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; LIN⁻, negative for lineage-specific markers; M-CSF, macrophage colony-stimulating factor.

division of the HSCs. HSCs give rise to two kinds of multipotent cells, the common lymphoid and common myeloid progenitors. The common lymphoid progenitor is the source of committed single-lineage precursors of T cells, B cells, or NK cells. Most of the steps in B cell maturation take place in the bone marrow, but the final events may occur after the cells leave the marrow and enter secondary lymphoid organs, particularly the spleen. T cell maturation occurs entirely in the thymus and therefore requires that common lymphoid progenitors or some poorly characterized progeny of these cells migrate out of the marrow into the blood and then into the thymus. NK cell maturation is thought to take place entirely in the bone marrow. The common myeloid progenitors give rise to committed single-lineage progenitors of the erythroid, megakaryocytic, granulocytic, and monocytic lineages, which give rise, respectively, to mature red cells, platelets, granulocytes (neutrophils, eosinophils, basophils), and monocytes. Most dendritic cells arise from the monocytic lineage.

The proliferation and maturation of precursor cells in the bone marrow are stimulated by cytokines (see

TABLE 2–4 Hematopoietic Cytokines

Cytokine	Size	Principal Cellular Sources	Principal Cellular Targets	Principal Cell Populations Induced
Stem cell factor (c-Kit ligand)	24 kD	Bone marrow stromal cells	Hematopoietic stem cells	All
Interleukin-7 (IL-7)	25 kD	Fibroblasts, bone marrow stromal cells	Immature lymphoid progenitors	B and T lymphocytes
Interleukin-3 (IL-3)	20-26 kD	T cells	Immature progenitors	All
Granulocyte-monocyte colony-stimulating factor (GM-CSF)	18-22 kD	T cells, macrophages, endothelial cells, fibroblasts	Immature and committed myeloid progenitors, mature macrophages	Granulocytes and monocytes, macrophage activation
Monocyte colony-stimulating factor (M-CSF)	Dimer of 70-90 kD; 40-kD subunits	Macrophages, endothelial cells, bone marrow cells, fibroblasts	Committed progenitors	Monocytes
Granulocyte colony-stimulating factor (G-CSF)	19 kD	Macrophages, fibroblasts, endothelial cells	Committed granulocyte progenitors	Granulocytes

Fig. 2-9). Many of these cytokines are called colony-stimulating factors because they were originally assayed by their ability to stimulate the growth and development of various leukocytic or erythroid colonies from marrow cells. Hematopoietic cytokines are produced by stromal cells and macrophages in the bone marrow, thus providing the local environment for hematopoiesis. They are also produced by antigen-stimulated T lymphocytes and cytokine-activated or microbe-activated macrophages, providing a mechanism for replenishing leukocytes that may be consumed during immune and inflammatory reactions. The names and properties of the major hematopoietic cytokines are listed in Table 2-4.

In addition to self-renewing stem cells and their differentiating progeny, the marrow contains numerous antibody-secreting plasma cells. These plasma cells are generated in peripheral lymphoid tissues as a consequence of antigenic stimulation of B cells and then migrate to the marrow, where they may live and continue to produce antibodies for many years. Some long-lived memory T lymphocytes also migrate to and may reside in the bone marrow.

Thymus

The thymus is the site of T cell maturation. The thymus is a bilobed organ situated in the anterior mediastinum. Each lobe is divided into multiple lobules by fibrous septa, and each lobule consists of an outer cortex and an inner medulla (Fig. 2-10). The cortex contains a dense collection of T lymphocytes, and the lighter-staining medulla is more sparsely populated with lymphocytes. Bone marrow–derived macrophages and dendritic cells are found almost exclusively in the medulla. Scattered throughout the thymus are nonlymphoid epithelial cells, which have abundant cytoplasm. Thymic cortical epithelial cells provide IL-7 required early in T cell development. A subset of these epithelial cells found only in the medulla, called thymic medullary epithelial cells (often abbreviated as TMEC), play a special role in presenting self antigens to developing T cells and causing their deletion. This is one mechanism of ensuring that the immune system remains tolerant to self and is discussed in detail in Chapter 14. In the medulla are structures called Hassall's corpuscles, which are composed of tightly packed whorls of epithelial cells that may be remnants of degenerating cells. The thymus has a rich vascular supply and efferent lymphatic vessels that drain into mediastinal lymph nodes. The epithelial component of the thymus is derived from invaginations of the ectoderm in the developing neck and chest of the embryo, forming structures called branchial pouches. Dendritic cells, macrophages, and lymphocyte precursors are derived from the bone marrow.

Humans with DiGeorge syndrome suffer from T cell deficiency because of mutations in genes required for thymus development. In the "nude" mouse strain, which has been widely used in immunology research, a mutation in the gene encoding a transcription factor causes a failure of differentiation of certain types of epithelial cells that are required for normal development of the thymus and hair follicles. Consequently, these mice lack T cells and hair.

The lymphocytes in the thymus, also called **thymocytes**, are T lymphocytes at various stages of maturation. Cells that are committed to the T cell lineage are believed to develop in the bone marrow from common lymphoid progenitor cells, enter the circulation, and home to the thymic cortex through the blood vessels. Further maturation in the thymus begins in the cortex, and as thymocytes mature, they migrate toward the medulla, so that the medulla contains mostly mature T cells. Only mature T cells exit the thymus and enter the blood and peripheral lymphoid tissues. The details of thymocyte maturation are described in Chapter 8.

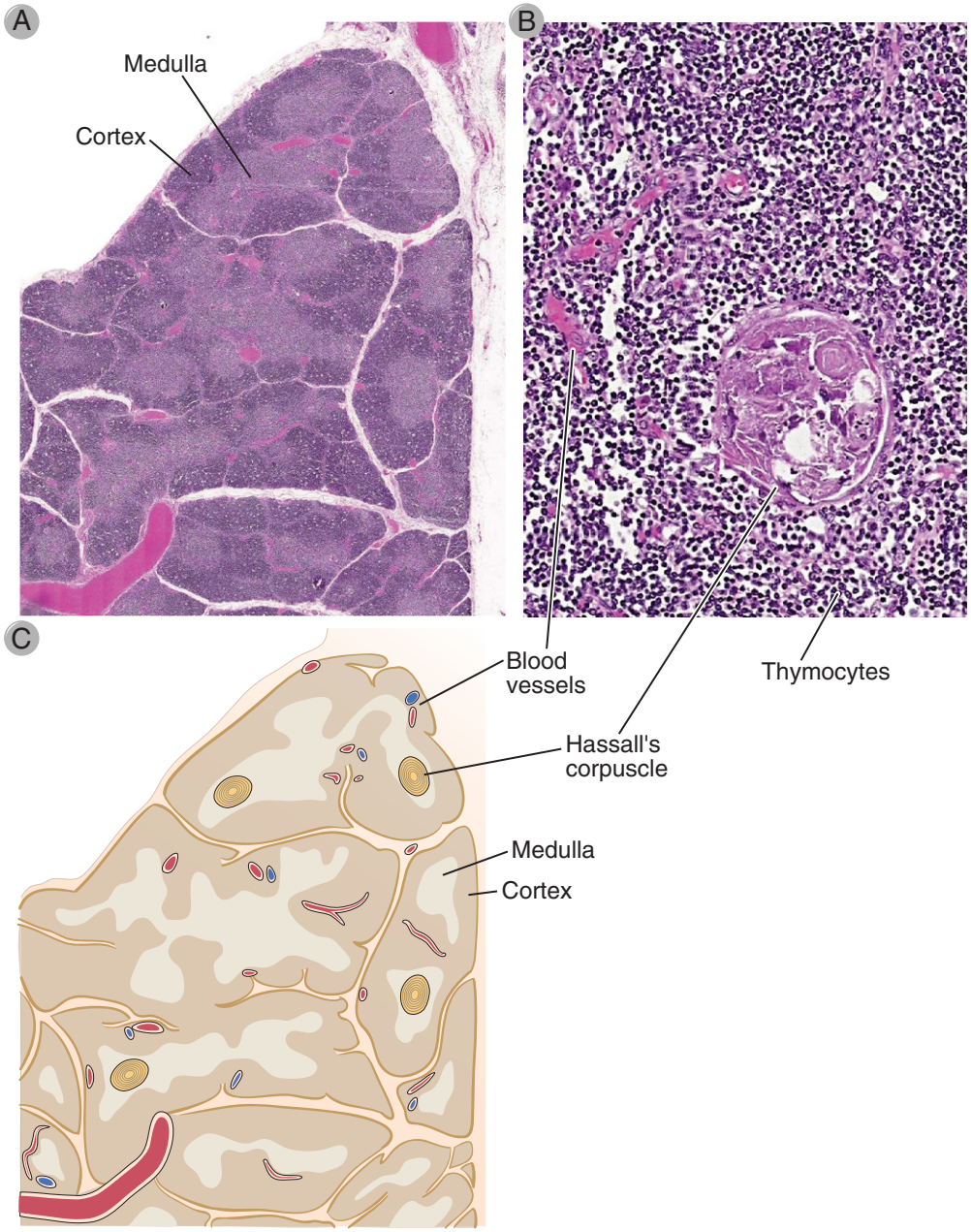


FIGURE 2–10 Morphology of the thymus. A, Low-power light micrograph of a lobe of the thymus showing the cortex and medulla. The darker blue-stained outer cortex and paler blue inner medulla are apparent. **B,** High-power light micrograph of the thymic medulla. The numerous small blue-staining cells are developing T cells called thymocytes, and the larger pink structure is Hassall's corpuscle, uniquely characteristic of the thymic medulla but whose function is poorly understood. **C,** Schematic diagram of the thymus illustrating a portion of a lobe divided into multiple lobules by fibrous trabeculae.

The Lymphatic System

The lymphatic system, which consists of specialized vessels that drain fluid from tissues into and out of lymph nodes and then into the blood, is essential for tissue fluid homeostasis and immune responses (Fig. 2-11). Interstitial fluid is constitutively formed in all vascularized tissues by movement of a filtrate of plasma out of capillaries, and the rate of local formation can increase dramatically when tissue is injured or infected. The skin, epithelia, and parenchymal organs contain numerous lymphatic capillaries that absorb this fluid from spaces between tissue cells. The lymphatic capillaries are blind-ended vascular channels lined by overlapping endothelial cells without the tight intercellular junctions or basement membrane that are typical of blood vessels. These distal lymphatic capillaries permit free uptake of interstitial fluid, and the overlapping arrangement of the endothelial cells and one-way valves within their lumens prevents backflow of the fluid. The absorbed fluid, called **lymph** once it is within the lymphatic vasculature, is pumped into convergent, ever larger lymphatic vessels by the contraction of perilymphatic smooth muscle cells and the pressure exerted by movement of the musculoskeletal tissues.

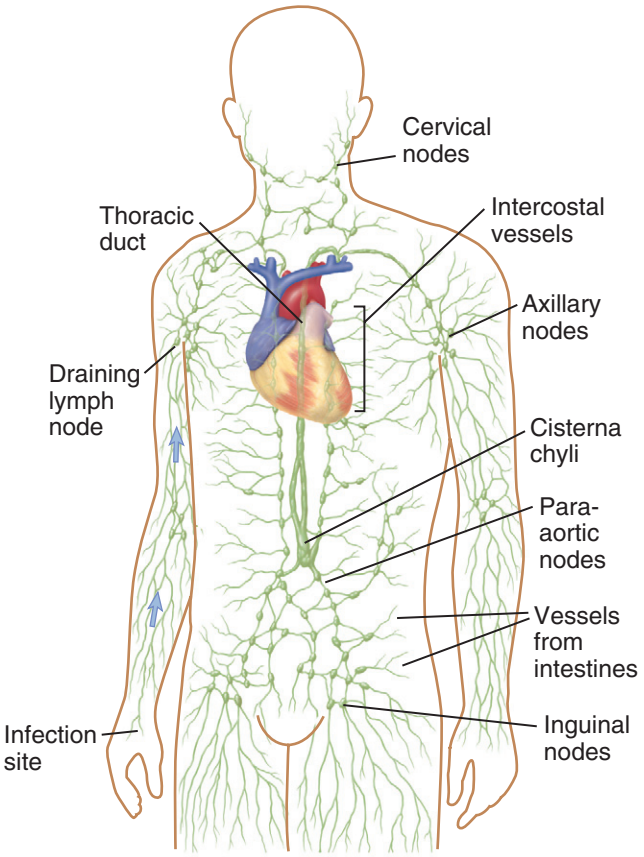


FIGURE 2–11 The lymphatic system. The major lymphatic vessels, which drain into the inferior vena cava (and superior vena cava, not shown), and collections of lymph nodes are illustrated. Antigens are captured from a site of infection and the draining lymph node to which these antigens are transported and where the immune response is initiated.

These vessels merge into afferent lymphatics that drain into lymph nodes, and the lymph drains out of the nodes through efferent lymphatics. Because lymph nodes are connected in series by lymphatics, an efferent lymphatic exiting one node may serve as the afferent vessel for another. The efferent lymph vessel at the end of a lymph node chain joins other lymph vessels, eventually culminating in a large lymphatic vessel called the thoracic duct. Lymph from the thoracic duct is emptied into the superior vena cava, thus returning the fluid to the blood stream. Lymphatics from the right upper trunk, right arm, and right side of the head drain into the right lymphatic duct, which also drains into the superior vena cava. About 2 liters of lymph are normally returned to the circulation each day, and disruption of the lymphatic system may lead to rapid tissue swelling.

The lymphatic system collects microbial antigens from their portals of entry and delivers them to lymph nodes, where they can stimulate adaptive immune responses. Microbes enter the body most often through the skin and the gastrointestinal and respiratory tracts. All these tissues are lined by epithelia that contain dendritic cells, and all are drained by lymphatic vessels. The dendritic cells capture some microbial antigens and enter lymphatic vessels. Other microbes and soluble antigens enter the lymphatics independently of dendritic cells. In addition, soluble inflammatory mediators, such as chemokines, produced at sites of infection enter the lymphatics. The lymph nodes are interposed along lymphatic vessels and act as filters that sample the soluble and dendritic cell–associated antigens in the lymph before it reaches the blood and permit the antigens to be seen by the adaptive immune system.

Lymph Nodes

Lymph nodes are encapsulated, vascularized secondary lymphoid organs with anatomic features that favor the initiation of adaptive immune responses to antigens carried from tissues by lymphatics (Fig. 2-12). Lymph nodes are situated along lymphatic channels throughout the body and therefore have access to antigens encountered at epithelia and originating in interstitial fluid in most tissues. A lymph node is surrounded by a fibrous capsule, beneath which is a sinus system lined by reticular cells, cross-bridged by fibrils of collagen and other extracellular matrix proteins and filled with lymph, macrophages, dendritic cells, and other cell types. Afferent lymphatics empty into the **subcapsular (marginal) sinus**, and lymph may drain from there directly into the connected medullary sinus and then out of the lymph node through the efferent lymphatics. Beneath the inner floor of the subcapsular sinus is the lymphocyte-rich **cortex**. The outer cortex contains aggregates of cells called **follicles**. Some follicles contain central areas called **germinal centers**, which stain lightly with commonly used histologic stains. Follicles without germinal centers are called primary follicles, and those with germinal centers are secondary follicles. The cortex around the follicles is called the parafollicular cortex or paracortex and is organized into cords, which are regions with a complex microanatomy of matrix proteins, fibers, lymphocytes, dendritic cells, and mononuclear phagocytes.

Anatomic Organization of B and T Lymphocytes

B and T lymphocytes are sequestered in distinct regions of the cortex of lymph nodes, each region with its own unique architecture of reticular fibers and stromal cells (Figs. 2-13 and 2-14). Follicles are the B cell zones. They are located in the lymph node cortex and are organized around FDCs, which have processes that interdigitate to form a dense reticular network. Primary follicles contain mostly mature, naive B lymphocytes. Germinal centers develop in response to antigenic stimulation. They are sites of remarkable B cell proliferation, selection of B cells producing high-affinity antibodies, and generation of memory B cells and long-lived plasma cells. The T lymphocytes are located mainly beneath and more central to the follicles, in the paracortical cords. These T cell–rich zones contain a network of **fibroblastic reticular cells (FRCs)**, which are arranged to form the outer layer of tube-like structures called FRC conduits. The conduits range in diameter from 0.2 to 3 μm and contain organized arrays of extracellular matrix molecules, including innermost parallel bundles of collagen fibers embedded in a meshwork of fibrillin microfibers, all tightly

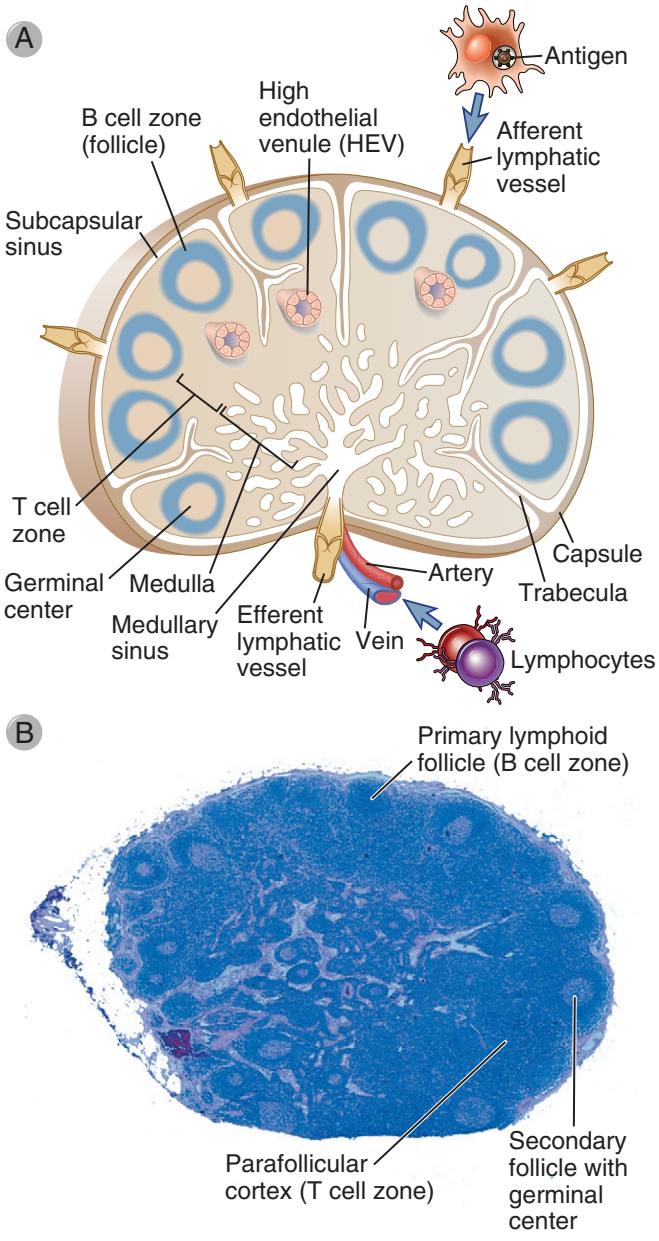


FIGURE 2–12 Morphology of a lymph node. A, Schematic diagram of a lymph node illustrating the T cell–rich and B cell–rich zones and the routes of entry of lymphocytes and antigen (shown captured by a dendritic cell). **B,** Light micrograph of a lymph node illustrating the T cell and B cell zones. *(Courtesy of Dr. James Gulizia, Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts.)*

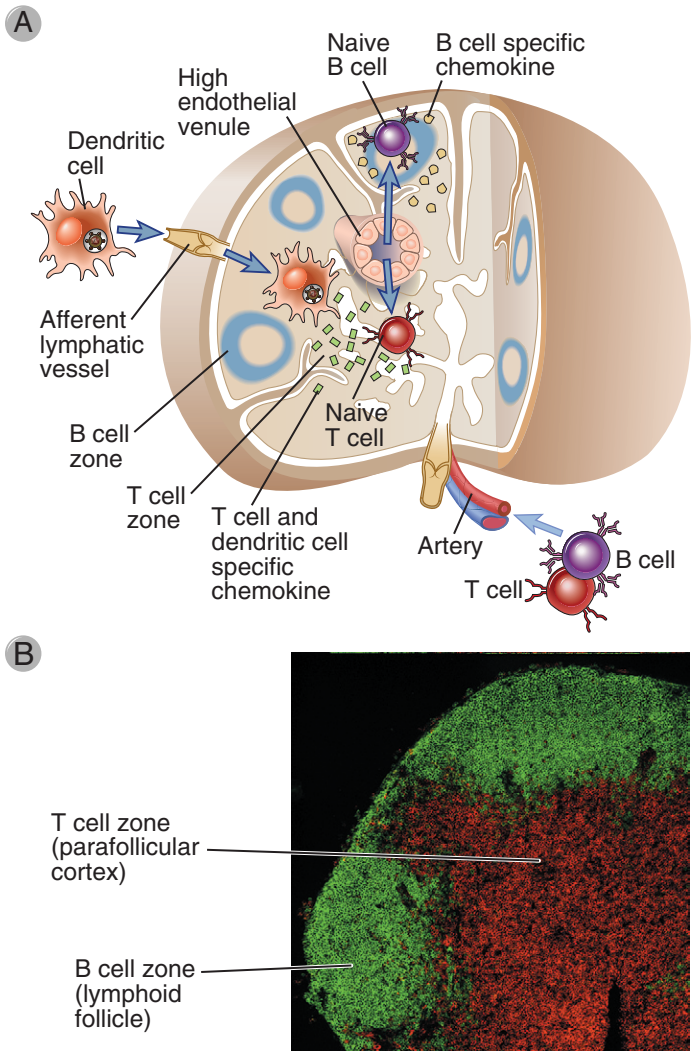


FIGURE 2–13 Segregation of B cells and T cells in a lymph node. A, The schematic diagram illustrates the path by which naive T and B lymphocytes migrate to different areas of a lymph node. The lymphocytes enter through an artery and reach a high endothelial venule, shown in cross section, from where naive lymphocytes are drawn to different areas of the node by chemokines that are produced in these areas and bind selectively to either cell type. Also shown is the migration of dendritic cells, which pick up antigens from the sites of antigen entry, enter through afferent lymphatic vessels, and migrate to the T cell–rich areas of the node. **B,** In this section of a lymph node, the B lymphocytes, located in the follicles, are stained green; the T cells, in the parafollicular cortex, are red. The method used to stain these cells is called immunofluorescence (see Appendix IV for details). *(Courtesy of Drs. Kathryn Pape and Jennifer Walter, University of Minnesota School of Medicine, Minneapolis.)* The anatomic segregation of T and B cells is also seen in the spleen (see Fig. 2-15).

surrounded by a basement membrane produced by a continuous sleeve of FRCs. These conduits begin at the subcapsular sinus and extend to both medullary sinus lymphatic vessels and cortical blood vessels, called high endothelial venules (HEVs). Naive T cells enter the T cell zones through the HEVs, as described in detail in Chapter 3. T cells are densely packed around the conduits in the lymph node cortex. Most (~70%) of the cortical T cells are $CD4^+$ helper T cells, intermingled with relatively sparse $CD8^+$ cells. These proportions can change dramatically during the course of an infection. For example, during a viral infection, there may be a marked increase in $CD8^+$ T cells. Dendritic cells are also concentrated in the paracortex of the lymph nodes, many of which are closely associated with the FRC conduits.

The anatomic segregation of B and T lymphocytes in distinct areas of the node is dependent on cytokines that are secreted by lymph node stromal cells in each area and that direct the migration of the lymphocytes (see Fig. 2-13). Naive T and B lymphocytes are delivered to a node through an artery and leave the circulation and enter the

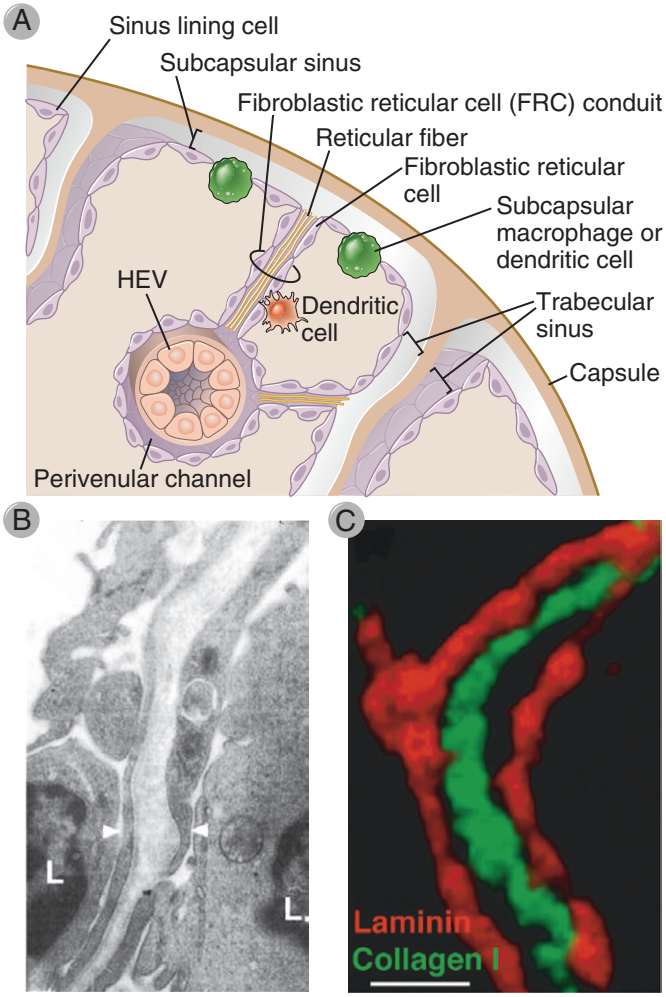


FIGURE 2–14 Microanatomy of the lymph node cortex. **A,** Schematic of the microanatomy of a lymph node depicting the route of lymph drainage from the subcapsular sinus, through fibroreticular cell conduits, to the perivenular channel around the high endothelial venule (HEV). **B,** Transmission electron micrograph of an FRC conduit surrounded by fibroblast reticular cells *(arrowheads)* and adjacent lymphocytes (L). *(From Gretz JE, CC Norbury, AO Anderson, AEI Proudfoot, and S Shaw. Lymph-borne chemokines and other low molecular weight molecules reach high endothelial venules via specialized conduits while a functional barrier limits access to the lymphocyte microenvironments in lymph node cortex. The Journal of Experimental Medicine 192:1425-1439, 2000.)* **C,** Immunofluorescent stain of an FRC conduit formed of the basement membrane protein laminin (red) and collagen fibrils (green). *(From Sixt M, K Nobuo, M Selg, T Samson, G Roos, DP Reinhardt, R Pabst, M Lutz, and L Sorokin. The conduit system transports soluble antigens from the afferent lymph to resident dendritic cells in the T cell area of the lymph node. Immunity 22:19-29, 2006. Copyright ©2005 by Elsevier Inc.)*

stroma of the node through the HEVs, which are located in the center of the cortical cords. The type of cytokines that determine where B and T cells reside in the node are called **chemokines** (chemoattractant cytokines), which bind to chemokine receptors on the lymphocytes. Chemokines include a large family of 8- to 10-kD cytokines that are involved in a wide variety of cell motility functions in development, maintenance of tissue architecture, and immune and inflammatory responses. We will discuss the general properties of chemokines and their receptors in Chapter 3. Naive T cells express a receptor called CCR7 that binds the chemokines CCL19 and CCL21 produced by stromal cells in the T cell zones of the lymph node. These chemokines attract naive T cells to move from the blood, through the HEVs, into the T cell zone. Dendritic cells that drain into the node through lymphatics also express CCR7, and this is why they migrate from the subcapsular sinus to the same area of the node as do naive T cells (see Chapter 6). Naive B cells express another chemokine receptor, CXCR5, that recognizes a chemokine, CXCL13, produced only in follicles by FDCs. Thus, B cells are attracted into the follicles, which are the B cell zones of lymph nodes. Another cytokine (which is not a chemokine) called lymphotoxin plays a role in stimulating CXCL13 production, especially in the follicles. The functions of chemokines and other cytokines in regulating where lymphocytes are located in lymphoid organs and in the formation of these organs have been established by numerous studies in mice. For example, CXCR5 knockout mice lack B cell–containing follicles in lymph nodes and spleen. Similarly, CCR7 knockout mice lack T cell zones.

The development of lymph nodes as well as of other peripheral lymphoid organs requires the coordinated actions of several cytokines, chemokines, transcription factors, and lymphoid tissue inducer cells. During fetal life, lymphoid tissue inducer cells, which are cells of hematopoietic origin with phenotypic features of both lymphocytes and NK cells, stimulate the development of lymph nodes and other secondary lymphoid organs. This function is mediated by various proteins expressed by the inducer cells, the most thoroughly studied being the cytokines lymphotoxin-α (LTα) and lymphotoxin-β (LTβ). Knockout mice lacking either of these cytokines do not develop lymph nodes or secondary lymphoid organs in the gut. Splenic white pulp development is also disorganized in these mice. The LTβ produced by the inducer cells acts on stromal cells in different locations of a developing secondary lymphoid organ, and these stromal cells are activated to produce the chemokines CXCL13 or CCL19 and CCL21. In areas where CXCL13 is induced, circulating B cells are recruited into nascent B cell follicles; and in the areas where CCL19 and CCL21 are induced, T cells and dendritic cells are recruited to form T cell zones. There are several other proteins expressed by lymphoid tissue inducer cells that are required for their function, including transcription factors, but their roles in lymphoid organogenesis are not well defined.

The anatomic segregation of T and B cells ensures that each lymphocyte population is in close contact with the appropriate APCs, that is, T cells with dendritic cells and B cells with FDCs. Furthermore, because of this precise segregation, B and T lymphocyte populations are kept apart until it is time for them to interact in a functional way. As we will see in Chapter 11, after stimulation by antigens, T and B cells lose their anatomic constraints and begin to migrate toward one another. Activated T cells may either migrate toward follicles to help B cells or exit the node and enter the circulation, whereas activated B cells migrate into germinal centers and, after differentiation into plasma cells, may home to the bone marrow.

Antigen Transport Through Lymph Nodes

Lymph-borne substances that enter the subcapsular sinus of the lymph node are sorted by molecular size and delivered to different cell types to initiate different types of immune responses. The floor of the subcapsular sinus is constructed in a way that permits cells in the sinus to contact or migrate into the underlying cortex but does not allow movement of soluble molecules in the lymph to freely pass into the cortex. Viruses and other high-molecular-weight antigens are taken up by sinus macrophages and presented to cortical B lymphocytes just beneath the cortical sinus. This is the first step in antibody responses to these antigens. Low-molecular-weight soluble antigens are transported out of the sinus through the FRC conduits and passed to resident cortical dendritic cells located adjacent to the conduits. The resident dendritic cells extend processes between the cells lining the conduits and into the lumen and capture and pinocytose the soluble antigens inside the conduits. The contribution of this pathway of antigen delivery may be important for initial T cell immune responses to some microbial antigens, but larger and sustained responses require delivery of antigens to the node by tissue dendritic cells, as discussed in Chapter 6. In addition to antigens, there is evidence that soluble inflammatory mediators, such as chemokines and other cytokines, are transported in the lymph that flows through the conduits; some of these may act on the penetrating dendritic cells, and others may be delivered to HEVs into which the conduits drain. This is a possible way in which tissue inflammation can be sensed in the lymph node and thereby influence recruitment and activation of lymphocytes in the node.

Spleen

The spleen is a highly vascularized organ whose major functions are to remove aging and damaged blood cells and particles (such as immune complexes and opsonized microbes) from the circulation and to initiate adaptive immune responses to blood-borne antigens. The spleen weighs about 150 g in adults and is located in the left upper quadrant of the abdomen. The splenic parenchyma is anatomically and functionally divided into the **red pulp**, composed mainly of blood-filled vascular sinusoids, and the lymphocyte-rich **white pulp**. Blood enters the spleen through a single splenic artery, which pierces the capsule at the hilum and divides into progressively smaller branches that remain surrounded by protective and supporting fibrous trabeculae (Fig. 2-15). Some of the arteriolar branches of the splenic artery end in extensive vascular sinusoids, which form the red pulp, lined by macrophages and filled with large numbers of erythrocytes. The sinusoids end in venules that drain into the splenic vein, which carries blood out of the spleen and into the portal circulation. The red pulp macrophages serve as an important filter for the blood, removing microbes, damaged cells, and antibody-coated (opsonized) cells and microbes. Individuals lacking a spleen are highly susceptible to infections with encapsulated bacteria such as pneumococci and meningococci. This may be because such organisms are normally cleared by opsonization and phagocytosis, and this function is defective in the absence of the spleen.

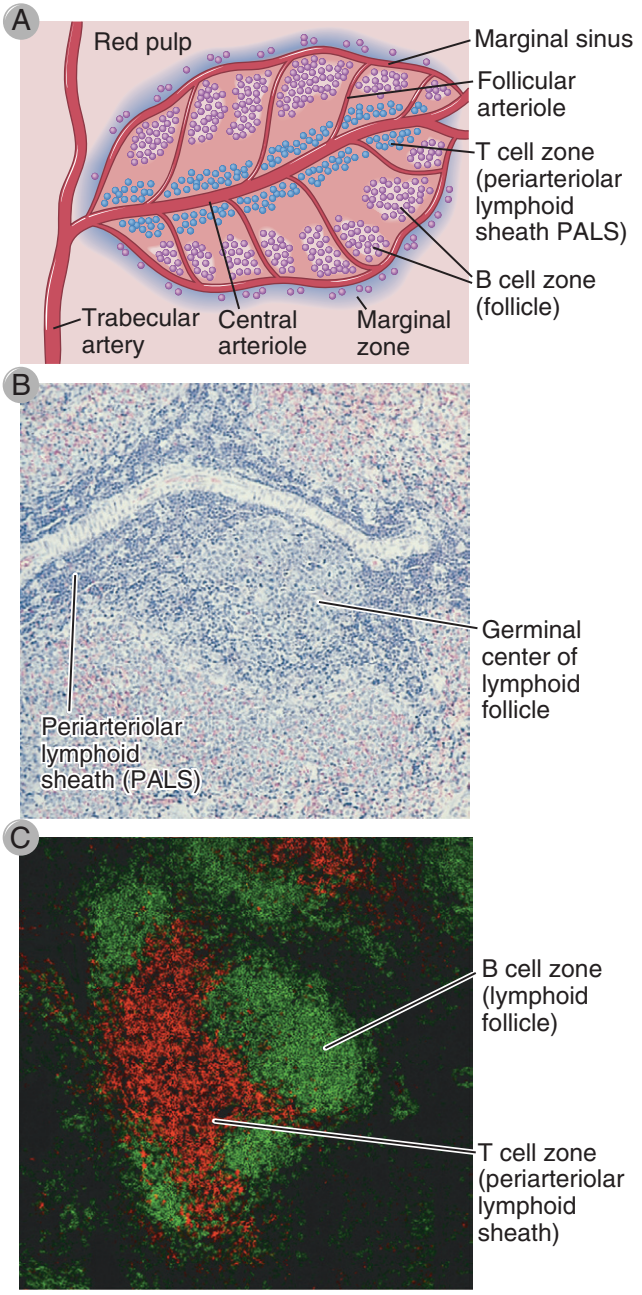


FIGURE 2–15 Morphology of the spleen. A, Schematic diagram of the spleen illustrating T cell and B cell zones, which make up the white pulp. **B,** Photomicrograph of a section of human spleen showing a trabecular artery with adjacent periarteriolar lymphoid sheath and a lymphoid follicle with a germinal center. Surrounding these areas is the red pulp, rich in vascular sinusoids. **C,** Immunohistochemical demonstration of T cell and B cell zones in the spleen, shown in a cross section of the region around an arteriole. T cells in the periarteriolar lymphoid sheath are stained red, and B cells in the follicle are stained green. *(Courtesy of Drs. Kathryn Pape and Jennifer Walter, University of Minnesota School of Medicine, Minneapolis.)*

The function of the white pulp is to promote adaptive immune responses to blood-borne antigens. The white pulp consists of many collections of densely packed lymphocytes, which appear as white nodules against the

background of the red pulp. The white pulp is organized around central arteries, which are branches of the splenic artery distinct from the branches that form the vascular sinusoids. Several smaller branches of each central artery pass through the lymphocyte-rich area and drain into a **marginal sinus**. A region of specialized cells surrounding the marginal sinus, called the **marginal zone**, forms the boundary between the red and white pulp. The architecture of the white pulp is analogous to the organization of lymph nodes, with segregated T cell and B cell zones. In the mouse spleen, the central arteries are surrounded by cuffs of lymphocytes, most of which are T cells. Because of their anatomic location, morphologists call these T cell zones **periarteriolar lymphoid sheaths**. B cell–rich follicles occupy the space between the marginal sinus and the periarteriolar sheath. As in lymph nodes, the T cell areas in the spleen contain a network of complex conduits composed of matrix proteins lined by FRC-like cells, although there are ultrastructural differences between the conduits in nodes and spleen. The marginal zone just outside the marginal sinus is a distinct region populated by B cells and specialized macrophages. The B cells in the marginal zone, known as marginal zone B cells, are functionally distinct from follicular B cells and have a limited repertoire of antigen specificities. The architecture of the white pulp is more complex in humans than in mice, with both inner and outer marginal zones and a perifollicular zone. Antigens in the blood are delivered into the marginal sinus by circulating dendritic cells or are sampled by the macrophages in the marginal zone. The anatomic arrangements of the APCs, B cells, and T cells in the splenic white pulp promote the interactions required for the efficient development of humoral immune responses, as will be discussed in Chapter 11. The segregation of T lymphocytes in the periarteriolar lymphoid sheaths and B cells in follicles and marginal zones is a highly regulated process, dependent on the production of different cytokines and chemokines by the stromal cells in these different areas, analogous to the case for lymph nodes. The chemokine CXCL13 and its receptor CXCR5 are required for B cell migration into the follicles, and CCL19 and CCL21 and their receptor CCR7 are required for naive T cell migration into the periarteriolar sheath. The production of these chemokines by nonlymphoid stromal cells is stimulated by the cytokine lymphotoxin.

Regional Immune Systems

Each major epithelial barrier of the body, including the skin, gastrointestinal mucosa, and bronchial mucosa, has its own system of lymph nodes, nonencapsulated lymphoid structures, and diffusely distributed immune cells, which work in coordinated ways to provide specialized immune responses against the pathogens that enter at those barriers. The skin-associated immune system has evolved to respond to a wide variety of environmental microbes. The components of the immune systems associated with the gastrointestinal and bronchial mucosa are called the mucosa-associated lymphoid tissue (MALT) and are involved in immune responses to ingested and inhaled antigens and microbes. The skin and MALT contain a major proportion of the cells of the innate and adaptive immune systems. We will discuss the special features of these regional immune systems in Chapter 13.

SUMMARY

- The anatomic organization of the cells and tissues of the immune system is of critical importance for the generation of effective innate and adaptive immune responses. This organization permits the rapid delivery of innate effector cells, including neutrophils and monocytes, to sites of infection and permits a small number of lymphocytes specific for any one antigen to locate and respond effectively to that antigen regardless of where in the body the antigen is introduced.
- The cells that perform the majority of effector functions of innate and adaptive immunity are phagocytes (including neutrophils and macrophages), APCs (including macrophages and dendritic cells), and lymphocytes.
- Neutrophils, the most abundant blood leukocyte with a distinctive multilobed segmented nucleus and abundant cytoplasmic lysosomal granules, are rapidly recruited to sites of infection and tissue injury, where they perform phagocytic functions.
- Monocytes are the circulating precursors of tissue macrophages. All tissues contain resident macrophages, which are phagocytic cells that ingest and kill microbes and dead host cells and secrete cytokines and chemokines that promote the recruitment of leukocytes from the blood.
- APCs function to display antigens for recognition by lymphocytes and to promote the activation of lymphocytes. APCs include dendritic cells, mononuclear phagocytes, and FDCs.
- B and T lymphocytes express highly diverse and specific antigen receptors and are the cells responsible for the specificity and memory of adaptive immune responses. NK cells are a distinct class of lymphocytes that do not express highly diverse antigen receptors and whose functions are largely in innate immunity. Many surface molecules are differentially expressed on different subsets of lymphocytes as well as on other leukocytes, and these are named according to the CD nomenclature.
- Both B and T lymphocytes arise from a common precursor in the bone marrow. B cell development proceeds in the bone marrow, whereas T cell precursors migrate to and mature in the thymus. After maturing, B and T cells leave the bone marrow and thymus, enter the circulation, and populate peripheral lymphoid organs.
- Naive B and T cells are mature lymphocytes that have not been stimulated by antigen. When they encounter antigen, they differentiate into effector lymphocytes that have functions in protective immune responses. Effector B lymphocytes are

antibody-secreting plasma cells. Effector T cells include cytokine-secreting $CD4^+$ helper T cells and $CD8^+$ CTLs.

- Some of the progeny of antigen-activated B and T lymphocytes differentiate into memory cells that survive for long periods in a quiescent state. These memory cells are responsible for the rapid and enhanced responses to subsequent exposures to antigen.
- The organs of the immune system may be divided into the generative organs (bone marrow and thymus), where lymphocytes mature, and the peripheral organs (lymph nodes and spleen), where naive lymphocytes are activated by antigens.
- Bone marrow contains the stem cells for all blood cells, including lymphocytes, and is the site of maturation of all of these cell types except T cells, which mature in the thymus.
- Extracellular fluid (lymph) is constantly drained from tissues through lymphatics into lymph nodes and eventually into the blood. Microbial antigens are carried in soluble form and within dendritic cells in the lymph to lymph nodes, where they are recognized by lymphocytes.
- Lymph nodes are encapsulated secondary lymphoid organs located throughout the body along lymphatics, where naive B and T cells respond to antigens that are collected by the lymph from peripheral tissues. The spleen is an encapsulated organ in the abdominal cavity where senescent or opsonized blood cells are removed from the circulation, and in which lymphocytes respond to blood-borne antigens. Both lymph nodes and the white pulp of the spleen are organized into B cell zones (the follicles) and T cell zones. The T cell areas are also the sites of residence of mature dendritic cells, which are APCs specialized for the activation of naive T cells. FDCs reside in the B cell areas and serve to activate B cells during humoral immune responses to protein antigens. The development of secondary lymphoid tissues depends on cytokines and lymph node inducer cells.

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Tissues of the Immune System

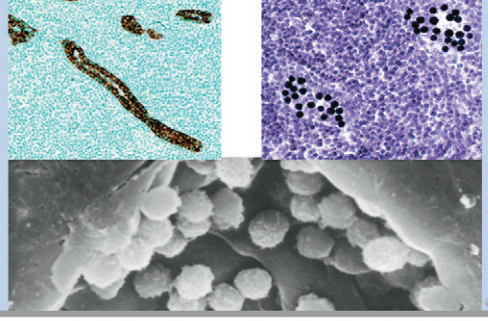
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CHAPTER

3

Leukocyte Migration into Tissues

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A unique property of the immune system that distinguishes it from all other tissue systems in the body is the constant and highly regulated movement of its major cellular components through the blood, into tissues, and often back into the blood again. This movement accomplishes three main functions (Fig. 3-1):

- Delivery of leukocytes of myeloid lineage (mainly neutrophils and monocytes) from their bone marrow site of maturation into tissue sites of infection or injury, where the cells perform their protective functions of eliminating infectious pathogens, clearing dead tissues, and repairing the damage.
- Delivery of lymphocytes from their sites of maturation (bone marrow or thymus) to secondary lymphoid organs, where they encounter antigens and differentiate into effector lymphocytes.
- Delivery of effector lymphocytes from the secondary lymphoid organs in which they were produced to sites of infection in any tissue, where they perform their protective functions.

The migration of a particular type of leukocyte into a restricted type of tissue, or a tissue with an ongoing infection or injury, is often called leukocyte homing, and the general process of leukocyte movement from blood into tissues is called recruitment. The migration of leukocytes to tissues follows several general principles.

- Leukocytes that have not been activated by external stimuli (i.e., are considered to be in a resting state) are normally located in the circulation and lymphoid organs. Only after activation are these cells rapidly recruited to where they are needed. The activating stimuli typically are products of microbes and dead cells (during innate immune responses) and antigens (during adaptive immune responses).
- Endothelial cells at sites of infection and tissue injury are also activated, mostly in response to cytokines secreted by macrophages and other tissue cells at these sites. Endothelial activation results in increased adhesiveness of endothelial cells for circulating leukocytes; the molecular basis of this adhesiveness is described later.
- The recruitment of leukocytes and plasma proteins from the blood to sites of infection and tissue injury is called **inflammation**. Inflammation is triggered by recognition of microbes and dead tissues in innate immune responses and is refined and prolonged during adaptive immune responses. This process delivers the cells and molecules of host defense to the sites where offending agents need to be combated. The same process is responsible for causing tissue damage and underlies many important diseases. We will return to inflammation in the context of innate immunity in Chapter 4 and in the discussion of inflammatory diseases in Chapter 18.

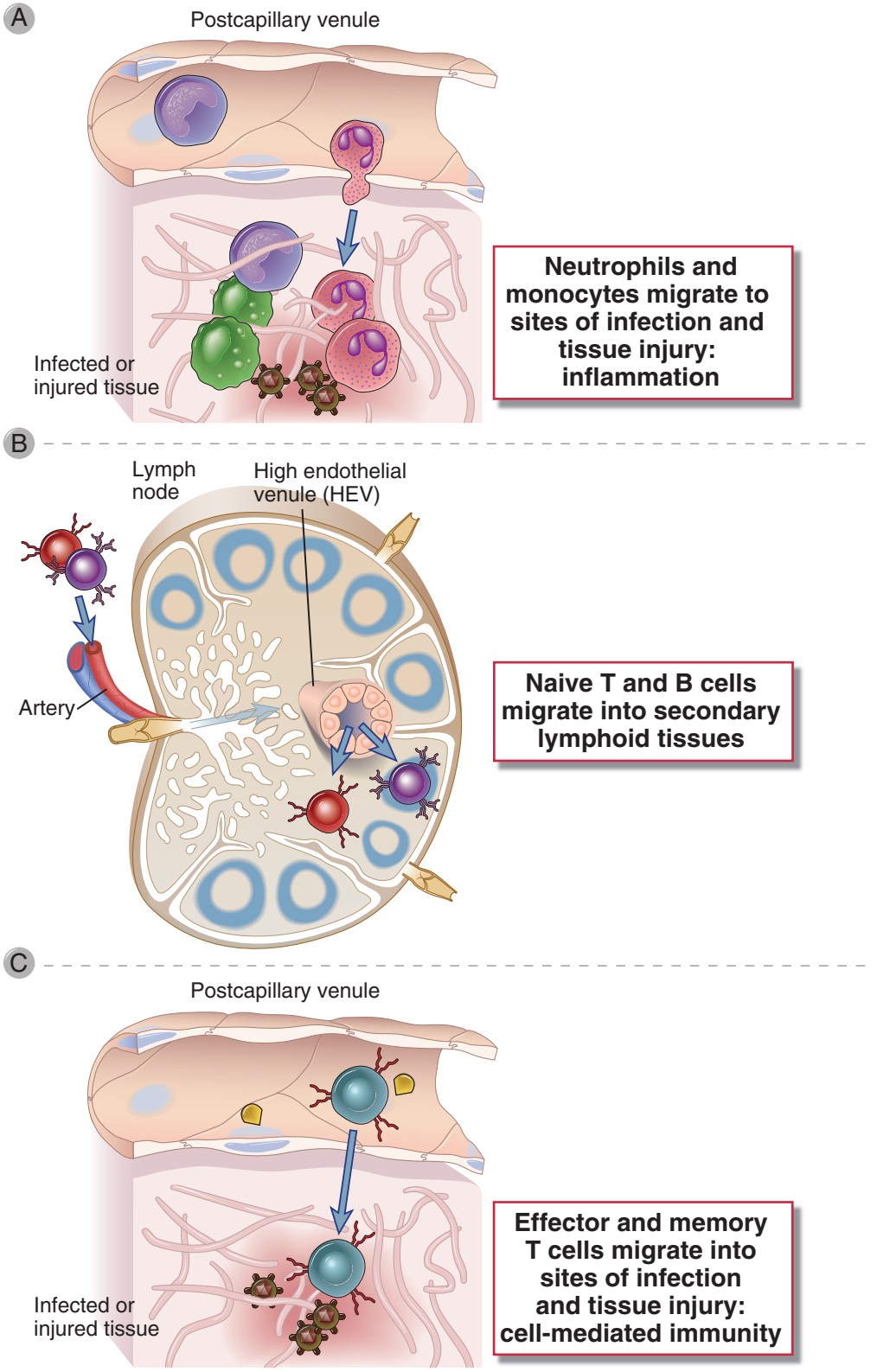


FIGURE 3–1 The main functions served by leukocyte migration from blood into tissues. A, Neutrophils and monocytes that arise in the bone marrow are recruited into tissue sites of infection or injury, where they eliminate infectious pathogens, clear dead tissues, and repair the damage. **B,** Naive lymphocytes that arise in bone marrow or thymus home to secondary lymphoid organs, such as lymph nodes (or spleen, not shown), where they become activated by antigens and differentiate into effector lymphocytes. **C,** Effector lymphocytes arising in secondary lymphoid organs migrate into tissue sites of infection, where they participate in microbial defense.

Leukocyte recruitment from the blood into tissues depends first on adhesion of the leukocytes to the endothelial lining of postcapillary venules and then movement through the endothelium and underlying basement membrane into the extravascular tissue. This is a multistep process in which each step is orchestrated by different types of molecules, including chemokines and adhesion molecules. The same basic process occurs for different types of leukocytes (neutrophils, monocytes, and naive and effector lymphocytes) homing to different types of tissues (secondary lymphoid organs, infected tissues), although the specific chemokines and adhesion molecules vary in ways that result in different migration properties for each cell type. Before describing the process, we discuss the properties and functions of the adhesion molecules and chemokines that are involved in leukocyte recruitment.

ADHESION MOLECULES ON LEUKOCYTES AND ENDOTHELIAL CELLS INVOLVED IN LEUKOCYTE RECRUITMENT

The migration of leukocytes from the blood into tissues involves adhesion between the circulating leukocytes and vascular endothelial cells as a prelude to the movement of the leukocytes out of the vessels into the tissues. This adhesion is mediated by two classes of molecules, called selectins and integrins, and their ligands. The expression of these molecules varies among different types of leukocytes and in blood vessels at different locations. We next describe the major selectins and integrins and their ligands and their roles in leukocyte recruitment into tissues.

Selectins and Selectin Ligands

Selectins are plasma membrane carbohydrate-binding adhesion molecules that mediate an initial step of low-affinity adhesion of circulating leukocytes to endothelial cells lining postcapillary venules (Table 3-1). The extracellular domains of selectins are similar to C-type lectins, so called because they bind carbohydrate structures (the definition of lectins) in a calcium-dependent manner. Selectins and their ligands are expressed on leukocytes and endothelial cells.

Two types of selectins are expressed by endothelial cells, called **P-selectin** (CD62P) and **E-selectin** (CD62E). P-selectin, so called because it was first found in platelets, is stored in cytoplasmic granules of endothelial cells and is rapidly redistributed to the surface in response to microbial products, cytokines, histamine from mast cells, and thrombin generated during blood coagulation. E-selectin is synthesized and expressed on the endothelial cell surface within 1 to 2 hours in response to the cytokines interleukin-1 (IL-1) and tumor necrosis factor (TNF) and microbial products such as lipopolysaccharide (LPS). We will discuss IL-1, TNF, and LPS in our discussion of inflammation in Chapter 4.

The ligands on leukocytes that bind to E-selectin and P-selectin on endothelial cells are complex sialylated carbohydrate groups related to the Lewis X or Lewis A family, present on various surface glycoproteins of granulocytes, monocytes, and some previously activated effector and memory T cells. The best defined of these is the tetrasaccharide sialyl Lewis X (sLeX). A leukocyte membrane glycoprotein called P-selectin glycoprotein ligand 1 (PSGL-1) is post-translationally modified to display the carbohydrate ligands for P-selectin. Several different

TABLE 3–1 Major Leukocyte-Endothelial Adhesion Molecules

Family	Molecule	Distribution	Ligand (molecule; cell type)
Selectin	P-selectin (CD62P)	Endothelium activated by cytokines (TNF, IL-1), histamine, or thrombin	Sialyl Lewis X on PSGL-1 and other glycoproteins; neutrophils, monocytes, T cells (effector, memory)
	E-selectin (CD62E)	Endothelium activated by cytokines (TNF, IL-1)	Sialyl Lewis X (e.g., CLA-1) on glycoproteins; neutrophils, monocytes, T cells (effector, memory)
	L-selectin (CD62L)	Neutrophils, monocytes, T cells (naive and central memory), B cells (naive)	Sialyl Lewis X/PNAd on GlyCAM-1, CD34, MadCAM-1, others; endothelium (HEV)
Integrin	LFA-1 (CD11aCD18)	Neutrophils, monocytes, T cells (naive, effector, memory)	ICAM-1 (CD54), ICAM-2 (CD102); endothelium (upregulated when cytokine activated)
	Mac-1 (CD11bCD18)	Monocytes, dendritic cells	ICAM-1 (CD54), ICAM-2 (CD102); endothelium (upregulated when cytokine activated)
	VLA-4 (CD49aCD29)	Monocytes, T cells (naive, effector, memory)	VCAM-1 (CD106); endothelium (upregulated when cytokine activated)
	$\alpha_4\beta_7$ (CD49dCD29)	Monocytes, T cells (gut homing, naive, effector, memory)	VCAM-1 (CD106), MadCAM-1; endothelium in gut and gut-associated lymphoid tissues

CLA-1, cutaneous lymphocyte antigen 1; GlyCAM-1, glycan-bearing cell adhesion molecule 1; HEV, high endothelial venule; ICAM-1, intracellular adhesion molecule 1; IL-1, interleukin-1; LFA-1, leukocyte function-associated antigen 1; MadCAM-1, mucosal addressin cell adhesion molecule 1; PNAd, peripheral node addressin; PSGL-1, P-selectin glycoprotein ligand 1; TNF, tumor necrosis factor; VCAM-1, vascular cell adhesion molecule 1; VLA-4, very late antigen 4.

molecules may display the carbohydrate ligands for E-selectin, including the glycoproteins PSGL-1 and E-selectin ligand 1 and some glycolipids.

A third selectin, called **L-selectin** (CD62L), is expressed on leukocytes but not on endothelial cells. The ligands for L-selectin are sialomucins displayed on high endothelial venules, collectively called peripheral node addressin (PNAd). A major recognition determinant that L-selectin binds to on these sialomucins is sialyl 6-sulfo Lewis X. The expression of these ligands is increased by cytokine activation of endothelial cells. L-selectin on neutrophils serves to bind these cells to endothelial cells that are activated by IL-1, TNF, and other cytokines produced at sites of inflammation. In adaptive immunity, L-selectin is important for naive T lymphocytes to home into lymph nodes through high endothelial venules. Leukocytes express L-selectin and the carbohydrate ligands for P-selectin and E-selectin at the tips of their microvilli, facilitating interactions with molecules on the endothelial cell surface.

Integrins and Integrin Ligands

Integrins are heterodimeric cell surface proteins composed of two noncovalently linked polypeptide chains that mediate adhesion of cells to other cells or to extracellular matrix, through specific binding interactions with various ligands. There are more than 30 different integrins, all with the same basic structure, containing one of more than 15 types of α chains and one of seven types of β chains. The extracellular globular heads of both chains contribute to interchain linking and to divalent cation-dependent ligand binding. The cytoplasmic domains of the integrins interact with cytoskeletal components (including vinculin, talin, actin, α-actinin, and tropomyosin). The name of this family of proteins derives from the idea that they coordinate (i.e., integrate) signals generated when they bind extracellular ligands with cytoskeleton-dependent motility, shape change, and phagocytic responses.

In the immune system, the most important integrins are two that are expressed on leukocytes, called LFA-1 (leukocyte function-associated antigen 1, more precisely named $\beta_2\alpha_L$ or CD11aCD18) and VLA-4 (very late antigen 4, or $\beta_1\alpha_4$, or CD49dCD29) (see Table 3-1). One important ligand for LFA-1 is intercellular adhesion molecule 1 (ICAM-1, CD54), a membrane glycoprotein expressed on cytokine-activated endothelial cells and on a variety of other cell types, including lymphocytes, dendritic cells, macrophages, fibroblasts, and keratinocytes. The extracellular portion of ICAM-1 is composed of globular domains that share some sequence homology and tertiary structural features of domains found in immunoglobulin (Ig) molecules and are called Ig domains. (Many proteins in the immune system contain Ig domains and belong to the Ig superfamily, which is discussed in more detail in Chapter 5.) LFA-1 binding to ICAM-1 is important for leukocyte-endothelial interactions (discussed later) and T cell interactions with antigen-presenting cells (see Chapter 6). Two other Ig superfamily ligands for LFA-1 are ICAM-2, which is expressed on endothelial cells, and ICAM-3, which is expressed on lymphocytes. VLA-4 binds to vascular cell adhesion molecule 1 (VCAM-1, CD106), an Ig superfamily protein expressed on cytokine-activated endothelial cells in some tissues, and this interaction is important for leukocyte recruitment into inflammatory sites. Other integrins also play roles in innate and adaptive immune responses. For example, Mac-1 ($\beta_2\alpha_m$, CD11bCD18) on circulating monocytes binds to ICAM-1 and mediates adhesion to endothelium. Mac-1 also functions as a complement receptor, binding particles opsonized with a product of complement activation called the inactivated C3b (iC3b) fragment, and thereby enhances phagocytosis of microbes.

An important feature of integrins is their ability to respond to intracellular signals by rapidly increasing their affinity for their ligands (Fig. 3-2). This is referred to as activation and occurs in response to signals generated from chemokine binding to chemokine receptors and in lymphocytes by intracellular signals generated when antigen binds to antigen receptors. The process of changes in the binding functions of the extracellular domain of integrins induced by intracellular signals is called inside-out signaling. Chemokine- and antigen

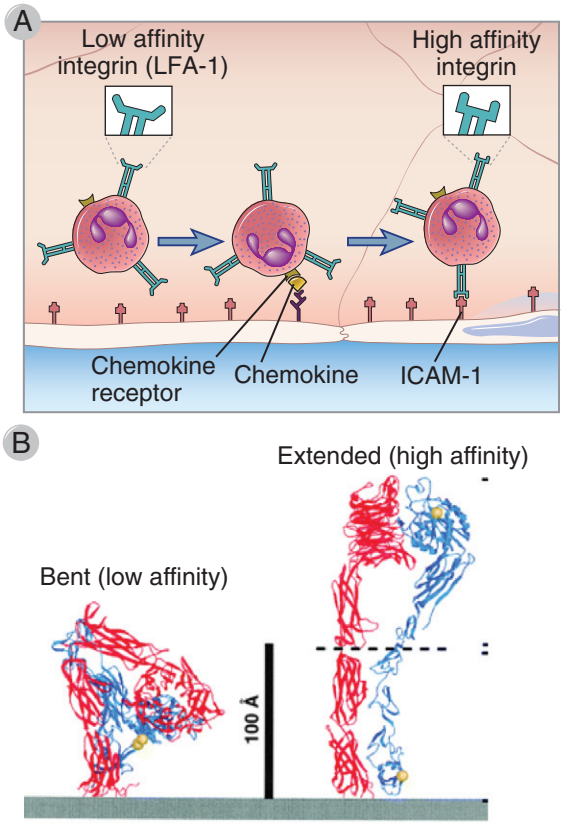


FIGURE 3–2 Integrin activation. A, The integrins on blood leukocytes are normally in a low-affinity state. If a leukocyte comes close to endothelial cells, such as when selectin-dependent rolling of leukocytes occurs, then chemokines displayed on the endothelial surface can bind chemokine receptors on the leukocyte. Chemokine receptor signaling then occurs, which activates the leukocyte integrins, increasing their affinity for their ligands on the endothelial cells. **B,** Ribbon diagrams are shown of bent and extended conformations of a leukocyte integrin, corresponding to low- and high-affinity states, respectively. *(**B** From Takagi J, and TA Springer. Integrin activation and structural rearrangement. Immunological Reviews 186:141-163, 2002.)*

receptor–induced inside-out signaling involves GTP-binding proteins (described in more detail later), eventually leading to the association of RAP family molecules and cytoskeleton-interacting proteins with the cytoplasmic tails of the integrin proteins. The resulting affinity changes are a consequence of conformational changes in the extracellular domains. In the low-affinity state, the stalks of the extracellular domains of each integrin subunit appear to be bent over, and the ligand-binding globular heads are close to the membrane. In response to alterations in the cytoplasmic tail, the stalks extend in switchblade fashion, bringing the globular heads away from the membrane to a position where they more effectively interact with their ligands (see Fig. 3-2).

Chemokines also induce membrane clustering of integrins. This results in increased avidity of integrin interactions with ligands on the endothelial cells, and therefore tighter binding of the leukocytes to the endothelium.

CHEMOKINES AND CHEMOKINE RECEPTORS

Chemokines are a large family of structurally homologous cytokines that stimulate leukocyte movement and regulate the migration of leukocytes from the blood to tissues. The name *chemokine* is a contraction of "chemotactic cytokine." We have already referred to the role of chemokines in the organization of lymphoid tissue and now we will describe the general properties of this family of cytokines and summarize their multiple roles in innate and adaptive immunity. Table 3-2 summarizes the major features of individual chemokines and their receptors.

Chemokine Structure, Production, and Receptors

There are about 50 human chemokines, all of which are 8- to 12-kD polypeptides that contain two internal disulfide loops. The chemokines are classified into four families on the basis of the number and location of N-terminal cysteine residues. The two major families are the CC (also called β) chemokines, in which the cysteine residues are adjacent, and the CXC (or α) family, in which these residues are separated by one amino acid. These differences correlate with organization of the subfamilies into separate gene clusters. A small number of chemokines have a single cysteine (C family) or two cysteines separated by three amino acids (CX_3C). Chemokines were originally named on the basis of how they were identified and what responses they triggered. More recently, a standard nomenclature, based in part on which receptors the chemokines bind to (see Table 3-2), is being used. Although there are exceptions, most of the CC chemokines and their receptors mediate recruitment of neutrophils and lymphocytes, and most of the CXC chemokines and their receptors recruit monocytes and lymphocytes.

The chemokines of the CC and CXC subfamilies are produced by leukocytes and by several types of tissue cells, such as endothelial cells, epithelial cells, and fibroblasts. In many of these cells, secretion of chemokines is induced by recognition of microbes through various cell receptors of the innate immune system discussed in Chapter 4. In addition, inflammatory cytokines, mainly TNF and IL-1, induce chemokine production. Several CC chemokines are also produced by antigen-stimulated T cells, providing a link between adaptive immunity and recruitment of inflammatory leukocytes.

The receptors for chemokines belong to the seven-transmembrane, guanosine triphosphate (GTP)–binding (G) protein–coupled receptor (GPCR) superfamily. These receptors initiate intracellular responses through associated trimeric G proteins. In a resting cell, the receptor-associated G proteins form a stable inactive complex containing guanosine diphosphate (GDP) bound to Gα subunits. Occupancy of the receptor by ligand results in an exchange of GTP for GDP. The GTP-bound form of the G protein activates numerous cellular enzymes, including an isoform of phosphatidylinositol-specific phospholipase C that functions to increase intracellular calcium and activate protein kinase C. The G proteins stimulate cytoskeletal changes and polymerization of actin and myosin filaments, resulting in increased cell motility. These signals also change the conformation of cell surface integrins and increase the affinity of the integrins for their ligands. Chemokine receptors may be rapidly downregulated by exposure to the chemokine, and this is a likely mechanism for termination of responses.

TABLE 3–2 Chemokines and Chemokine Receptors

Chemokine	Original Name	Chemokine Receptor	Major Function
CC chemokines			
CCL1	I-309	CCR8	Monocyte recruitment and endothelial cell migration
CCL2	MCP-1	CCR2	Mixed leukocyte recruitment
CCL3	MIP-1α	CCR1, CCR5	Mixed leukocyte recruitment
CCL4	MIP-1β	CCR5	T cell, dendritic cell, monocyte, and NK recruitment; HIV coreceptor
CCL5	RANTES	CCR1, CCR3, CCR5	Mixed leukocyte recruitment
CCL7	MCP-3	CCR1, CCR2, CCR3	Mixed leukocyte recruitment
CCL8	MCP-2	CCR3, CCR5	Mixed leukocyte recruitment

Continued

TABLE 3–2 Chemokines and Chemokine Receptors—cont'd

Chemokine	Original Name	Chemokine Receptor	Major Function
CCL9/CCL10		CCR1	
CCL11	Eotaxin	CCR3	Eosinophil, basophil, and T_H2 recruitment
CCL12	Unknown	CCR2	Mixed leukocyte recruitment
CCL13	MCP-4	CCR2, CCR3	Mixed leukocyte recruitment
CCL14	HHC-1	CCR1, CCR5	
CCL15	MIP-1δ	CCR1, CCR3	Mixed leukocyte recruitment
CCL16	HHC-4	CCR1, CCR2	
CCL17	TARC	CCR4	T cell and basophil recruitment
CCL18	DC-CK1	?	Lymphocyte and dendritic cell homing
CCL19	MIP-3β/ELC	CCR7	T cell and dendritic cell migration into parafollicular zones of lymph nodes
CCL20	MIP-3α	CCR6	
CCL21	SLC	CCR7	T cell and dendritic cell migration into parafollicular zones of lymph nodes
CCL22	MDC	CCR4	T cell and basophil recruitment
CCL23	MPIF-1	CCR1	
CCL24	Eotaxin-2	CCR3	Eosinophil, basophil, and T_H2 recruitment
CCL25	TECK	CCR9	Astrocyte migration
CCL26	Eotaxin-3	CCR3	Eosinophil, basophil, and T_H2 recruitment
CCL27	CTACK	CCR10	Dermal cell migration
CCL28	MEC	CCR10	Dermal cell migration
CXC chemokines			
CXCL1	GROα	CXCR2	Neutrophil recruitment
CXCL2	GROβ	CXCR2	Neutrophil recruitment
CXCL3	GROγ	CXCR2	Neutrophil recruitment
CXCL4	PF4	CXC3B	Platelet aggregation
CXCL5	ENA-78	CXCR2	Neutrophil recruitment
CXCL6	GCP-2	CXCR1, CXCR2	Neutrophil recruitment
CXCL7	NAP-2	CXCR2	Neutrophil recruitment
CXCL8	IL-8	CXCR1, CXCR-2	Neutrophil recruitment
CXCL9	Mig	CXCR3	Effector T cell recruitment
CXCL10	IP-10	CXC3, CXCR3B	Effector T cell recruitment
CXCL11	I-TAC	CXC3	Effector T cell recruitment
CXCL12	SDF-1αβ	CXCR4	Mixed leukocyte recruitment; HIV coreceptor
CXCL13	BCA-1	CXCR5	B cell migration into follicles
CXCL14	BRAK		
CXCL16	—	CXCR5	CXCL16
C chemokines			
XCL1	Lymphotactin	XCR1	T cell and NK cell recruitment
XCL2	SCM-1β	XCL1	
CX_3C chemokines			
CX_3CL1	Fractalkine	CX_3CR1	T cell, NK cell, and macrophage recruitment; CTL and NK cell activation

Different combinations of more than 17 different chemokine receptors are expressed on different types of leukocytes, which results in distinct patterns of migration of the leukocytes. There are 10 distinct receptors for CC chemokines (called CCR1 through CCR10), six for CXC chemokines (called CXCR1 through CXCR6), and one for CX_3CL1 (called CX_3CR1) (see Table 3-2). Chemokine receptors are expressed on all leukocytes, with the greatest number and diversity seen on T cells. The receptors exhibit overlapping specificity for chemokines within each family, and the pattern of cellular expression of the receptors determines which cell types respond to which chemokines. Certain chemokine receptors, notably CCR5 and CXCR4, act as coreceptors for the human immunodeficiency virus (HIV) (see Chapter 20). Some activated T lymphocytes secrete chemokines that bind to CCR5 and block infection with HIV by competing with the virus.

Biologic Actions of Chemokines

Some chemokines are produced by leukocytes and other cells in response to external stimuli and are involved in inflammatory reactions, and other chemokines are produced constitutively in tissues and play a role in tissue organization. Chemokines were discovered on the basis of their activity as leukocyte chemoattractants, and this action is the main basis of their functional roles.

- ***Chemokines are essential for the recruitment of circulating leukocytes from blood vessels into extravascular sites.*** Leukocyte recruitment, including naive lymphocytes entering lymph nodes through high endothelial venules and effector lymphocytes, monocytes, and neutrophils entering into tissue sites of infection, is regulated by the actions of several chemokines. Chemokines produced in the tissues bind to heparan sulfate proteoglycans on endothelial cells that line postcapillary venules and are displayed in this way to circulating leukocytes that have bound to the endothelial surfaces through adhesion molecule interactions. The endothelial display provides a high local concentration of chemokines, which bind to chemokine receptors on the leukocytes. Signals from chemokine receptors lead to enhanced integrin affinity, which results in firm adhesion of the leukocyte, a critical step for migration of leukocytes out of blood vessels into extravascular tissue. Different chemokines act on different cells and, in coordination with the types of adhesion molecules expressed, thus control the nature of the inflammatory infiltrate.
- ***Extravascular chemokines stimulate movement of leukocytes and their migration toward the chemical gradient of the secreted protein, a process called chemokinesis.*** In this way, leukocytes can be directed toward infected cells in tissues or toward particular regions within lymphoid organs.
- ***Chemokines are involved in the development of lymphoid organs, and they regulate the traffic of lymphocytes and other leukocytes through peripheral lymphoid tissues.*** The function of chemokines in the anatomic organization of lymphoid tissues has been discussed in Chapter 2.
- ***Chemokines are required for the migration of dendritic cells from sites of infection into draining lymph nodes.*** Dendritic cells play a key role in bridging innate and adaptive immunity. They use various receptors to recognize and respond to microbes in peripheral tissues, and they then migrate to lymph nodes to inform T lymphocytes of the presence of infection (discussed in Chapter 6). The migration is dependent on upregulation of CCR7 on the dendritic cell in response to recognition of microbes. CCR7 allows the dendritic cell to respond to CCL19 and CCL21, two chemokines that are made in the lymph nodes. Recall that CCR7 is also the chemokine receptor on naive T cells, which explains how dendritic cells and naive T cells localize to the same place in lymph nodes, enabling the dendritic cells to present antigen to the T cells.

LEUKOCYTE-ENDOTHELIAL INTERACTIONS AND LEUKOCYTE EXTRAVASATION

Selectins, integrins, and chemokines work in concert to govern the leukocyte-endothelial interactions that are required for migration of leukocytes into tissues (Fig. 3-3). Studies of these interactions under flow conditions in vitro, and in vivo, by use of intravital microscopic techniques, have established a sequence of events common to migration of most leukocytes into most tissues. These events include the following.

- ***Selectin-mediated rolling of leukocytes on endothelium.*** In response to microbes and cytokines produced by cells (e.g., macrophages) that encounter the microbes, endothelial cells lining postcapillary venules at the site of infection rapidly increase surface expression of selectins. Leukocytes move close to the endothelium-lined walls of venules in sites of innate immune responses as a result of vasodilation and slowing of blood flow, and the selectin ligands on the microvilli of the leukocytes bind to the selectins on the endothelial cells. Because selectin–selectin ligand interactions are of low affinity (K_d ~100 μm) with a fast off-rate, they are easily disrupted by the shear force of the flowing blood. As a result, the leukocytes repetitively detach and bind again and thus roll along the endothelial surface. This slowing of leukocytes on the endothelium allows the next set of stimuli in the multistep process to act on the leukocytes.
- ***Chemokine-mediated increase in affinity of integrins.*** As discussed earlier, chemokines are produced at an infection site by various cell types in response to a variety of pathogens or endogenous stimuli. Once secreted, they are transported to the luminal surface of the endothelial cells of postcapillary venules, where they are bound by heparan sulfate glycosaminoglycans and are displayed at high concentrations. At this location, the chemokines bind to specific chemokine receptors on the surface of the rolling leukocytes. Leukocyte integrins are in a low-affinity state in unactivated cells and ineffective in mediating adhesion interactions. Two consequences of chemokine receptor signaling are enhanced affinity of leukocyte integrins

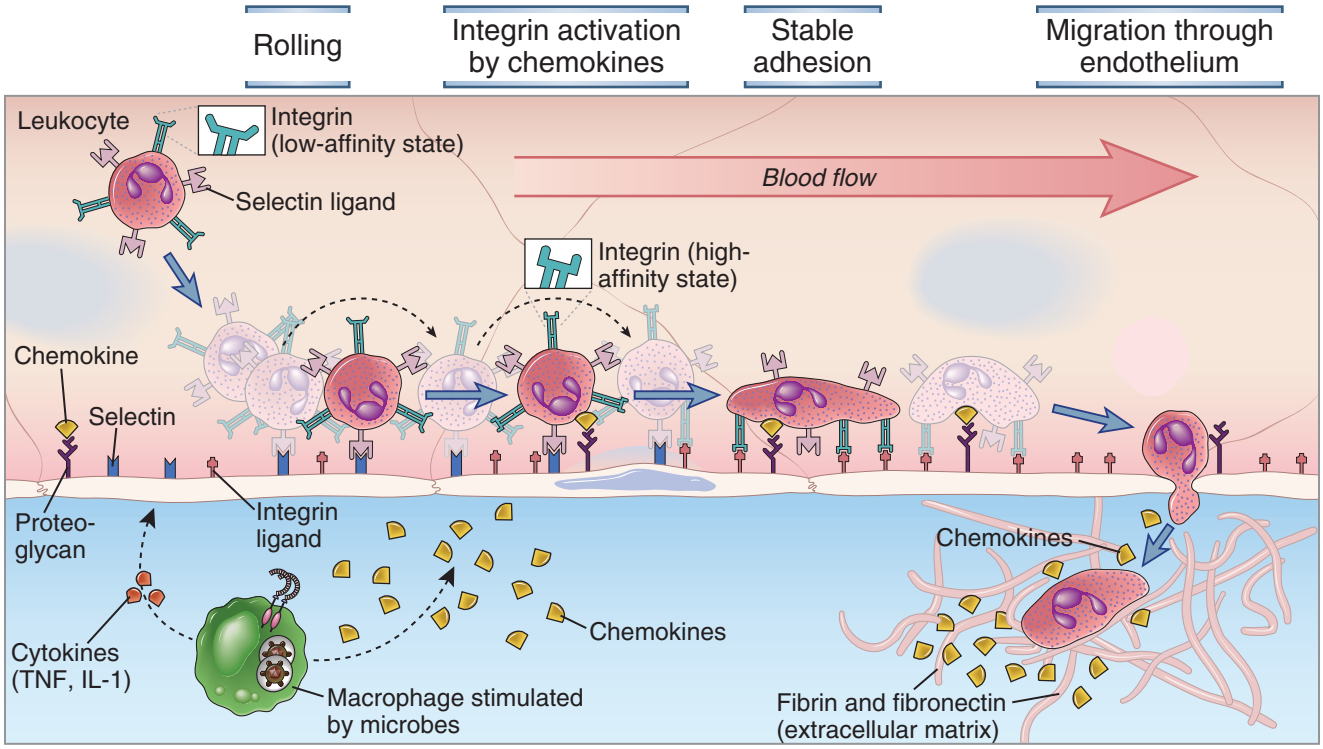


FIGURE 3–3 Multistep leukocyte-endothelial interactions mediating leukocyte recruitment into tissues. At sites of infection, macrophages that have encountered microbes produce cytokines (such as TNF and IL-1) that activate the endothelial cells of nearby venules to produce selectins, ligands for integrins, and chemokines. Selectins mediate weak tethering and rolling of blood leukocytes on the endothelium, and the shear force of blood flow causes the leukocytes to roll along the endothelial surface. Chemokines produced in the surrounding infected tissues or by the endothelial cells are displayed on the endothelial surface and bind to receptors on the rolling leukocytes, which results in activation of the leukocyte integrins to a high-affinity binding state. The activated integrins bind to their Ig superfamily ligands on the endothelial cells, and this mediates firm adhesion of leukocytes. The leukocytes then crawl to junctions between endothelial cells and migrate through the venular wall. Neutrophils, monocytes, and T lymphocytes use essentially the same mechanisms to migrate out of the blood.

for their ligands and membrane clustering of the integrins, resulting in increased avidity of binding of leukocyte integrins to their ligands on the endothelial surface.

- ***Stable integrin-mediated adhesion of leukocytes to endothelium.*** In parallel with the activation of integrins and their conversion to the high-affinity state, cytokines (TNF and IL-1) also enhance endothelial expression of integrin ligands, mainly VCAM-1, the ligand for the VLA-4 integrin, and ICAM-1, the ligand for the LFA-1 and Mac-1 integrins. The net result of these changes is that the leukocytes attach firmly to the endothelium, their cytoskeleton is reorganized, and they spread out on the endothelial surface.
- ***Transmigration of leukocytes through the endothelium.*** Most often, leukocytes transmigrate between the borders of endothelial cells, a process called paracellular transmigration, to reach extravascular tissues. Paracellular transmigration depends on leukocyte integrins and their ligands on the endothelial cells as well as other proteins, notably CD31, which is expressed on the leukocytes and endothelial cells. This process requires a transient and reversible disruption of adherens junction proteins that hold postcapillary endothelial cells together, primarily the VE-cadherin complex. The mechanism responsible for disruption of the VE-cadherin complex is thought to involve activation of kinases when leukocyte integrins bind ICAM-1 or VCAM-1. The kinases phosphorylate the cytoplasmic tail of VE-cadherin and lead to reversible disruption of the adherens complex. Less often, leukocytes have been observed to move through endothelial cells rather then between them, by a less well understood process called transcellular migration.

There is specificity in this process of leukocyte migration based on the expression of distinct combinations of adhesion molecule and chemokine receptors on neutrophils, monocytes, and different subsets of lymphocytes, as we will discuss in more detail later.

Evidence for the essential role of selectins, integrins, and chemokines in leukocyte migration has come from gene knockout mice and rare human diseases caused by gene mutations. For example, mice lacking fucosyltransferases, which are enzymes required to synthesize the carbohydrate ligands that bind to selectins, have marked defects in leukocyte migration and immune responses. Humans who lack one of the enzymes needed to express the carbohydrate ligands for E-selectin and P-selectin on neutrophils have similar problems, resulting in a

syndrome called type 2 leukocyte adhesion deficiency (LAD-2) (see Chapter 20). Similarly, an autosomal recessive inherited deficiency in the CD18 gene, which encodes the β subunit of LFA-1 and Mac-1, is the cause of an immune deficiency disease called type 1 leukocyte adhesion deficiency (LAD-1). These disorders are characterized by recurrent bacterial and fungal infections, lack of neutrophil accumulation at sites of infection, and defects in adherence-dependent lymphocyte functions. Rare human mutations in the signaling pathways linking chemokine receptors to integrin activation also result in impaired leukocyte adhesion and recruitment into tissues and therefore ineffective leukocyte defense against infections.

MIGRATION OF NEUTROPHILS AND MONOCYTES TO SITES OF INFECTION OR TISSUE INJURY

After maturing in the bone marrow, neutrophils and monocytes enter the blood and circulate throughout the body. Although these cells can perform some phagocytic functions within the blood, their main functions, including phagocytosis of microbes and dead tissue cells, take place in extravascular sites of infection virtually anywhere in the body.

Blood neutrophils and monocytes are recruited to tissue sites of infection and injury by a selectin-, integrin-, and chemokine-dependent multistep process, which follows the basic sequence common to the migration of all leukocytes into tissues, discussed before. Cytokines (TNF and IL-1) secreted during the innate immune response to microbes induce the expression of adhesion molecules (selectins and integrin ligands) on endothelial cells and the local production of chemokines. Neutrophils and monocytes in the circulation bind to these adhesion molecules and respond to the chemokines, resulting in recruitment of the leukocytes into the tissues.

Neutrophils and monocytes express distinct sets of adhesion molecules and chemokine receptors and therefore migrate into different inflammatory sites or into the same inflammatory site at different times. As we will discuss in detail in Chapter 4, neutrophils are the first type of leukocyte to be recruited from the blood into a site of infection or tissue injury. Monocyte recruitment follows hours later and continues, perhaps for days, after neutrophil recruitment stops. Furthermore, in some inflammatory sites, neutrophils are not recruited at all, but monocytes are. These different migratory behaviors reflect variations in expression of adhesion molecules and chemokine receptors on neutrophils and monocytes and the fact that different chemokines are expressed in different sites or at different times in the same site. Both monocytes and neutrophils express L-selectin and P- and E-selectin ligands and use all three selectins to mediate initial rolling interactions with cytokine-activated endothelial cells. Neutrophils express the integrins LFA-1 and Mac-1, which, on activation, bind to endothelial ICAM-1 and mediate stable arrest of the cells on the vessel wall. Monocytes express the integrins LFA-1 and VLA-4, which bind to endothelial ICAM-1 and VCAM-1, causing stable arrest of these leukocytes.

The chemokine receptors expressed on neutrophils and monocytes are also different, which is probably the major determinant of the divergent migratory behavior of each cell type. Neutrophils express CXCR1 and CXCR2, which bind GRO family chemokines including CXCL8 (IL-8), the major chemokine supporting neutrophil migration into tissues (see Table 3-2). Thus, early neutrophil recruitment reflects early and abundant CXCL8 production by tissue resident macrophages in response to infections. There are at least two populations of monocytes in the blood, and in both humans and mice, the populations are defined, in part, by chemokine receptor expression. Inflammatory monocytes, which are the main type recruited to inflammatory sites, express CCR2 in both mice and humans. This receptor binds several chemokines, but the most important one for monocyte recruitment is CCL2 (MCP-1). Thus, monocyte recruitment occurs when resident tissue cells express CCL2 in response to infection. The other population of monocytes, sometimes called nonclassical, lacks CCR2 but expresses CX_3CR1. The ligand for this receptor, CX_3CL1, is expressed both in soluble form and as a membrane-bound molecule that can support adhesion of monocytes to endothelium.

Once neutrophils enter inflammatory sites, they perform various effector functions, which we will describe in Chapter 4, and die within hours. Monocytes become macrophages in the tissues and perform their effector functions during the course of many days to weeks. Some macrophages may migrate into lymph nodes through draining lymphatics.

MIGRATION AND RECIRCULATION OF T LYMPHOCYTES

Lymphocytes are continuously moving through the blood stream, lymphatics, secondary lymphoid tissues, and peripheral nonlymphoid tissues, and functionally distinct populations of lymphocytes show different trafficking patterns through these sites (Fig. 3-4). When a mature naive T cell emerges from the thymus and enters the blood, it homes to lymph nodes, spleen, or mucosal lymphoid tissues and migrates into the T cell zones of these secondary lymphoid tissues. If the T cell does not recognize antigen in these sites, it remains naive and leaves the nodes or mucosal tissue through lymphatics and eventually drains back into the blood stream. Naive T cells leave the spleen directly through the circulation. Once back in the blood, a naive T cell repeats its homing to other secondary lymph nodes. This traffic pattern of naive lymphocytes, called **lymphocyte recirculation**, maximizes the chances that the limited number of naive lymphocytes emerging from the thymus that are specific for a particular foreign antigen will encounter the antigen if it shows up anywhere in the body. Lymphocytes that have recognized and become activated by antigen proliferate and differentiate to produce thousands of effector and memory cells within secondary lymphoid tissues. The effector and memory lymphocytes may move back into the blood stream and then migrate into sites of infection or inflammation in peripheral (nonlymphoid) tissues. Some effector lymphocyte subsets preferentially

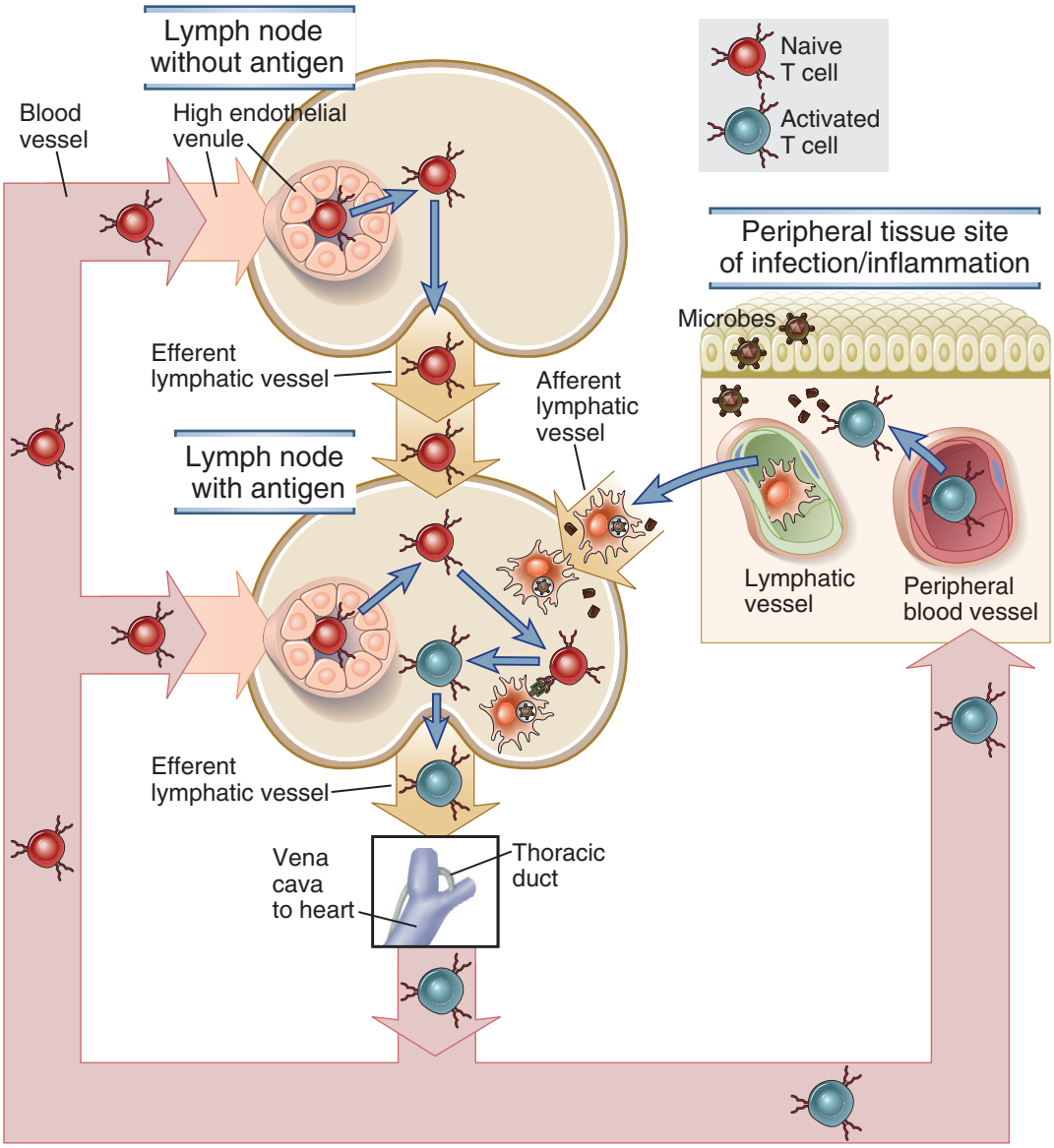


FIGURE 3–4 Pathways of T lymphocyte recirculation. Naive T cells preferentially leave the blood and enter lymph nodes across the high endothelial venules. Dendritic cells bearing antigen enter the lymph node through lymphatic vessels. If the T cells recognize antigen, they are activated, and they return to the circulation through the efferent lymphatics and the thoracic duct, which empties into the superior vena cava, then into the heart, and ultimately into the arterial circulation. Effector and memory T cells preferentially leave the blood and enter peripheral tissues through venules at sites of inflammation. Recirculation through peripheral lymphoid organs other than lymph nodes is not shown.

migrate into a particular tissue, such as skin or gut. The process by which particular populations of lymphocytes selectively enter lymph nodes or particular tissues but not others is called lymphocyte **homing**. The existence of different homing patterns ensures that different subsets of lymphocytes are delivered to the tissue microenvironments where they are required to combat different types of microbes and not, wastefully, to places where they would serve no purpose. In the following section, we describe the mechanisms and pathways of lymphocyte recirculation and homing. Our discussion emphasizes T cells because much more is known about their movement through tissues than is known about B cell recirculation.

Recirculation of Naive T Lymphocytes between Blood and Secondary Lymphoid Organs

T lymphocyte recirculation depends on mechanisms that control entry of naive T cells from the blood into lymph nodes as well as molecular signals that control when naive T cells exit the nodes. We will discuss these two mechanisms separately.

Migration of Naive T Cells into Lymph Nodes

The homing mechanisms that bring naive T cells into lymph nodes are very efficient, resulting in a net flux of lymphocytes through lymph nodes of up to 25×10^9 cells each day. Each lymphocyte goes through one node once

a day on average. Peripheral tissue inflammation, which usually accompanies infections, causes a significant increase of blood flow into lymph nodes and consequently an increase in T cell influx into lymph nodes draining the site of inflammation. At the same time, egress of the T cells into efferent lymphatics is transiently reduced by mechanisms we will discuss later, so that T cells stay in lymph nodes that drain sites of inflammation longer than in other lymph nodes. Antigens are concentrated in the secondary lymphoid organs, including lymph nodes, mucosal lymphoid tissues, and spleen, where they are presented by mature dendritic cells, the type of antigen-presenting cell that is best able to initiate responses of naive T cells (see Chapter 6). Thus, movement and transient retention of naive T cells in the secondary lymphoid organs maximizes the chances of specific encounter with antigen and initiation of an adaptive immune response.

Homing of naive T cells into lymph nodes and mucosa-associated lymphoid tissues occurs through specialized postcapillary venules called high endothelial venules (HEVs) located in the T cell zones. Naive T lymphocytes are delivered to secondary lymphoid tissues through arterial blood flow, and they leave the circulation and migrate into the stroma of lymph nodes through HEVs. These vessels are lined by plump endothelial cells and not the flat endothelial cells that are typical of other venules (Fig. 3-5). HEVs are also present in mucosal lymphoid tissues, such as Peyer's patches in the gut, but not in the spleen. The endothelial cells of HEVs are specialized to display certain adhesion molecules and chemokines on their surfaces, discussed later, which support the selective homing of only certain populations of lymphocytes. Certain cytokines, such as lymphotoxin, are required for HEV development. In fact, HEVs may develop in extralymphoid sites of chronic inflammation where such cytokines are produced for prolonged periods.

Naive T cell migration out of the blood through the HEVs into the lymph node parenchyma is a multistep process consisting of selectin-mediated rolling of the cells, chemokine-induced integrin activation, integrin-mediated firm adhesion, and transmigration through the vessel wall (Fig. 3-6). This process is similar to the migration of other leukocytes, described earlier. The adhesion molecules expressed on lymphocytes are often called homing receptors, and the adhesion molecules that the homing receptors bind to on the endothelial cells are called addressins. The sequential events involved in homing of naive T cells to lymph nodes and the molecules involved are the following.

- The rolling of naive T cells on HEVs in peripheral lymphoid organs is mediated by L-selectin on the lymphocytes binding to its carbohydrate ligand on HEVs called peripheral node addressin (PNAd). The PNAd carbohydrate groups that bind L-selectin may be attached to different sialomucins on the HEV in different tissues. For example, on lymph node HEVs, the PNAd is displayed by two sialomucins, called GlyCAM-1 (glycan-bearing cell adhesion molecule 1) and CD34. In Peyer's patches in the intestinal wall, the L-selectin ligand is

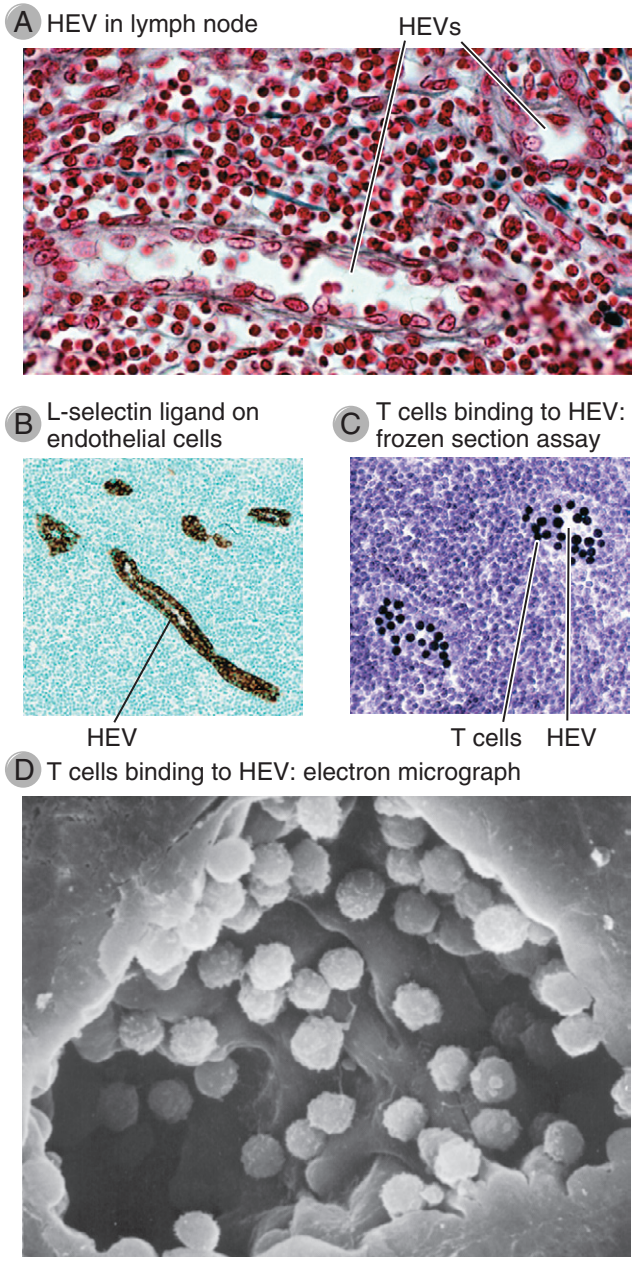
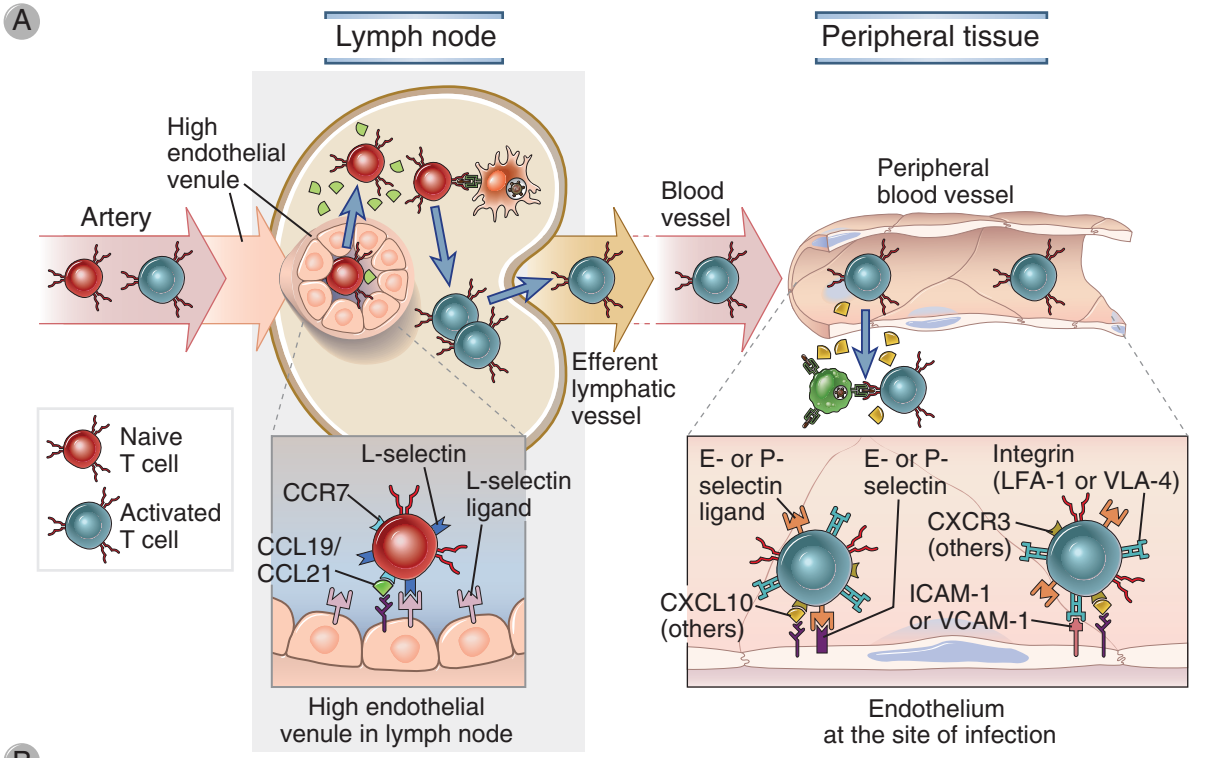


FIGURE 3–5 High endothelial venules. A, Light micrograph of an HEV in a lymph node illustrating the tall endothelial cells. *(Courtesy of Dr. Steve Rosen, Department of Anatomy, University of California, San Francisco.)* **B,** Expression of L-selectin ligand on HEVs, stained with a specific antibody by the immunoperoxidase technique. (The location of the antibody is revealed by a brown reaction product of peroxidase, which is coupled to the antibody; see Appendix IV for details.) The HEVs are abundant in the T cell zone of the lymph node. *(Courtesy of Drs. Steve Rosen and Akio Kikuta, Department of Anatomy, University of California, San Francisco.)* **C,** A binding assay in which lymphocytes are incubated with frozen sections of a lymph node. The lymphocytes (stained dark blue) bind selectively to HEVs. *(Courtesy of Dr. Steve Rosen, Department of Anatomy, University of California, San Francisco.)* **D,** Scanning electron micrograph of an HEV with lymphocytes attached to the luminal surface of the endothelial cells. *(Courtesy of J. Emerson and T. Yednock, University of California, San Francisco, School of Medicine. From Rosen SD, and LM Stoolman. Potential role of cell surface lectin in lymphocyte recirculation. In Olden K, and J Parent [eds]. Vertebrate Lectins. Van Nostrand Reinhold, New York, 1987.)*



B

T cell homing receptor	Ligand on endothelial cell	Function of receptor: ligand pair
Naive T cells		
L-selectin	L-selectin ligand	Initial weak adhesion of naive T cells to high endothelial venule in lymph node
CCR7	CCL19 or CCL21	Activation of integrins and chemokinesis
LFA-1 (β2-integrin)	ICAM-1	Stable arrest on high endothelial venule in lymph node
Activated (effector and memory) T cells		
E- and P-selectin ligand	E- or P-selectin	Initial weak adhesion of effector and memory T cells to cytokine activated endothelium at peripheral site of infection
CXCR3	CXCL10 (others)	Activation of integrins and chemokinesis
CCR5	CCL4 (others)	Activation of integrins and chemokinesis
LFA-1 (β2-integrin) or VLA-4 (β1 integrin)	ICAM-1 or VCAM-1	Stable arrest on cytokine activated endothelium at peripheral site of infection

FIGURE 3–6 Migration of naive and effector T lymphocytes. A, Naive T lymphocytes home to lymph nodes as a result of L-selectin binding to its ligand on high endothelial venules, which are present only in lymph nodes, and as a result of binding chemokines (CCL19 and CCL21) displayed on the surface of the high endothelial venule. Activated T lymphocytes, including effector cells, home to sites of infection in peripheral tissues, and this migration is mediated by E-selectin and P-selectin, integrins, and chemokines that are produced at sites of infection. Additional chemokines and chemokine receptors, besides the ones shown, are involved in effector/memory T cell migration. **B,** The adhesion molecules, chemokines, and chemokine receptors involved in naive and effector/memory T cell migration are described.

a molecule called MadCAM-1 (mucosal addressin cell adhesion molecule 1).

- The subsequent firm adhesion of the T cells to the HEVs is mediated by integrins, mainly LFA-1. The affinity of these integrins on naive T cells is rapidly increased by CCL19 and CCL21, which we introduced in Chapter 2 as chemokines required for the maintenance of T cell zones in lymph nodes. CCL19 is constitutively produced by HEVs and is bound to glycosaminoglycans on the cell surface for display to rolling lymphocytes. CCL21 is produced by other cell types in the lymph node and is displayed by HEVs in the same fashion as CCL19. Recall that both these chemokines bind to the chemokine receptor called CCR7, which is highly expressed on naive lymphocytes. This interaction of the chemokines with CCR7 ensures that naive T cells increase integrin avidity and are able to adhere firmly to HEVs.
- The firmly adherent T cells are no longer subject to dislodgment by the blood flow, but they are capable of crawling on the endothelial surfaces toward intercellular junctions. At these junctions, the T cells move through the vessel wall into the extravascular tissue. This process is likely dependent on other adhesion molecules on the T cell binding to HEV adhesion molecules whose expression is restricted to intercellular junctions.

The important role for L-selectin and chemokines in naive T cell homing to secondary lymphoid tissues is supported by many different experimental observations. Lymphocytes from L-selectin knockout mice do not bind to peripheral lymph node HEVs, and the mice have a marked reduction in the number of lymphocytes in peripheral lymph nodes. There are very few naive T cells in the lymph nodes of mice with genetic deficiencies in CCL19 and CCL21, or CCR7, but the B cell content of these lymph nodes is relatively normal.

Exit of Naive T Cells from Lymph Nodes

Naive T cells that have homed into lymph nodes but fail to recognize antigen and to become activated will eventually return to the blood stream. This return to the blood completes one recirculation loop and provides the naive T cells another chance to enter secondary lymphoid tissues and search for the antigens they can recognize. The major route of reentry into the blood is through the efferent lymphatics, perhaps via other lymph nodes in the same chain, and then through the lymphatic vasculature to the thoracic or right lymphatic duct, and finally into the superior vena cava or right subclavian vein.

The exit of naive T cells from lymph nodes is dependent on a lipid chemoattractant called sphingosine 1-phosphate (S1P), which binds to a signaling receptor on T cells called sphingosine 1-phosphate receptor 1 (S1PR1) (Fig. 3-7). S1P is present at relatively high concentrations in the blood and lymph compared with tissues. This concentration gradient is maintained because an S1P-degrading enzyme, S1P lyase, is ubiquitously present in tissues, so the tissue concentration of the lipid is less than in the lymph and blood. S1PR1 is a G protein–coupled receptor. Signals generated by S1P binding to S1PR1 stimulate directed movement of the naive T cells along the S1P concentration gradient out of the lymph node parenchyma. Circulating naive T cells have very little surface S1PR1 because the high blood concentration of S1P causes internalization of the receptor. Once a naive T cell enters a lymph node, where S1P concentrations are low, it may take several hours for the surface S1P1R to be re-expressed. This allows time for a naive T cell to interact with antigen-presenting cells before it is directed down the S1P concentration gradient into the efferent lymphatic. S1P and the S1P1R are also required for mature naive T cell egress from the thymus, migration of activated T cells out of lymph nodes, and migration of antibody-secreting B cells from secondary lymphoid organs.

Our understanding of the role of S1P and S1PR1 in T cell trafficking is based in large part on studies of the effects of a drug called fingolimod (FTY720), which binds to S1P1R and causes its down-modulation from the cell surface. Fingolimod blocks T cell egress from lymphoid organs and thereby acts as an immunosuppressive drug. It is now approved for the treatment of multiple sclerosis, an autoimmune disease of the central nervous system, and there is great interest in use of fingolimod and other drugs with a similar mechanism of action to treat various autoimmune diseases or graft rejection. Additional experimental evidence for the central role of S1P in naive T cell trafficking comes from studies of mice with genetic ablation of S1PR1. In these mice, there is failure of T cells to leave the thymus and populate secondary lymphoid organs. If naive T cells from S1PR1 knockout are injected into the circulation of other mice, the cells enter lymph nodes but are unable to exit.

Recirculation of T Cells through Other Lymphoid Tissues

Naive T cell homing into gut-associated lymphoid tissues, including Peyer's patches and mesenteric lymph nodes, is fundamentally similar to homing to other lymph nodes and relies on interactions of the T cells with HEVs, which are mediated by selectins, integrins, and chemokines. One particular feature of naive T cell homing to mesenteric lymph nodes and Peyer's patches is the contribution of an Ig superfamily molecule called MadCAM-1 (mucosal addressin cell adhesion molecule 1), which is expressed on HEVs in these sites but not typically elsewhere in the body. Naive T cells express two ligands that bind to MadCAM-1, L-selectin and an integrin called $\alpha_4\beta_7$, and both contribute to the rolling step of naive T cell homing into gut-associated lymphoid tissues.

Naive T cell migration into the spleen is not as finely regulated as homing into lymph nodes. The spleen does not contain HEVs, and it appears that naive T cells are delivered to the marginal zone and red pulp sinuses by passive mechanisms that do not involve selectins, integrins, or chemokines. However, CCR7-binding chemokines do participate in directing the naive T cells into the white pulp. Even though homing of naive T cells to the spleen appears to be less tightly regulated than homing into lymph nodes, the rate of lymphocyte passage through

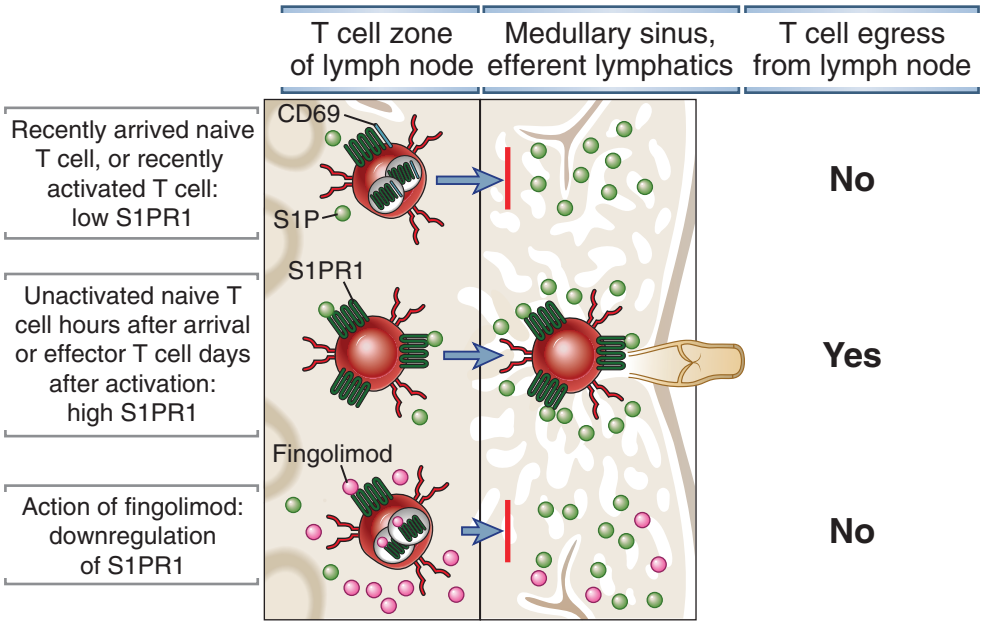


FIGURE 3–7 Mechanism of egress of lymphocytes from lymphoid organs. T cell egress from thymus and lymph node requires the expression of a signaling receptor called S1PR1 that binds to the chemoattractant lipid sphingosine 1-phosphate (S1P). S1P concentrations in blood and lymph are much higher than in lymphoid tissues because of the action of the S1P-degrading enzyme S1P lyase in the tissues. Circulating naive T cells have low levels of S1PR1 because the receptor is internalized after binding S1P in the blood. Therefore, naive T cells that have recently entered a lymph node cannot sense the S1P concentration gradient between the T cell zone of the node and the lymph in the medullary sinus and efferent lymphatics, and these T cells cannot exit the node. After activation of a naive T cell by antigen, S1PR1 is not re-expressed for several days, and the activated cells will also not leave the node. After several hours for naive T cells or days for activated and differentiated effector T cells, S1PR1 is re-expressed, and these cells can then sense the S1P gradient and exit the node. The immunosuppressive drug fingolimod is an agonist of S1PR1 that binds S1PR1 and causes its downmodulation, and is not degraded by S1P lyase. Therefore, this drug interferes with the sensing of the S1P concentration gradient and will block exit of naive and effector cells from the lymph node and reentry into the circulation.

the spleen is very high, about half the total circulating lymphocyte population every 24 hours.

Migration of Effector T Lymphocytes to Sites of Infection

Effector T cells that have been generated by antigen-induced activation of naive T cells exit secondary lymphoid tissues through lymphatic drainage and return to the circulating blood. Many of the protective, antimicrobial functions of effector T cells must be performed locally at sites of infections, and therefore these cells must be able to leave lymphoid tissues. During differentiation of naive T cells into effector cells, which occurs in the peripheral lymphoid organs, the cells undergo a change in expression of chemokine receptors, S1PR1, and adhesion molecules, which determine the migratory behavior of these cells. The expression of S1PR1 is suppressed for several days after antigen-mediated activation of naive T cells, and therefore the ability of the cells to leave the lymphoid tissue in response to an S1P gradient is impaired. This suppression of S1PR1 is controlled in part by cytokines called type I interferons that are expressed during innate immune responses to infections, as we will discuss. Antigenic stimulation and interferons together increase the expression of a T cell membrane protein called CD69, which binds to S1PR1 and blocks its cell surface expression. Thus, the activated T cell becomes transiently insensitive to the S1P gradient. This allows the antigen-activated T cells to remain in the lymphoid organ and undergo clonal expansion and differentiation into effector T cells, a process that takes several days. When differentiation into effector cells is complete, the cells re-express S1PR1 and therefore become responsive to the concentration gradient of S1P, which is low in the lymphoid tissue and high in the draining lymph. CCR7 expression is also greatly reduced in the effector T cells, and therefore these cells are not constrained to remain in the T cell zones where the CCR7 ligands CCL19 and CCL20 are produced. These changes in S1PR1 and CCR7 expression favor egress of the effector T cells out of the lymphoid tissue into efferent lymphatics and subsequent return to the circulating blood. L-selectin expression, which is required for entry of naive T cells into secondary lymphoid tissues, is also reduced in recently differentiated effector T cells. Therefore, two essential molecules needed for reentry of T cells into secondary lymphoid organs through HEV (CCR7 and L-selectin) are lacking on the effector T cells, which prevents these cells from reentering lymphoid tissues and keeps them available for migration into infected tissues.

Circulating effector T cells preferentially home to peripheral tissue sites of infection by a selectin-, integrin-, and chemokine-dependent multistep process (see Fig. 3-6). As with neutrophils and monocytes, selective recruitment of effector T cells into sites of infection,

but not into healthy tissues, is initially dependent on the innate immune response to microbes, leading to cytokine-induced expression of E-selectin, P-selectin, and integrin ligands on postcapillary venular endothelial cells, and local production of various chemokines, which are displayed on the endothelial lining of postcapillary venules. Effector T cells in the circulation express selectin ligands, integrins, and chemokine receptors that bind to the types of selectins, integrin ligands, and chemokines, respectively, that are induced by innate immune responses. The net result is enhanced T cell adhesion to endothelium and transmigration through the venule wall. Because naive T cells do not express ligands for E-selectin and P-selectin nor the chemokine receptors that bind inflammatory chemokines, they are not recruited efficiently to these sites of infection (see Fig. 3-6). Antigen-induced activation of the effector T cells in the inflamed tissues and the continued presence of chemokines keep the integrins on these cells in high-affinity states, and this favors retention of the effector T cells at these sites. Most effector cells that enter a site of infection eventually die at these sites, after performing their effector functions.

Different subsets of effector T cells exist, each with distinct functions, and these subsets have different although often overlapping patterns of migration. Effector T cells include $CD8^+$ cytotoxic T cells and $CD4^+$ helper T cells. Helper T cells include T_H1, T_H2, and T_H17 subsets, each of which expresses different types of cytokines and protects against different types of microbes. The characteristics and functions of these subsets will be discussed in detail in Chapters 9 and 10. For now, it is important to know that the migration of each subset is different. This is because the array of chemokine receptors and adhesion molecules expressed by each subset differs in ways that result in preferential recruitment of each subset into inflammatory sites elicited by different types of infections.

Some effector cells have a propensity to migrate to particular types of tissues. This selective migration capacity is acquired during the differentiation of the effector T cells from naive precursors in secondary lymphoid tissues. By enabling distinct groups of effector T cells to migrate to different sites, the adaptive immune system directs cells with specialized effector functions to the locations where they are best suited to deal with particular types of infections. The clearest examples of populations of effector T cells that specifically home to different tissues are skin-homing and gut-homing T cells. Skin-homing effector T cells express a carbohydrate ligand for E-selectin called CLA-1 (cutaneous lymphocyte antigen 1) and the CCR4 and CCR10 chemokine receptors, which bind CCL17 and CCL27, chemokines that are commonly expressed in inflamed skin. Gut-homing effector T cells express the $\alpha_4\beta_7$ integrin, which binds to MadCAM-1 on gut endothelial cells, and CCR9, which binds to CCL25, a chemokine expressed in inflamed bowel. Remarkably, these distinct migratory phenotypes of skin- and gut-homing effector T cells may be induced by distinct signals delivered to naive T cells at the time of antigen presentation by dendritic cells in either subcutaneous lymph nodes or gut-associated lymphoid tissues, respectively. Although the molecular basis for this imprinting of migratory phenotype is not known, there is evidence that dendritic cells in Peyer's patches produce retinoic acid, which promotes the expression of $\alpha_4\beta_7$ and CCR9 by responding T cells. Similarly, dendritic cells in the skin draining lymph nodes produce vitamin D, which instructs T cells to express CLA-1, CCR4, and CCR10. Other T cells express an integrin called CD103 ($\alpha_E\beta_7$) that can bind to E-cadherin molecules on epithelial cells, allowing T cells to maintain residence as intraepithelial lymphocytes in both skin and gut. We will discuss tissue-specific lymphocyte homing further in Chapter 13.

Memory T Cell Migration

Memory T cells are heterogeneous in their patterns of expression of adhesion molecules and chemokine receptors and in their propensity to migrate to different tissues. Because the ways of identifying memory T cells are still imperfect (see Chapter 2), the distinction between effector and memory T cells in experimental studies and humans is often not precise. Two subsets of memory T cells, namely, central memory and effector memory T cells, were initially identified on the basis of differences in CCR7 and L-selectin expression. Central memory T cells were defined as human $CD45RO^+$ blood T cells that express high levels of CCR7 and L-selectin; effector memory T cells were defined as $CD45RO^+$ blood T cells that express low levels of CCR7 and L-selectin but express other chemokine receptors that bind inflammatory chemokines. These phenotypes suggest that central memory T cells home to secondary lymphoid organs, whereas effector memory T cells home to peripheral tissues. Although central and effector memory T cell populations can also be detected in mice, experimental homing studies have indicated that CCR7 expression is not a definitive marker to distinguish central and effector memory T cell subpopulations. Nonetheless, it is clear that some memory T cells either remain in or tend to home to secondary lymphoid organs, whereas others migrate into peripheral tissues, especially mucosal tissues. In general, the peripheral tissue–homing effector memory T cells respond to antigenic stimulation by rapidly producing effector cytokines, whereas the lymphoid tissue–based central memory cells tend to proliferate more (providing a pool of cells for recall responses) and provide helper functions for B cells.

MIGRATION OF B LYMPHOCYTES

Naive B cells use the same basic mechanisms as do naive T cells to home to secondary lymphoid tissues throughout the body, which enhances their likelihood of responding to microbial antigens in different sites. Immature B cells leave the bone marrow through the blood and enter the red pulp of the spleen, migrate to the periphery of the white pulp, and then, as they mature further, move into the white pulp in response to a chemokine called CXCL13, which binds to the chemokine receptor CXCR5 expressed by the B cell. Once the maturation is completed within the white pulp, naive follicular B cells reenter the circulation and home to lymph nodes and mucosal lymphoid

tissues. Homing of naive B cells from the blood into lymph nodes involves rolling interactions on HEVs, chemokine activation of integrins, and stable arrest, as described earlier for naive T cells. Once naive B cells enter the stroma of secondary lymphoid organs, they migrate into follicles, the site where they may encounter antigen and become activated. This migration of naive B cells into follicles is mediated by CXCL13, which is produced in follicles and binds to the CXCR5 receptor on naive B cells. Homing of naive B cells into Peyer's patches involves CXCR5 and the integrin $\alpha_4\beta_7$, which binds to MadCAM-1. During the course of B cell responses to protein antigens, B cells and helper T cells must directly interact, and this is made possible by highly regulated movements of both cell types within the secondary lymphoid organs. These local migratory events, and the chemokines that orchestrate them, will be discussed in detail in Chapter 11.

Egress of B cells from secondary lymphoid organs depends on S1P1. This has been most clearly shown for differentiated antibody-secreting B cells, which leave the secondary lymphoid organs in which they were generated from naive B cells by antigen activation and home to bone marrow or tissue sites. S1PR1-deficient antibody-secreting cells have diminished ability to home from spleen to bone marrow or to form gut-associated lymphoid tissues. Presumably, naive B cells that have entered secondary lymphoid tissues but do not become activated by antigen reenter the circulation, like naive T cells do, but how this process is controlled is not clear.

Subsets of B cells committed to producing particular types of antibodies migrate from secondary lymphoid organs into specific tissues. As we will describe in later chapters, different populations of activated B cells may secrete different types of antibodies, called isotypes, each of which performs a distinct set of effector functions. Many antibody-producing plasma cells migrate to the bone marrow, where they secrete antibodies for long periods. Most bone marrow–homing plasma cells produce IgG antibodies, which are then distributed throughout the body through the blood stream. B cells within mucosa-associated lymphoid tissues usually become committed to expression of the IgA isotype of antibody, and these committed cells may home specifically to epithelium-lined mucosal tissues. This homing pattern, combined with the local differentiation within the mucosa of B cells into IgA-secreting plasma cells, serves to optimize IgA responses to mucosal infections. As we will discuss in more detail in Chapter 13, IgA is efficiently excreted into the lumen of tissues lined by mucosal epithelia, such as the gut and respiratory tract. The mechanisms by which different B cell populations migrate to different tissues are, not surprisingly, similar to the mechanisms we described for tissue-specific migration of effector T cells and depend on expression of distinct combinations of adhesion molecules and chemokine receptors on each B cell subset. For example, bone marrow–homing IgG-secreting plasma cells express VLA-4 and CXCR4, which bind respectively to VCAM-1 and CXCL12 expressed on bone marrow sinusoidal endothelial cells. In contrast, mucosa-homing IgA-secreting plasma cells express $\alpha_4\beta_7$, CCR9, and CCR10, which bind respectively to MadCAM-1, CCL25, and CCL28, expressed on mucosal endothelial cells. IgG-secreting B cells are also recruited to chronic inflammatory sites in various tissues, and this pattern of homing can be attributed to CXCR3 and VLA-4 on these B cells binding to VCAM-1, CXCL9, and CXCL10, which are often found on the endothelial surface at sites of chronic inflammation.

SUMMARY

- Leukocyte migration from blood into tissues occurs through postcapillary venules and depends on adhesion molecules expressed on the leukocytes and vascular endothelial cells as well as chemokines.
- Selectins are carbohydrate-binding adhesion molecules that mediate low-affinity interactions of leukocytes with endothelial cells, as the first step in leukocyte migration from blood into tissues. E-selectin and P-selectin are expressed on activated endothelial cells and bind to selectin ligands on leukocytes, and L-selectin is expressed on leukocytes and binds ligands on endothelial cells.
- Integrins are a large family of adhesion molecules, some of which mediate tight adhesion of leukocytes with activated endothelium, as a critical step in leukocyte migration from blood into tissues. The important leukocyte integrins include LFA-1 and VLA-4, which bind to ICAM-1 and VCAM-1, respectively, on endothelial cells.
- Leukocyte migration from blood into tissues involves a series of sequential steps of interactions with endothelial cells, starting with low-affinity leukocyte binding to and rolling along the endothelial surface (mediated by selectins and selectin ligands). Next, the leukocytes become firmly bound to the endothelium, through interactions of leukocyte integrins binding to Ig superfamily ligands on the endothelium. Integrin binding is enhanced by chemokines, produced at the site of infection, that bind to receptors on the leukocytes.
- Lymphocyte recirculation is the process by which naive lymphocytes continuously migrate from blood into secondary lymphoid organs through HEVs, back into the blood through lymphatics, and into other secondary lymphoid organs. This process maximizes the chance of naive T cell encounter with the antigen it recognizes and is critical for the initiation of immune responses.
- Naive B and T cells migrate preferentially to lymph nodes; this process is mediated by binding of L-selectin on lymphocytes to peripheral lymph node addressin on HEVs in lymph nodes and by the CCR7 receptor on the lymphocytes that binds to the chemokines CCL19 and CCL21, which are produced in lymph nodes.
- The effector and memory lymphocytes that are generated by antigen stimulation of naive cells exit the lymph node by a process dependent on the

sphingosine-1 phosphate receptor on the lymphocytes and a gradient of sphingosine 1 phosphate. Effector T cells have decreased L-selectin and CCR7 expression but increased expression of integrins and E-selectin and P-selectin ligands, and these molecules mediate binding to endothelium at peripheral inflammatory sites. Effector and memory lymphocytes also express receptors for chemokines that are produced in infected peripheral tissues.

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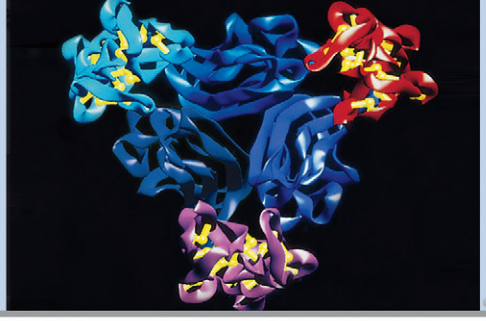
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CHAPTER

4

Innate Immunity

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Innate immunity is the first line of defense against infections. The cells and soluble molecules of innate immunity either exist in a fully functional state before encounter with microbes or are rapidly activated by microbes, faster than the development of adaptive immune responses (see Chapter 1, Fig. 1-1). Innate immunity coevolved with microbes to protect all multicellular organisms from infections. Some components of the mammalian innate immune system are remarkably similar to components in plants and insects, suggesting that these appeared in common ancestors long ago in evolution. For example, peptides that are toxic to bacteria and fungi, called defensins, are found in plants and mammals and have essentially the same tertiary structure in both life forms. A family of receptors that we will discuss in significant detail later in this chapter, called Toll-like receptors, are proteins that respond to the presence of pathogenic microbes by activating antimicrobial defense mechanisms in the cells in which they are expressed. Toll-like receptors are found in every life form in the evolutionary tree from insects up to mammals. The major signal transduction pathway that Toll-like receptors engage to activate cells, called the NF-κB pathway in mammals, also shows remarkable evolutionary conservation. In fact, most of the mechanisms of innate immune defense that we will discuss in this chapter appeared very early in evolution, after the development of complex multicultural organisms, about 750 million years ago. An adaptive immune system, in contrast, is clearly recognizable only in vertebrates about 500 million years ago. Adaptive immunity improves on some of the antimicrobial mechanisms of innate immunity by making them more powerful. In addition, adaptive immunity can recognize a much broader range of substances and, unlike innate immunity, displays memory of antigen encounter and specialization of effector mechanisms.

In this chapter, we describe the components, specificity, and anti-microbial mechanisms of the innate immune system. The remainder of this book is largely devoted to the role of the adaptive immune response in host defense and disease.

Innate immunity serves three important functions.

- ***Innate immunity is the initial response to microbes that prevents, controls, or eliminates infection of the***

host by many microbes. The importance of innate immunity in host defense is illustrated by studies showing that inhibition or elimination of any of several mechanisms of innate immunity markedly increases susceptibility to infections, even when the adaptive immune system is intact and functional. We will review examples of such studies later in this chapter and in Chapter 15 when we discuss immunity to different types of microbes. Many pathogenic microbes have evolved strategies to resist innate immunity, and these strategies are crucial for the virulence of the microbes. In infection by such microbes, innate immune defenses may keep the infection in check until the adaptive immune responses are activated. Adaptive immune responses, being more potent and specialized, are able to eliminate microbes that resist the defense mechanisms of innate immunity.

Different innate immune mechanisms work at different stages of infections. Epithelial barriers impair microbial entry into the host. Resident and recruited phagocytes in subepithelial and other tissues provide protection if the barriers are breached, and plasma proteins and circulating phagocytes provide protection if microbes reach the blood stream.

- ***Innate immune mechanisms recognize the products of damaged and dead host cells and serve to eliminate these cells and to initiate the process of tissue repair.*** The innate immune system also reacts against a variety of substances that are non-microbial but should not be present in healthy tissues, such as intracellular crystals.
- ***Innate immunity to microbes stimulates adaptive immune responses and can influence the nature of the adaptive responses to make them optimally effective against different types of microbes.*** Thus, innate immunity not only serves defensive functions early after infection but also provides the "warning" that an infection is present against which a subsequent adaptive immune response has to be mounted. Moreover, different components of the innate immune response often react in distinct ways to different microbes (e.g., bacteria versus viruses) and thereby influence the type of adaptive immune response that develops. We will return to this concept at the end of the chapter.

The two major types of responses of the innate immune system that protect against microbes are inflammation and antiviral defense. Inflammation is the process by which leukocytes and circulating plasma proteins are brought into sites of infection and activated to destroy and eliminate the offending agents. Inflammation is also the major reaction to damaged or dead cells and to accumulations of abnormal substances in cells and tissues. Antiviral defense consists of changes in cells that prevent virus replication and increase susceptibility to killing by lymphocytes, thus eliminating reservoirs of viral infection. In addition to these reactions, innate immune mechanisms include physical and chemical defense at epithelial barriers and activation of several circulating cells and proteins that can eliminate microbes in the blood independent of inflammation. The mechanisms by which the innate immune system works to protect against infections are described later in the chapter.

Many cells and tissues in higher organisms are endowed with the ability to contribute to innate immune reactions. Some components of innate immunity function at all times, even before infection; these components include barriers to microbial entry provided by epithelial surfaces, such as the skin and lining of the gastrointestinal and respiratory tracts. Other components of innate immunity are normally inactive but poised to respond rapidly to the presence of microbes and damaged cells; these components include phagocytes and the complement system. We begin our discussion of innate immunity by describing how the innate immune system recognizes microbes and host cells that are damaged by microbial infection. We will then proceed to the individual components of innate immunity and their functions in host defense.

RECOGNITION OF MICROBES AND DAMAGED SELF BY THE INNATE IMMUNE SYSTEM

The specificities of innate immune recognition have evolved to combat microbes and are different from the specificities of the adaptive immune system in several respects (Table 4-1).

The innate immune system recognizes molecular structures that are characteristic of microbial pathogens but not mammalian cells. The microbial substances that stimulate innate immunity are called **pathogen-associated molecular patterns (PAMPs)**. Different classes of microbes (e.g., viruses, gram-negative bacteria, gram-positive bacteria, fungi) express different PAMPs. These structures include nucleic acids that are unique to microbes, such as double-stranded RNA found in replicating viruses and unmethylated CpG DNA sequences found in bacteria; features of proteins that are found in microbes, such as initiation by *N*-formylmethionine, which is typical of bacterial proteins; and complex lipids and carbohydrates that are synthesized by microbes but not by mammalian cells, such as lipopolysaccharide (LPS) in gram-negative bacteria, lipoteichoic acid in gram-positive bacteria, and mannose-rich oligosaccharides found in microbial but not in mammalian glycoproteins (Table 4-2). In actuality, there are only a limited number of fundamental differences between microbial molecules and the molecules that higher organisms produce. Thus, the innate immune system has evolved to recognize only a limited number of molecules, most of which are unique to microbes, whereas the adaptive immune system is capable of recognizing a much wider array of foreign substances whether or not they are products of microbes.

The innate immune system recognizes microbial products that are often essential for survival of the microbes. This feature of innate immune recognition is important because it ensures that the targets of innate immunity cannot be discarded by microbes in an effort to evade recognition by the host. An example of a target of innate

TABLE 4–1 Specificity of Innate and Adaptive Immunity

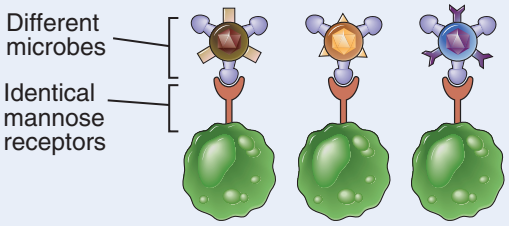
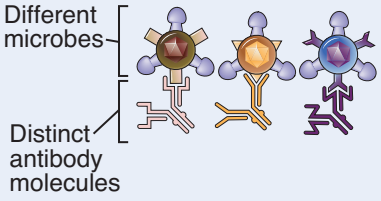
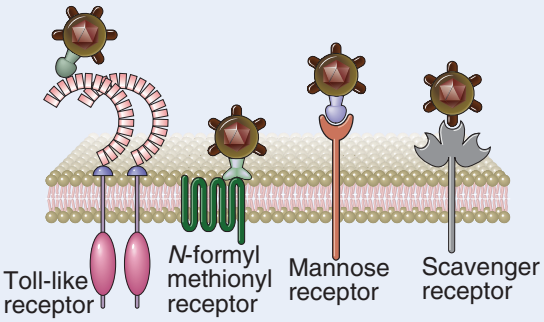
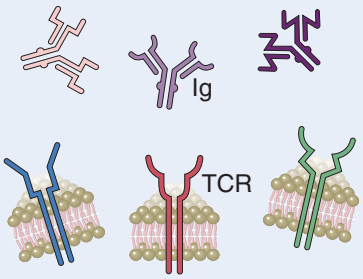
	Innate Immunity	Adaptive Immunity
Specificity	For structures shared by classes of microbes (pathogen-associated molecular patterns) 	For structural detail of microbial molecules (antigens); may recognize nonmicrobial antigens 
Receptors	Encoded in germline; limited diversity (pattern recognition receptors) 	Encoded by genes produced by somatic recombination of gene segments; greater diversity 
Distribution of receptors	Nonclonal: identical receptors on all cells of the same lineage	Clonal: clones of lymphocytes with distinct specificities express different receptors
Discrimination of self and non-self	Yes; healthy host cells are not recognized or they may express molecules that prevent innate immune reactions	Yes; based on elimination or inactivation of self-reactive lymphocytes; may be imperfect (giving rise to autoimmunity)

TABLE 4–2 Examples of PAMPs and DAMPs

Pathogen-Associated Molecular Patterns		Microbe Type
Nucleic acids	ssRNA	Virus
	dsRNA	Virus
	CpG	Virus, bacteria
Proteins	Pilin	Bacteria
	Flagellin	Bacteria
Cell wall lipids	LPS	Gram-negative bacteria
	Lipoteichoic acid	Gram-positive bacteria
Carbohydrates	Mannan	Fungi, bacteria
	Dectin glucans	Fungi
Damage-Associated Molecular Patterns		
Stress-induced proteins	HSPs	
Crystals	Monosodium urate	
Nuclear proteins	HMGB1	

CpG, cytidine-guanine dinucleotide; dsRNA, double-stranded RNA; HMGB1, high-mobility group box 1; HSPs, heat shock proteins; LPS, lipopolysaccharide; ssRNA, single-stranded RNA.

immunity that is essential for microbes is double-stranded viral RNA, which plays a critical role in the replication of certain viruses. Similarly, LPS and lipoteichoic acid are structural components of bacterial cell walls that are recognized by innate immune receptors; both are required for bacterial survival and cannot be discarded. In contrast, as we shall see in Chapter 15, microbes may mutate or lose many of the antigens that are recognized by the adaptive immune system, thereby enabling the microbes to evade host defense without compromising their own survival.

The innate immune system also recognizes endogenous molecules that are produced by or released from damaged and dying cells. These substances are called **damage-associated molecular patterns (DAMPs)** (see Table 4-2). DAMPs may be produced as a result of cell damage caused by infections, but they may also indicate sterile injury to cells caused by any of myriad reasons, such as chemical toxins, burns, trauma, or decreased blood supply. DAMPs are generally not released from cells dying by apoptosis. In some cases, healthy cells of the immune system are stimulated to produce and release DAMPs, which enhances an innate immune response to infections.

The innate immune system uses several types of cellular receptors, present in different locations in cells, and soluble molecules in the blood and mucosal secretions to recognize PAMPs and DAMPs (Table 4-3). Cell-associated recognition molecules of the innate immune system are expressed by phagocytes (primarily macrophages and neutrophils), dendritic cells, epithelial cells that compose the barrier interface between the body and the external environment, and many other types of cells that occupy tissues and organs. These cellular receptors for pathogens and damage-associated molecules are often called **pattern recognition receptors**. They are expressed on the plasma membrane or endosomal membranes of various cell types and also in the cytoplasm of these cells. These various locations of the receptors ensure that the innate immune system can respond to microbes that may be present outside cells or within different cellular compartments (Fig. 4-1). When these cell-associated pattern recognition molecules bind to PAMPs and DAMPs, they activate signal transduction events that promote the antimicrobial and proinflammatory functions of the cells in which they are expressed. In addition, there are many proteins present in the blood and extracellular fluids (see Table 4-3) that recognize PAMPs. These soluble molecules are responsible for facilitating the clearance of microbes from blood and extracellular fluids by enhancing uptake into cells or by activating extracellular killing mechanisms.

The receptors of the innate immune system are encoded in the germline, whereas the receptors of adaptive immunity are generated by somatic recombination of receptor genes in the precursors of mature lymphocytes. As a result, the repertoire of specificities of innate immune system receptors is small compared with that of B and T cells of the adaptive immune system. It is estimated that the innate immune system can recognize about 10^3 molecular patterns. In contrast, the adaptive immune system is capable of recognizing 10^7 or more distinct antigens. Furthermore, whereas the adaptive immune system can distinguish between antigens of different microbes of the same class and even different antigens of one microbe, innate immunity can distinguish only classes of microbes, or only damaged cells from healthy cells, but not particular species of microbes or cell types.

The innate immune system does not react against normal, healthy cells and tissues. This characteristic is, of course, essential for the health of the organism. It is determined in part by the specificity of innate immune mechanisms for PAMPs and DAMPs and in part by regulatory proteins expressed by normal cells that prevent activation of various components of innate immunity. We will discuss examples of such regulation later in the chapter.

CELL-ASSOCIATED PATTERN RECOGNITION RECEPTORS OF INNATE IMMUNITY

With this introduction, we can proceed to a discussion of the large variety of molecules in the body that are capable of recognizing PAMPs and DAMPs, focusing on their specificity, location, and functions. We will begin with cell-associated molecules expressed on membranes or in the cytoplasm of cells. The soluble recognition and effector molecules of innate immunity, found in the blood and extracellular fluids, are described later.

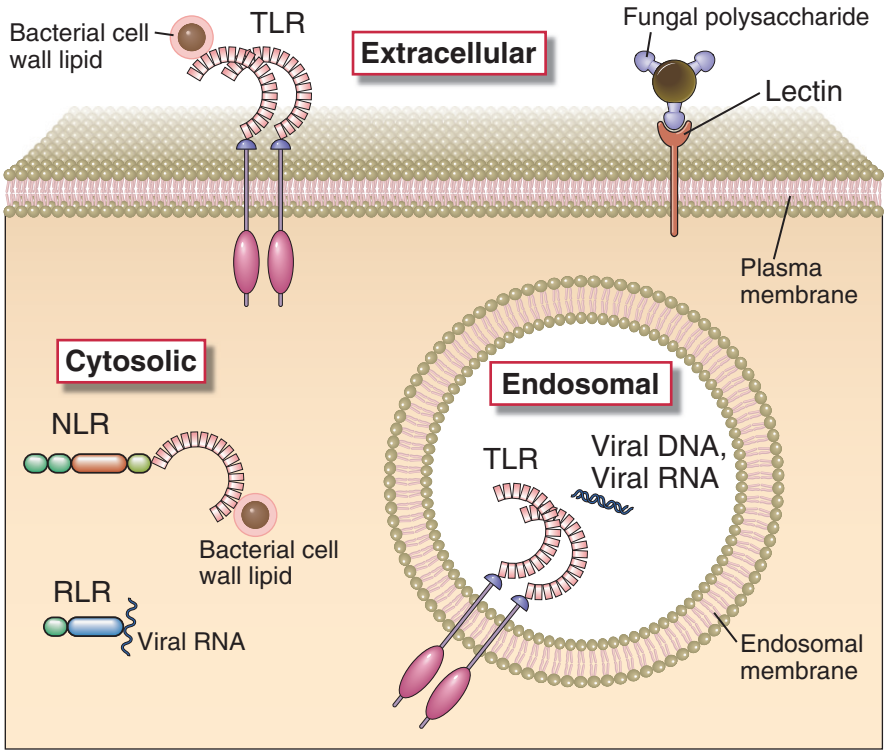
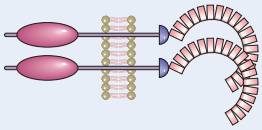
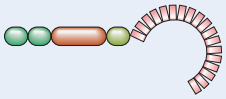
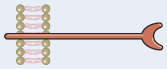
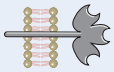
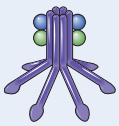
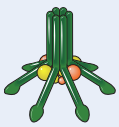
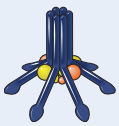
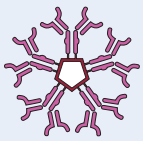


FIGURE 4–1 Cellular locations of pattern recognition molecules of the innate immune system. Some pattern recognition molecules of the TLR family (see Fig. 4-2) are expressed on the cell surface, where they may bind extracellular pathogen-associated molecular patterns. Other TLRs are expressed on endosomal membranes and recognize nucleic acids of microbes that have been phagocytosed by cells. Cells also contain cytoplasmic sensors of microbial infection (discussed later in the chapter), including the NLR family of proteins, which recognize bacterial peptidoglycans, RIG-like receptors, which bind viral RNA, and plasma membrane lectin-like receptors that recognize fungal glycans. Cytoplasmic receptors that recognize products of damaged cells as well as some microbes are shown in Fig. 4-4.

TABLE 4–3 Pattern Recognition Molecules of the Innate Immune System

Cell-Associated Pattern Recognition Receptors	Location	Specific Examples	PAMP/DAMP Ligands
Toll-like receptors (TLRs) 	Plasma membrane and endosomal membranes of dendritic cells, phagocytes, B cells endothelial cells, and many other cell types	TLRs 1-9	Various microbial molecules including bacterial LPS and peptidoglycans, viral nucleic acids
NOD-like receptors (NLRs) 	Cytoplasm of phagocytes epithelial cells, and other cells	NOD1/2 NALP family (inflammasomes)	Bacterial cell wall peptidoglycans Flagellin, muramyl dipeptide, LPS; urate crystals; products of damaged cells
RIG-like receptors (RLRs) 	Cytoplasm of phagocytes and other cells	RIG-1, MDA-5	Viral RNA
C-type lectin–like receptors 	Plasma membranes of phagocytes	Mannose receptor Dectin	Microbial surface carbohydrates with terminal mannose and fructose Glucans present in fungal cell walls
Scavenger receptors 	Plasma membranes of phagocytes	CD36	Microbial diacylglycerides
N-Formyl met-leu-phe receptors 	Plasma membranes of phagocytes	FPR and FPRL1	Peptides containing *N*-formylmethionyl residues
Soluble Recognition Molecules	**Location**	**Specific Examples**	**PAMP Ligands**
Pentraxins 	Plasma	C-reactive protein	Microbial phosphorylcholine and phosphatidylethanolamine
Collectins 	Plasma Alveoli	Mannose-binding lectin Surfactant proteins SP-A and SP-D	Carbohydrates with terminal mannose and fructose Various microbial structures
Ficolins 	Plasma	Ficolin	*N*-Acetylglucosamine and lipoteichoic acid components of the cell walls of gram-positive bacteria
Complement 	Plasma	C3	Microbial surfaces
Natural antibodies 	Plasma	IgM	Phosphorylcholine on bacterial membranes and apoptotic cell membranes

Most cell types express pattern recognition receptors and therefore are capable of participating in innate immune responses. Phagocytes, including neutrophils and macrophages, and dendritic cells express the widest variety and greatest amount of these receptors, which is in keeping with their fundamental role in detecting microbes and damaged cells and ingesting them for destruction (as the neutrophils and macrophages do) or reacting in ways that elicit inflammation and subsequent adaptive immunity (which is an important function of dendritic cells). Pattern recognition receptors are linked to intracellular signal transduction pathways that activate various cellular responses, including the production of molecules that promote inflammation and defend against microbes.

We will organize our discussion around several distinct classes of cellular pattern recognition receptors, which differ in their structure and specificity for various types of microbes.

Toll-like Receptors

The Toll-like receptors (TLRs), an evolutionarily conserved family of pattern recognition receptors expressed on many cell types, recognize products of a wide variety of microbes. *Toll* was originally identified as a *Drosophila* gene involved in establishing the dorsal-ventral axis during embryogenesis of the fruit fly, but subsequently it was discovered that the Toll protein also mediated antimicrobial responses in these organisms. This discovery led to the identification of mammalian homologues of Toll, which were named *Toll-like receptors*. There are 9 different functional TLRs in humans, named TLR1 to TLR9 (Fig. 4-2). The TLRs are type I integral membrane glycoproteins that contain leucine-rich repeats flanked by characteristic cysteine-rich motifs in their extracellular regions, which are involved in ligand binding, and a Toll/IL-1 receptor (TIR) homology domain in their cytoplasmic tails, which is essential for signaling. TIR domains are also found in the cytoplasmic tails of the receptors for the cytokines IL-1 and IL-18, and similar signaling pathways are engaged by TLRs, IL-1, and IL-18.

Mammalian TLRs are involved in responses to a wide variety of molecules that are expressed by microbial but not by healthy mammalian cells. The ligands that the different TLRs recognize are structurally diverse and include products of all classes of microorganism (see Fig. 4-2). Examples of bacterial products that bind to TLRs are LPS and lipoteichoic acid, constituents of the cell walls of gram-negative bacteria and gram-positive bacteria, respectively, and flagellin, the protein subunit component of the flagella of motile bacteria. Examples of TLR ligands produced by viruses are double-stranded RNAs, which compose the genomes of some viruses and are generated during the life cycle of most RNA viruses but are not produced by eukaryotic cells, and single-stranded RNAs, which are distinguished from cellular cytoplasmic single-stranded RNA transcripts by their location within endosomes and by their high guanosine and uridine content. Fungal mannose polysaccharides (mannans) are also TLR ligands.

TLRs are also involved in response to endogenous molecules whose expression or location indicates cell damage. Examples of host molecules that engage TLRs include heat shock proteins (HSPs), which are chaperones induced in response to various cell stresses, and high-mobility group box 1 (HMGB1), an abundant DNA-binding protein involved in transcription and DNA repair. Both HSPs and HMGB1 are normally intracellular but may become extracellular when released from injured or dying cells. From their extracellular location, they activate TLR2 and TLR4 signaling in dendritic cells, macrophages, and other cell types.

The structural basis of TLR specificities resides in the multiple extracellular leucine-rich modules of these receptors, which bind directly to PAMPs or to adaptor molecules that bind the PAMPs. There are between 16 and 28 leucine-rich repeats in TLRs, and each of these modules is composed of 20 to 30 amino acids that include conserved LxxLxLxxN motifs (where L is leucine, x is any amino acid, and N is asparagine) and amino acid residues that vary between different TLRs. The ligand-binding variable residues of the modules are on the convex surface formed by α helices and β turns or loops. These repeats contribute to the ability of some TLRs to bind hydrophobic molecules such as bacterial LPS. Ligand binding to the leucine-rich domains causes physical interactions between TLR molecules and the formation of TLR dimers. The repertoire of specificities of the TLR system is extended by the ability of TLRs to heterodimerize with one another. For example, dimers of TLR2 and TLR6 are required for responses to peptidoglycan.

Specificities of the TLRs are also influenced by various non-TLR accessory molecules. This is best defined for the TLR4 response to LPS. LPS first binds to soluble LPS-binding protein in the blood or extracellular fluid, and this complex serves to facilitate delivery of the LPS to the surface of the responding cell. An extracellular protein called MD2 (myeloid differentiation protein 2) binds to the lipid A component of LPS, forming a complex that then interacts with TLR4 and initiates signaling. Another protein called CD14 is also required for efficient LPS-induced signaling. CD14 is expressed by most cells (except endothelial cells) as a soluble protein or as a glycophosphatidylinositol-linked membrane protein. Both CD14 and MD2 can also associate with other TLRs. Thus, different combinations of accessory molecules in TLR complexes may serve to broaden the range of microbial products that can induce innate immune responses.

TLRs are found on the cell surface and on intracellular membranes and are thus able to recognize microbes in different cellular locations (see Fig 4-2). TLRs 1, 2, 4, 5, and 6 are expressed on the plasma membrane, where they recognize various PAMPs in the extracellular environment. Some of the most potent microbial stimuli for innate immune responses bind to these plasma membrane TLRs, such as bacterial LPS and lipoteichoic acid, which are recognized by TLRs 2 and 4, respectively. In contrast, TLRs 3, 7, 8, and 9 are mainly expressed inside cells on endoplasmic reticulum and endosomal membranes, where they detect several different nucleic acid ligands (see Fig. 4-2). Some of these nucleic acids are much more abundantly expressed by microbes than by mammals, such as double-stranded RNA, which is made by RNA viruses and binds to TLR3, and unmethylated

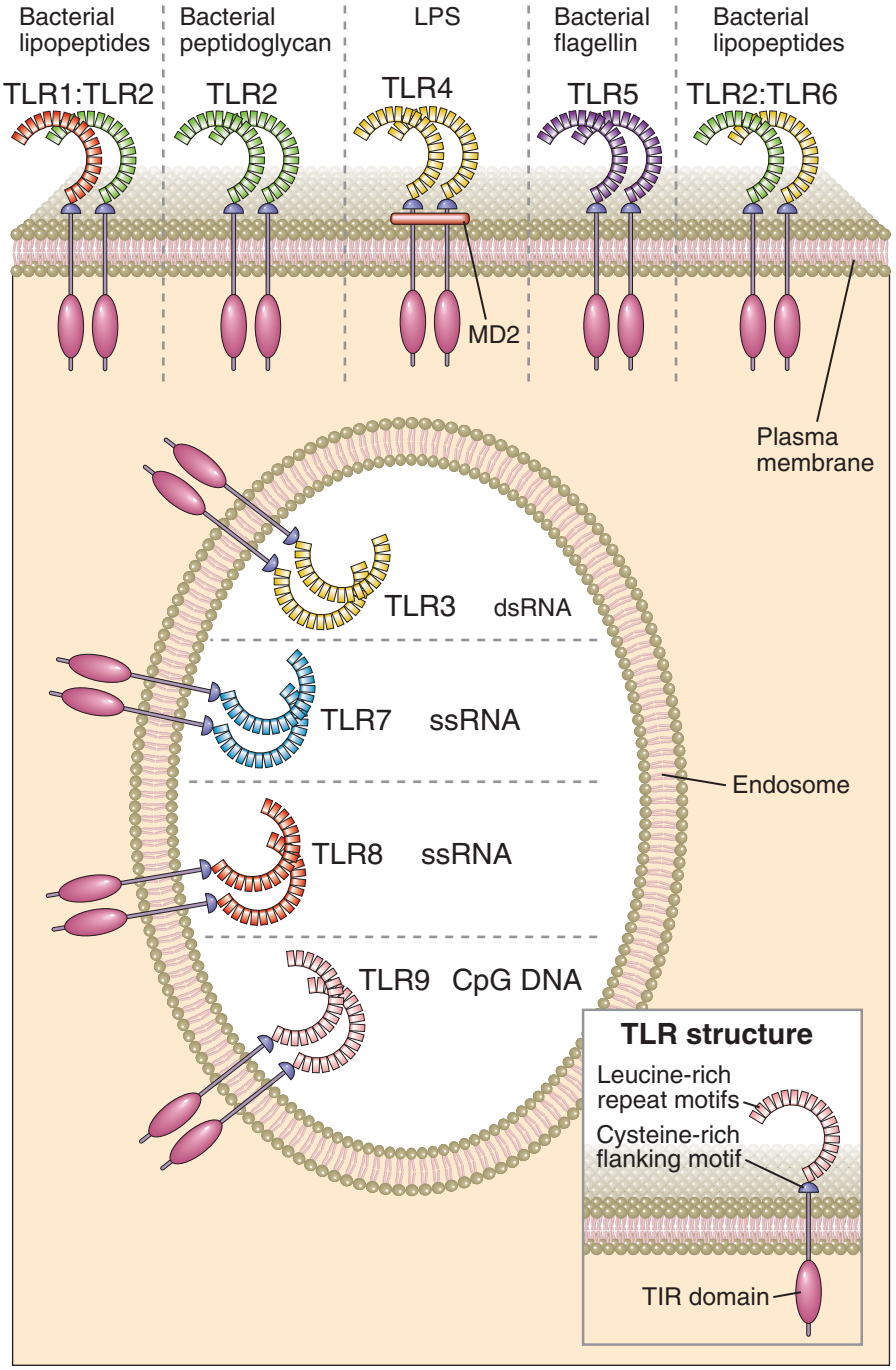


FIGURE 4–2 Structure, location, and specificities of mammalian TLRs. Note that some TLRs are expressed in endosomes and some on the cell surface.

CpG motifs common in prokaryotic DNA, which bind to TLR9. Single-stranded RNA, which binds to TLR8, and single- or double-stranded DNA, which binds to TLR9, are not uniquely expressed by microbes, but the relative specificity of these TLRs for microbial products is linked to their endosomal location. Host cell RNA and DNA are not normally present in endosomes, but microbial RNA and DNA may end up in endosomes of neutrophils, macrophages, or dendritic cells when the microbes are phagocytosed by these cells. Furthermore, host DNA from cells that have died because of infection or other causes may end up in the endosomes of the phagocytes. In other words, TLRs 3, 7, 8, and 9 may distinguish healthy self from foreign or unhealthy self on the basis, in part, of the cellular location of the nucleic acids they bind. A protein in the endoplasmic reticulum called UNC-93B is required for the endosomal localization and proper function of TLR3, 7, 8 and 9.

TLR recognition of microbial ligands results in the activation of several signaling pathways and ultimately transcription factors, which induce the expression of genes whose products are important for inflammatory and antiviral responses (Fig. 4-3). The signaling pathways are initiated by ligand binding to the TLR at the cell surface or in the endoplasmic reticulum or endosomes, leading to dimerization of the TLR proteins.

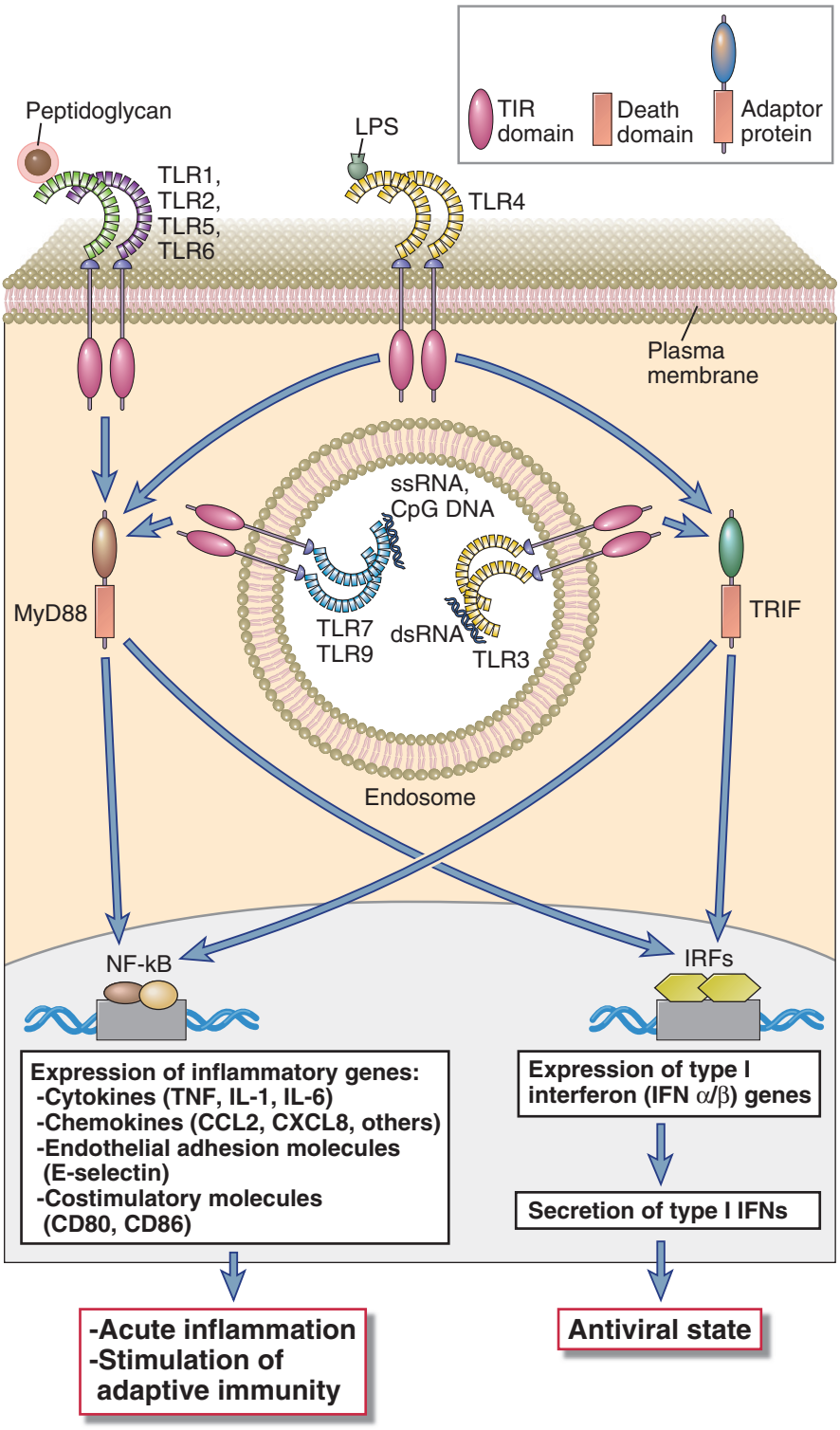


FIGURE 4–3 Signaling functions of TLRs. TLRs 1, 2, 5, and 6 use the adaptor protein MyD88 and activate the transcription factors NF-κB and AP-1. TLR3 uses the adaptor protein TRIF and activates the IRF3 and IRF7 transcription factors. TLR4 can activate both pathways. TLRs 7 and 9 in the endosome use MyD88 and activate both NF-κB and IRF7 (not shown).

Ligand-induced TLR dimerization is predicted to bring the TIR domains of the cytoplasmic tails of each protein close to one another. This is followed by recruitment of TIR domain–containing **adaptor proteins**, which facilitate the recruitment and activation of various protein kinases, leading to the activation of different transcription factors. The major transcription factors that are activated by TLR signaling pathways are nuclear factor κB (NF-κB), activation protein 1 (AP-1), interferon response factor 3 (IRF3), and IRF7. NF-κB and AP-1 stimulate the expression of genes encoding many of the molecules required for inflammatory responses including inflammatory cytokines (e.g., TNF and IL-1), chemokines (e.g., CCL2 and CXCL8), and endothelial adhesion

molecules (e.g., E-selectin) (discussed later). IRF3 and IRF7 promote production of type I interferons (IFN-α and IFN-β), important for anti-viral innate immune responses.

Different combinations of adaptors and signaling intermediates are used by different TLRs, which is the basis for common and unique downstream effects of the TLRs. For example, cell surface TLRs that engage the adaptor MyD88 lead to NF-κB activation, and TLR signaling that uses the adaptor called TRIF (TIR domain–containing adaptor inducing IFN-β) leads to IRF3 activation. All TLRs except TLR3 signal through MyD88 and are therefore capable of activating NF-κB and inducing an inflammatory response. TLR3 signals through TRIF and therefore activates IRF3 and induces expression of type I interferons. TLR4 signals through both MyD88 and TRIF and is able to induce both types of responses. Endosomal TLRs 7 and 9, which are most highly expressed in plasmacytoid dendritic cells, signal through a MyD88-dependent, TRIF-independent pathway that activates both NF-κB and IRF4. Therefore, TLR7 and TLR9, like TLR4, induce both inflammatory and antiviral responses. Details of NF-κB activation are discussed in Chapter 7.

Cytosolic Receptors for PAMPs and DAMPs

In addition to the membrane-bound TLRs, which sense pathogens outside cells or in endosomes, the innate immune system has evolved to equip cells with pattern recognition receptors that detect infection or cell damage in the cytoplasm (see Fig. 4-1 and Table 4-3). The two major classes of these cytoplasmic receptors are NOD-like receptors and RIG-like receptors. These cytoplasmic receptors, like TLRs, are linked to signal transduction pathways that promote inflammation or type I interferon production. The ability of the innate immune system to detect infection in the cytoplasm is important because parts of the normal life cycles of some microbes, such as viral gene translation and viral particle assembly, take place in the cytoplasm. Some bacteria and parasites have mechanisms to escape from phagocytic vesicles into the cytoplasm. Microbes can produce toxins that create pores in host cell plasma membranes, including endosomal membranes, through which microbial molecules can enter the cytoplasm. These pores can also result in changes in the concentration of endogenous molecules in the cytoplasm, which are reliable signs of infection and damage and are detected by the cytoplasmic receptors.

NOD-like Receptors

NOD-like receptors (NLRs) are a family of more than 20 different cytosolic proteins, some of which sense cytoplasmic PAMPs and DAMPs and recruit other proteins to form signaling complexes that promote inflammation. This family of proteins is named after NOD (nucleotide oligomerization domain–containing protein). Typical NLR proteins contain at least three different domains with distinct structures and functions. These include a leucine-rich repeat domain that senses the presence of ligand, similar to the leucine-rich repeats of TLRs; a NACHT (neuronal apoptosis inhibitory protein [NAIP], CIITA, HET-E, and TP1) domain, which allows NLRs to bind to one another and form oligomers; and an effector domain, which recruits other proteins to form signaling complexes. There are three NLR subfamilies, the members of which use different effector domains to initiate signaling, called CARD, Pyrin, and BIR domains. NLRs are found in a wide variety of cell types, although some NLRs have restricted tissue distributions. Some of the best studied NLRs are found in immune and inflammatory and epithelial barrier cells.

NOD1 and NOD2, members of the CARD domain–containing NOD subfamily of NLRs, are expressed in the cytoplasm of several cell types including mucosal epithelial cells and phagocytes, and they respond to bacterial cell wall peptidoglycans. NOD2 is particularly highly expressed in intestinal Paneth cells, where it stimulates expression of antimicrobial substances called defensins in response to pathogens. NOD1 recognizes substances derived mainly from gram-negative bacteria, whereas NOD2 recognizes a distinct molecule called muramyl dipeptide from both gram-negative and gram-positive organisms. These peptides are released from intracellular or extracellular bacteria; in the latter case, their presence in the cytoplasm requires specialized mechanisms of delivery of the peptides into host cells. These mechanisms include type III and type IV secretion systems, which have evolved in pathogenic bacteria as a means of delivering toxins into host cells. When oligomers of NODs recognize their peptide ligands, including bacterial toxins, a conformational change occurs that allows the CARD effector domains of the NOD proteins to recruit multiple copies of the kinase RIP2, forming a signaling complex that has been called the NOD signalosome. The RIP2 kinases in these complexes activate NF-κB, which promotes inflammatory gene expression, similar to TLRs that signal through MyD88, discussed earlier. Both NOD1 and NOD2 appear to be important in innate immune responses to bacterial pathogens in the gastrointestinal tract, such as *Helicobacter pylori* and *Listeria monocytogenes*. There is great interest in the finding that certain NOD2 polymorphisms increase the risk for an inflammatory disease of the bowel called Crohn's disease, probably because of a defective innate response to commensal and pathogenic organisms in the intestine. Also, mutations of NOD2 that cause increased NOD signaling lead to a systemic inflammatory disease called Blau's syndrome.

The NLRP subfamily of NLRs respond to cytoplasmic PAMPs and DAMPs by forming signaling complexes called inflammasomes, which generate active forms of the inflammatory cytokine IL-1 (Fig. 4-4). There are 14 NLRPs (NLR family, pyrin-domain-containing proteins), most of which share a Pyrin effector domain, named after the Greek root *pyro,* meaning heat, because it was first identified in a mutated gene that is associated with an inherited febrile illness. Inflammasomes containing only three of these NLRPs have been well studied, notably IPAF/NLRC4, NLRP3, and NLRP1. When these NLRPs are activated by the presence of microbial products or changes in the amount of endogenous molecules or ions in the cytoplasm, they bind other proteins through homotypic interactions between shared structural domains, thereby forming the **inflammasome** complex. For example, after binding of a ligand, multiple identical NLRP3

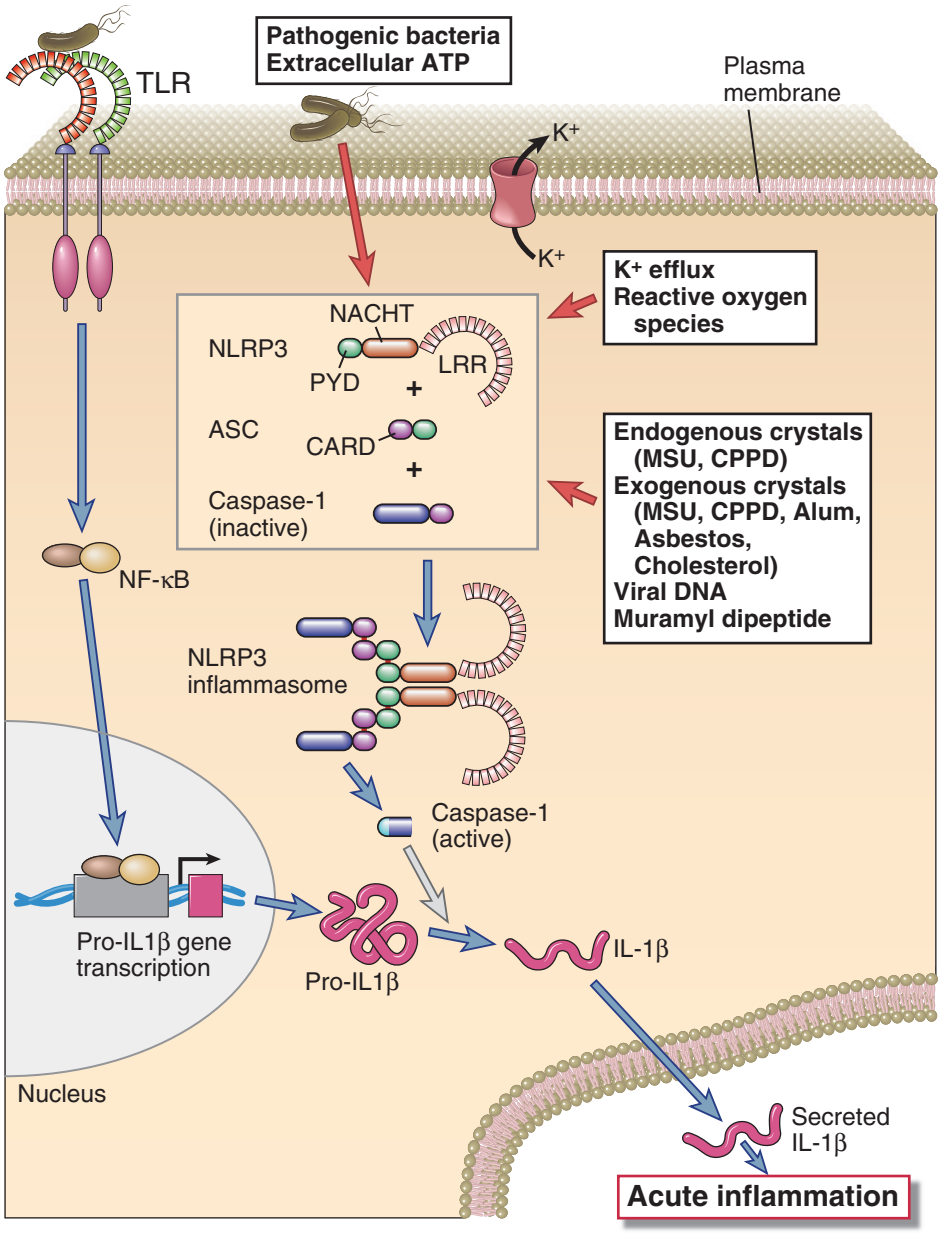


FIGURE 4–4 The inflammasome. The activation of the NLRP3 inflammasome, which processes pro–IL-1β to active IL-1, is shown. Inflammasomes with other NLRP proteins function in a similar way. Pro–IL-1β expression is induced by various PAMPs or DAMPs through pattern recognition receptor signaling, such as a TLR, as shown. CPPD, calcium pyrophosphate dihydrate; MSU, monosodium urate.

proteins interact to form an oligomer, and individual NLRP3 proteins in the oligomer each bind an adaptor protein called ASC. The adaptors then bind an inactive precursor form of the enzyme caspase-1 through interactions of caspase-recruitment domains on both proteins. Caspases are proteases with cysteine residues in their active site that cleave substrate proteins at aspartate residues. Caspase-1 becomes active only after recruitment to the inflammasome complex. Although several other caspases participate in a form of cell death called apoptosis (see Chapter 14), the main function of caspase-1 is to cleave the inactive cytoplasmic precursor forms of two homologous cytokines called IL-1β and IL-18. Caspase-1 cleavage generates active forms of these cytokines, which then leave the cell and perform various proinflammatory functions. We will describe the action of these cytokines and the inflammatory response in detail later in the chapter. Suffice it to say here that the inflammation induced by IL-1 serves a protective function against the microbes that incite the formation of the inflammasome. When inflammasome activity is abnormally stimulated, the abundant IL-1 that is produced can cause tissue damage. For example, some of the hereditary periodic fevers (also called autoinflammatory syndromes), which are rare diseases characterized by repeated bouts of fever, inflammation, and tissue destruction, are caused by gain-of-function mutations of the NLRP3 gene, and IL-1 antagonists are very effective in treating these diseases.

NLRP-inflammasome responses are induced by a wide variety of cytoplasmic stimuli, including microbial products, environmentally or endogenously derived crystals, and reduction in cytoplasmic potassium ion (K^+) concentrations, that are often associated with infections and cell stress (see Fig. 4-4). Microbial products that activate NLRP-inflammasomes include bacterial molecules such as flagellin, muramyl dipeptide, LPS, and pore-forming toxins as well as bacterial and viral RNA. Crystalline substances are also potent activators of inflammasomes, and these crystals can be derived from the environment, such as asbestos and silica, or they can be endogenously derived from dead cells, such as monosodium urate and calcium pyrophosphate dehydrate. Another endogenous stimulus of inflammasome activation is extracellular ATP, perhaps released from dead cells and transported into the cytoplasm of the responding cell. The structural diversity of the agents that activate the inflammasome suggests that they do not directly bind to NLRP proteins but may act by inducing a smaller set of changes in endogenous cytoplasmic conditions that activate the NLRPs. Reduced cytoplasm potassium ion concentrations may be one such common mechanism because reductions in cellular K^+ induced by some bacterial pore-forming toxins can activate inflammasomes, and many of the other known inflammasome activators cause increased K^+ efflux from cells. Another common mechanism implicated in inflammasome activation is the generation of reactive oxygen species, which are toxic free radicals of oxygen that are often produced during cell injury. A type of inflammasome that uses a protein called AIM2 (absent in melanoma-2) rather than an NLRP-family protein, recognizes cytosolic dsDNA.

The discovery that some crystalline substances are potent inflammasome activators has changed our understanding of certain inflammatory diseases. Gout is a painful inflammatory condition of the joints that has long been known to be caused by deposition of monosodium urate crystals in joints. Based on the understanding that urate crystals activate the inflammasome, there is interest in using IL-1 antagonists to treat cases of severe gout that are resistant to conventional anti-inflammatory drugs. Similarly, pseudogout is caused by deposition of calcium pyrophosphate crystals and inflammasome activation. Occupational inhalation of silica and asbestos can cause chronic inflammatory and fibrotic disease of the lung, and there is also interest in the potential of blocking the inflammasome or IL-1 to treat these diseases.

RIG-like Receptors

RIG-like receptors (RLRs) are cytosolic sensors of viral RNA that respond to viral nucleic acids by inducing the production of the antiviral type I interferons. RLRs can recognize double-stranded and single-stranded RNA, which includes the genomes of RNA viruses and RNA transcripts of RNA and DNA viruses. The two best characterized RLRs are RIG-I (retinoic acid–inducible gene I) and MDA5 (melanoma differentiation-associated gene 5). Both of these proteins contain two N-terminal caspase recruitment domains, which interact with other signaling proteins, and an RNA-helicase domain of unknown function. RIG-I and MDA5 display different specificities for viral RNA, partly based on length of double-stranded RNA genome, which may enhance sensitivity in detecting a wide range of viral double-stranded RNAs with heterogeneous lengths. RLRs also can discriminate viral single-stranded RNA from normal cellular single-stranded RNA transcripts. For example, RIG-I will only recognize RNA with a 5′ triphosphate moiety, which is not present in mammalian host cell cytoplasmic RNA because of addition of a 7-methylguanosine cap or removal of the 5′ triphosphate. RLRs are expressed in a wide variety of cell types, including bone marrow–derived leukocytes and various tissue cells. Therefore, these receptors enable the many cell types susceptible to infection by RNA viruses to participate in innate immune responses to these viruses.

On binding RNA, the RLRs initiate signaling events that lead to IRF3 and IRF7 activation, and these transcription factors induce production of type I interferons. In addition, RLR signaling can also activate NF-κB. Signaling of both RIG-I and MDA5 depends on their binding to adaptor proteins and activation of signaling cascades that lead to either IRF3/7 or NF-κB activation.

Other Cell-Associated Pattern Recognition Receptors

Several types of plasma membrane and cytoplasmic receptors other than the classes described before are expressed on the plasma membranes of various cell types and recognize microbial molecules (see Table 4-3). Some of these receptors transmit activating signals, similar to TLRs, that promote inflammatory responses and enhance killing of microbes. Other receptors mainly participate in the uptake of microbes into phagocytes.

Receptors for Carbohydrates

Receptors that recognize carbohydrates on the surface of microbes facilitate the phagocytosis of the microbes and stimulate subsequent adaptive immune responses. These receptors belong to the **C-type lectin** family, so called because they bind carbohydrates (hence, *lectins*) in a Ca^{++}-dependent manner (hence, *C-type*). Some of these are soluble proteins found in the blood and extracellular fluids (discussed later); others are integral membrane proteins found on the surfaces of macrophages, dendritic cells, and some tissue cells. All these molecules contain a conserved carbohydrate recognition domain. There are several types of plasma membrane C-type lectins with specificities for different carbohydrates, including mannose, glucose, *N*-acetylglucosamine, and β-glucans. In general, these cell surface lectins recognize carbohydrate structures found on the cell walls of microorganisms but not mammalian cells. Some of these C-type lectin receptors function in the phagocytosis of microbes, and others have signaling functions that induce protective responses of host cells to microbes.

- ***Mannose receptor.*** One of the most studied membrane C-type lectins is the **mannose receptor** (CD206), which is involved in phagocytosis of microbes. This receptor recognizes certain terminal sugars on microbial surface carbohydrates, including D-mannose, L-fucose, and *N*-acetyl-D-glucosamine. These terminal

sugars are often present on the surface of microorganisms, whereas eukaryotic cell carbohydrates are most often terminated by galactose and sialic acid. Thus, the terminal sugars on microbes can be considered PAMPs. Mannose receptors do not have any known intrinsic signaling functions and are thought to bind microbes as the first step in their ingestion by macrophages and dendritic cells. However, the overall importance of mannose receptor–mediated phagocytic clearance of microbes remains unknown.

- ***Dectins.*** Dectin-1 (dendritic cell–associated C-type lectin 1) and dectin-2 are dendritic cell receptors that serve as pattern recognition receptors for two life cycle stages of fungal organisms. Dectin-1 binds β-glucan, which is the major component of the yeast form of *Candida albicans*, a ubiquitous but potentially pathogenic fungus. Dectin-2 recognizes high-mannose oligosaccharides on the hyphal form of *Candida*. In response to binding of their ligands on the cell walls of fungi, both dectins induce signaling events in dendritic cells that stimulate the production of cytokines and other proteins that promote inflammation and enhance adaptive immune responses. Dectin stimulation of dendritic cells induces the production of some cytokines that promote the differentiation of naive $CD4^+$ T cells to a type of effector T cell called T_H17, which is particularly effective in defense against fungal infections. Other dendritic cell carbohydrate receptors include langerin (CD207), mainly expressed by epidermal Langerhans cells, and DC-SIGN, expressed on the majority of dendritic cells. DC-SIGN may play a pathogenic role in promoting HIV-1 infection of T cells. The HIV-1 gp120 envelope glycoprotein binds to DC-SIGN on dendritic cells in mucosal tissues, the dendritic cells carry the virus through lymphatics to draining lymph nodes, and the virus is then transferred to and infects $CD4^+$ T cells.

Scavenger Receptors

Scavenger receptors comprise a structurally and functionally diverse collection of cell surface proteins that were originally grouped on the basis of the common characteristic of mediating the uptake of oxidized lipoproteins into cells. Some of these scavenger receptors, including SR-A and CD36, are expressed on macrophages and mediate the phagocytosis of microorganisms. In addition, CD36 functions as a coreceptor in TLR2/6 recognition and response to bacterially derived lipoteichoic acid and diacylated lipopeptides. There is a wide range of molecular structures that bind to each scavenger receptor, including LPS, lipoteichoic acid, nucleic acids, β-glucan, and proteins. The significance of scavenger receptors in innate immunity is highlighted by increased susceptibility to infection in gene knockout mice lacking the receptors and by the observations that several microbial pathogens express virulence factors that block scavenger receptor–mediated recognition and phagocytosis.

N-Formyl Met-Leu-Phe Receptors

***N*-Formyl met-leu-phe receptors**, including FPR and FPRL1 expressed by neutrophils and macrophages, respectively, recognize bacterial peptides containing *N*-formylmethionyl residues and stimulate directed movement of the cells. Because all bacterial proteins and few mammalian proteins (only those synthesized within mitochondria) are initiated by *N*-formylmethionine, FPR and FPRL1 allow phagocytes to detect and respond preferentially to bacterial proteins. The bacterial peptide ligands that bind these receptors are some of the first identified and most potent chemoattractants for leukocytes. Chemoattractants include several types of diffusible molecules, often produced at sites of infection, that bind to specific receptors on cells and direct their movement toward the source of the chemoattractant. Other chemoattractants, such as the chemokines discussed in Chapter 3, are made by host cells. FPR and FPRL1, along with all other chemoattractant receptors, belong to the seven-transmembrane, guanosine triphosphate (GTP)–binding (G) protein–coupled receptor (GPCR) superfamily. These receptors initiate intracellular responses through associated trimeric G proteins (see Chapter 7). The G proteins stimulate many types of cellular responses, including cytoskeletal changes, resulting in increased cell motility.

CELLULAR COMPONENTS OF THE INNATE IMMUNE SYSTEM

The cells of the innate immune system perform several functions that are essential for defense against microorganisms. Some cells form physical barriers that impede infections. Several cell types express the various pattern recognition receptors we have just discussed, and after recognizing PAMPs and DAMPs, they respond by producing inflammatory cytokines and antiviral proteins and by killing microbes or infected cells. In addition, some of the cells of innate immunity are critical for stimulating subsequent adaptive immune responses. We will now discuss the cell types that perform these functions.

Epithelial Barriers

Intact epithelial surfaces form physical barriers between microbes in the external environment and host tissue, and epithelial cells produce antimicrobial chemicals that further impede the entry of microbes (Fig. 4-5). The main interfaces between the environment and the mammalian host are the skin and the mucosal surfaces of the gastrointestinal, respiratory, and genitourinary tracts. These interfaces are lined by continuous layers of specialized epithelial cells that serve many physiologic functions, including preventing the entry of microbes. Loss of the integrity of these epithelial layers by trauma or other reasons predisposes an individual to infections. The protective barrier function is in large part physical. The epithelial cells form tight junctions with one another, blocking passage of microbes between the cells. The outer layer of keratin, which accumulates as surface skin keratinocytes die, serves to block microbial penetration into deeper layers of the epidermis. Mucus, a viscous secretion containing glycoproteins called mucins, is produced by respiratory, gastrointestinal, and urogenital epithelial cells. Mucus physically impairs microbial invasion and

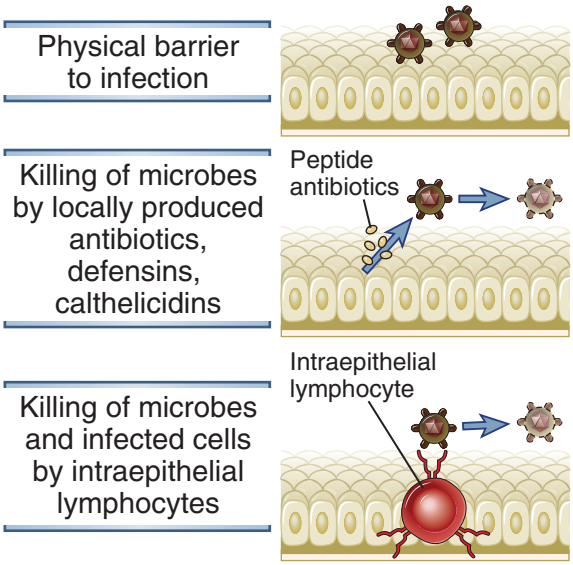


FIGURE 4–5 Epithelial barriers. Epithelia at the portals of entry of microbes provide physical barriers, produce antimicrobial substances, and harbor intraepithelial lymphocytes that are believed to kill microbes and infected cells.

facilitates microbe removal by ciliary action in the bronchial tree and peristalsis in the gut. Although these physical barrier properties alone are very important in host defense, other epithelial defense mechanisms have evolved to complement the physical barrier.

Epithelial cells as well as some leukocytes produce peptides that have antimicrobial properties. Two structurally distinct families of antimicrobial peptides are the defensins and the cathelicidins.

- **Defensins** are small cationic peptides, 29 to 34 amino acids long, that contain three intrachain disulfide bonds. Two families of human defensins, named α and β, are distinguished by the location of these bonds. Defensins are produced by epithelial cells of mucosal surfaces and by granule-containing leukocytes, including neutrophils, natural killer cells, and cytotoxic T lymphocytes. The set of defensin molecules produced differs between different cell types. Paneth cells within the crypts of the small bowel are a major producer of α defensins. Paneth cell defensins are sometimes called crypticidins; their function is to limit the amount of microbes in the lumen. Defensins are also produced elsewhere in the bowel, in respiratory mucosal cells, and in the skin. Some defensins are constitutively produced by some cell types, but their secretion may be enhanced by cytokines or microbial products. In other cells, defensins are produced only in response to cytokines and microbial products. The protective actions of the defensins include both direct toxicity to microbes, including bacteria and fungi, and the activation of cells involved in the inflammatory response to microbes. The mechanisms of direct microbicidal effects are poorly understood.
- **Cathelicidins** are produced by neutrophils and various barrier epithelia, including skin, gastrointestinal tract, and respiratory tract. Cathelicidin is synthesized as an 18-kD two-domain precursor protein and is proteolytically cleaved into two peptides, each with protective functions. Both precursor synthesis and proteolytic cleavage may be stimulated by inflammatory cytokines and microbial products. The active cathelicidins protect against infections by multiple mechanisms, including direct toxicity to a broad range of microorganisms and the activation of various responses in leukocytes and other cell types that promote eradication of microbes. The C-terminal fragment, called LL-37, can also bind and neutralize LPS, a toxic component of the outer wall of gram-negative bacteria that has been mentioned previously.

Barrier epithelia contain certain types of lymphocytes, including intraepithelial T lymphocytes, that recognize and respond to commonly encountered microbes. Intraepithelial T lymphocytes are present in the epidermis of the skin and in mucosal epithelia. Various subsets of intraepithelial lymphocytes are present in different proportions, depending on species and tissue location. These subsets are distinguished mainly by the type of T cell antigen receptors (TCRs) they express. Some intraepithelial T lymphocytes express the conventional αβ form of TCR, which is present on most T cells in lymphoid tissues. Other T cells in epithelia express a form of antigen receptor called the γδ receptor that may recognize peptide and nonpeptide antigens. A common characteristic of these T cells is the limited diversity of their antigen receptors compared with most T cells in the adaptive immune system. The intraepithelial T lymphocytes are believed to recognize a limited number of commonly encountered microbial structures (e.g., PAMPs). Intraepithelial lymphocytes may function in host defense by secreting cytokines, activating phagocytes, and killing infected cells.

Phagocytes

Cells that have specialized phagocytic functions, primarily macrophages and neutrophils, are the first line of defense against microbes that breach epithelial barriers. We have introduced these cell types in Chapter 2, and we will discuss many other details of their functions later in this chapter and in other chapters. For now, it is important to know that these phagocytic cells perform two general types of functions in defense against microbes. First, they are able to internalize and kill microbes. Neutrophils and macrophages are particularly good at this function. Second, phagocytes respond to microbes by producing various cytokines that promote inflammation and also enhance the antimicrobial function of host cells at the site of infection. Among the "professional phagocytes," macrophages are particularly good at this second function. Macrophages are also involved in the repair of damaged tissues, which is another function important in host defense. The essential role that phagocytes play in innate immune defense against microbes is demonstrated by the high rate of lethal bacterial and fungal infections in patients with low blood neutrophil counts caused by bone marrow cancers or cancer therapy and in patients with inherited deficiencies in the functions of phagocytes.

Dendritic Cells

Dendritic cells perform essential recognition and effector roles in innate immunity. We introduced dendritic cells in Chapter 2, and their role in antigen presentation to T cells is discussed in Chapter 6. Recall that this heterogeneous family of bone marrow–derived cells with long dendrite-like cytoplasmic processes are constitutively present in epithelia and most tissues of the body. Because of their placement and morphology, these cells are poised to detect invading microbes. Furthermore, dendritic cells express more different types of TLRs and cytoplasmic pattern recognition receptors than any other cell type, making them the most versatile sensors of PAMPs and DAMPs among all cell types in the body. One particular subset of dendritic cells, called **plasmacytoid dendritic cells** because their morphology is similar to antibody-producing plasma cells, is the major source of antiviral cytokines, type I interferons, produced in response to viral infections. This feature of plasmacytoid dendritic cells is due in part to the fact that these cells, more than other cell types, abundantly express the endosomal TLRs (TLRs 3, 7, 8, 9) that recognize nucleic acids of viruses that have been internalized into the cell. We will discuss the antiviral actions of type I interferons in more detail later in the chapter.

Dendritic cells are uniquely capable of triggering and directing adaptive T cell–mediated immune responses, and this is dependent on their innate immune responses to microbes. This capability reflects the ability of dendritic cells to take up microbial protein antigens, to transport them to lymph nodes where naive T cells home, and to alter and display the protein antigens in a way that the T cells can recognize. These functions will be discussed in great detail in Chapter 6. Importantly, the innate response of dendritic cells to PAMPs is essential for these functions, which are enhanced by TLR signaling. Furthermore, TLR signaling induces dendritic cell expression of molecules, including costimulatory molecules and cytokines, that are needed, in addition to antigen, for the activation of the naive T cells and their differentiation into effector T cells. Depending on the nature of the microbe that induces the innate response, a dendritic cell will direct naive T cell differentiation into distinct types of effector cells, such as IFN-γ–producing T_H1 cells or IL-17–producing T_H17 cells. The influence of dendritic cells on T cell activation and differentiation will be discussed further in Chapter 9.

Natural Killer Cells

Natural killer (NK) cells are lymphocytes distinct from T and B cells that play important roles in innate immune responses mainly against intracellular viruses and bacteria. The term *natural killer* derives from the fact that these cells are capable of performing their killing function without a need for clonal expansion and differentiation, which is required for effector responses of the immune system's other killer cells, the cytotoxic T lymphocytes (CTLs). NK cells constitute 5% to 15% of the mononuclear cells in the blood and spleen. They are rare in other lymphoid organs but are concentrated in certain organs such as the liver and gravid uterus. NK cells arise from bone marrow precursors and appear as large lymphocytes with numerous cytoplasmic granules. NK cells do not express highly diverse, clonally distributed antigen receptors typical of B and T cells. Rather, they use germline DNA-encoded receptors, discussed later, to distinguish pathogen-infected from healthy cells. They can be identified in the blood by expression of CD56 and the absence of CD3, two membrane proteins often found together on activated CTLs.

Recognition of Infected and Stressed Cells by NK Cells

NK cells distinguish infected and stressed cells from healthy cells, and NK cell activation is regulated by a balance between signals that are generated from activating receptors and inhibitory receptors. There are several families of these receptors (Fig. 4-6), some members of which we will discuss later. These receptors recognize molecules on the surface of other cells and generate activating or inhibitory signals that promote or inhibit NK responses. In general, the activating receptors recognize ligands on infected and injured cells, and the inhibitory receptors recognize healthy normal cells. When an NK cell interacts with another cell, the outcome is determined by the integration of signals generated from the array of inhibitory and activating receptors that are expressed by the NK cell and that interact with ligands on the other cell. Because of the stochastic nature of their expression, there is significant diversity in the array of activating and inhibitory receptors that different NK cells express in any one individual. The result of this is that an individual's NK cells will respond to different types of microbes or infected cells. Furthermore, the genes encoding many of these receptors are polymorphic, meaning that there are several variants of the genes in the population, so that one person will express a slightly different form of the receptors than another person.

Most NK cells express inhibitory receptors that recognize class I major histocompatibility complex (MHC) molecules, which are cell surface proteins normally expressed on almost all healthy cells in the body (Fig. 4-7). A major function of class I MHC molecules, distinct from their role in regulating NK cell activation, is to display peptides derived from cytoplasmic proteins, including microbial proteins, on the cell surface for recognition by $CD8^+$ T lymphocytes. We will describe the structure and function of MHC molecules in relation to $CD8^+$ T cell antigen recognition in Chapter 6. For now, it is important to understand that NK cells use fundamentally different types of receptors than do T cells to recognize class I MHC molecules. Unlike T cells, many of the NK receptors for class I MHC respond by inhibiting NK activation. This is useful because normal cells express class I MHC molecules, and many viruses and other causes of cell stress lead to a loss of cell surface expression of class I MHC. Thus, NK cells interpret the presence of class I MHC molecules as markers of normal, healthy self, and their absence is an indication of infection or damage. Conversely, NK cells will not receive inhibitory signals from infected or stressed cells. At the same time, the NK cells are likely to receive activating signals from the same

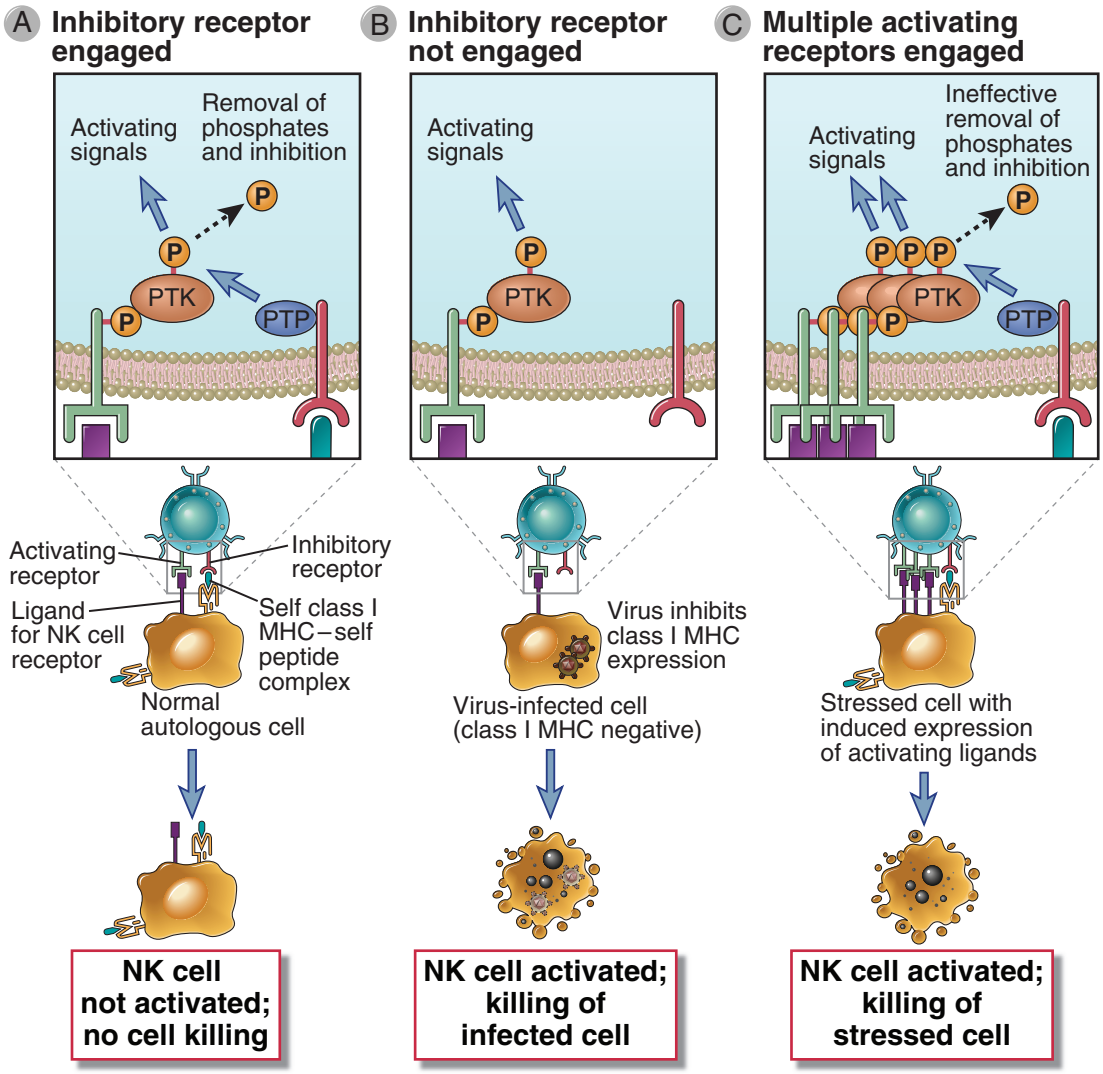


FIGURE 4–6 Functions of activating and inhibitory receptors of NK cells. A, Activating receptors of NK cells recognize ligands on target cells and activate protein tyrosine kinase (PTK), whose activity is inhibited by inhibitory receptors that recognize class I MHC molecules and activate protein tyrosine phosphatases (PTP). NK cells do not efficiently kill class I MHC–expressing healthy cells. **B,** If a virus infection or other stress inhibits class I MHC expression on infected cells and induces expression of additional activating ligands, the NK cell inhibitory receptor is not engaged and the activating receptor functions unopposed to trigger responses of NK cells, such as killing of target cells and cytokine secretion. **C.** Cells stressed by infection or neoplastic transformation may express increased amounts of activating ligands, which bind NK cell activating receptors and induce more tyrosine phosphorylation than can be removed by inhibitory receptor associated phosphatases, resulting in killing of the stressed cells. Structural details and ligands of inhibitory and activating NK cell receptors are shown in Figure 4-7.

infected cells through activating receptors. The net result will be activation of the NK cell to secrete cytokines and to kill the infected or stressed cell. This ability of NK cells to become activated by host cells that lack class I MHC has been called recognition of missing self.

Inhibitory receptors of NK cells share the common feature of a structural motif in their cytoplasmic tails, called an immunoreceptor tyrosine-based inhibition motif (ITIM), which engages molecules that block the signaling pathways of activating receptors (see Figs. 4-6 and 4-7). ITIMs contain tyrosine residues that are phosphorylated on ligand binding to the inhibitory receptor. This leads to the recruitment and activation of phosphatases, which remove phosphates from several signaling proteins or lipids generated by the signaling pathways downstream of NK activating receptors. The end result is blocking of the signaling functions of activating receptors. ITIMs are found in cytoplasmic tails of other receptors besides NK inhibitory receptors, and their structure and signaling functions are discussed in more detail in Chapter 7.

The largest group of NK inhibitory receptors are the killer cell immunoglobulin-like receptors (KIRs), which are members of the immunoglobulin (Ig) superfamily. Members of this family all contain a structural domain called an Ig fold, first identified in antibody (also known as Ig) molecules, discussed in Chapter 5. KIRs bind a variety of class I MHC molecules. A second important group of NK inhibitory receptors belong to the C-type lectin family, which includes proteins with carbohydrate-binding properties, as discussed earlier. One of these receptors is a heterodimer called CD94/NKG2A, which

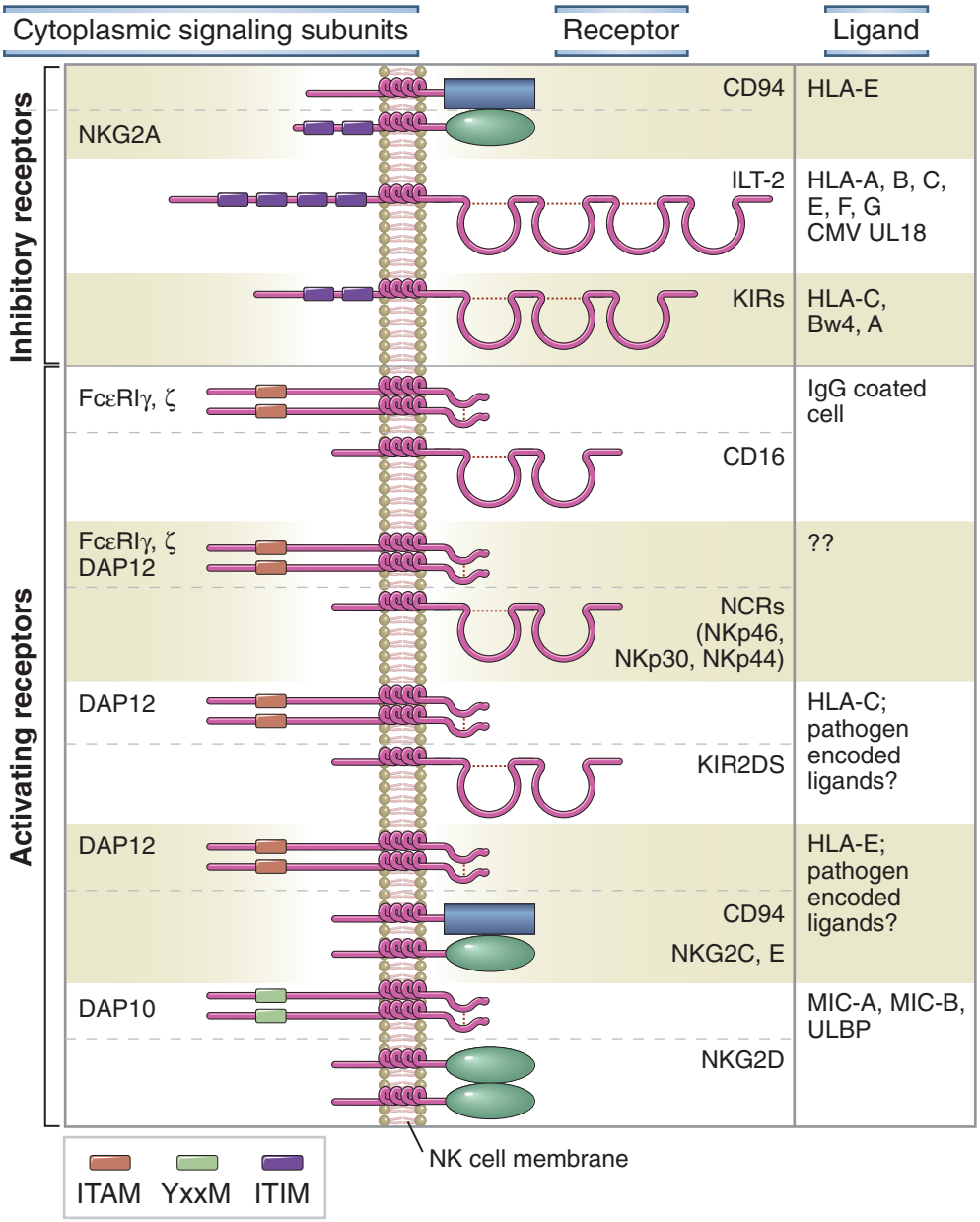


FIGURE 4–7 Structure and ligands of activating and inhibitory receptors of NK cells. Examples of inhibitory and activating NK cell receptors and their ligands. CD16 and the natural cytotoxic receptors (NCRs) associate with ζ chain homodimers, FcεRIγ homodimers, or ζ-FcεRIγ heterodimers. There are multiple different KIRs, with varying ligand specificities.

recognizes a class I MHC molecule called HLA-E. Interestingly, HLA-E displays peptides derived from other class I MHC molecules, so in essence, CD94/NKG2A is a surveillance receptor for several different class I MHC molecules. A third family of NK inhibitory receptors, called the leukocyte Ig-like receptors (LIRs), are also Ig superfamily members that bind class I MHC molecules, albeit with lower affinity than the KIRs, and are more highly expressed on B cells than on NK cells.

Activating receptors on NK cells recognize a heterogeneous group of ligands, some of which may be expressed on normal cells and others of which are expressed mainly on cells that have undergone stress, are infected with microbes, or are transformed. The molecular features of the ligands for many of these receptors are not well characterized. The induced expression of ligands on unhealthy cells that bind to activating receptors on NK cells may lead to signals that overwhelm the signals from inhibitory receptors, especially if class I MHC is also reduced or lost on the unhealthy cell (see Fig. 4-6).

Most activating NK receptors share the common feature of a structural motif in their cytoplasmic tails, called an immunoreceptor tyrosine-based activation motif (ITAM), which engages in signaling events that promote target cell killing and cytokine secretion (see Fig. 4-7). In some of these receptors, a single polypeptide chain contains the ITAM as well as the extracellular ligand-binding portion. In other receptors, the ITAMs are in separate polypeptide chains, such as FcεRIγ, ζ, and DAP12, that do not bind

ligand but are noncovalently associated with the ligand-binding chain. ITAMs are also found in cytoplasmic tails of other multichain signaling receptors in the immune system, including the antigen receptors on T and B cells. After ligand binding to the NK cell activating receptors, tyrosine residues within the ITAMs become phosphorylated by cytoplasmic kinases, other protein kinases are recruited to the modified ITAMs and become activated, and these kinases contribute to further signaling by phosphorylating additional proteins. The structure and signaling functions of ITAMs are discussed in more detail in Chapter 7.

Many of the NK cell activating receptors are members of the C-type lectin or KIR families, which also include inhibitory receptors, as discussed before. Some of the activating receptors appear to bind class I MHC molecules, like the inhibitory receptors, but it is not known how these receptors are preferentially activated by infected or damaged cells. It is also clear that the activating receptors recognize ligands other than classical MHC molecules. One well-studied NK cell activating receptor in the C-type lectin family is NKG2D, which binds class I MHC–like proteins, including MIC-A and MIC-B, that are found on virally infected cells and tumor cells but not normal cells. The NKG2D receptor associates with a signaling subunit named DAP10, which has a signaling motif different from the ITAMs in other activating receptors but also enhances NK cell cytotoxicity against target cells.

Another important activating receptor on NK cells is CD16 (FcγRIIIa), which is a low-affinity receptor for IgG antibodies. Antibody molecules have highly variable antigen-binding ends, and on the opposite end, they have an invariant structure, called the Fc region, that interacts with various other molecules in the immune system. We will describe the structure of antibodies in detail in Chapter 5 but, for now, it is sufficient to know that CD16 binds to the Fc regions of certain types of antibodies called IgG1 or IgG3. CD16 associates with one of three different ITAM-containing signaling proteins (e.g., FcεRIγ, ζ, and DAP12 proteins). During an infection, the adaptive immune system produces IgG1 and IgG3 antibodies that specifically bind to the infecting microbes and their antigens on infected cells, and CD16 on NK cells can bind to the Fc parts of these antibodies. As a result, CD16 generates activating signals, through the associated signaling partners, and the NK cells may kill the infected cells that have been coated with antibody molecules. This process is called **antibody-dependent cell-mediated cytotoxicity**; it is an effector function of adaptive immunity and will be discussed in Chapter 12 when we consider humoral immunity.

The ability of activating receptors to induce functional responses in NK cells is enhanced by cytokines. The major cytokines of the innate immune system that stimulate NK function are IL-12, IL-15, IL-18, and type I interferons (discussed later). Each of these cytokines enhances the cytotoxic activity of NK cells and the amount of the cytokine IFN-γ the NK cells secrete. IFN-γ has various antimicrobial effects and will be discussed in detail in Chapter 10. In addition, IL-12 and IL-15 are important growth factors for NK cells.

KIR genes are polymorphic, meaning that there are several allelic variants in the human population, and groups of KIR alleles are often inherited together from a single parent. These groups of linked genes are called KIR haplotypes. There are two major KIR haplotypes and some rarer ones. Haplotypes differ in the number of receptors encoded, and some have more or fewer activating receptors than others. Some haplotypes are associated with increased susceptibility to some diseases, including spontaneous abortion and uveitis.

Effector Functions of NK Cells

The effector functions of NK cells are to kill infected cells and to activate macrophages to destroy phagocytosed microbes (Fig. 4-8). The mechanism of NK cell–mediated cytotoxicity is essentially the same as that of $CD8^+$ CTLs, which we will describe in detail in Chapter 10. NK cells, like CTLs, have granules containing proteins that mediate killing of target cells. When NK cells are activated, granule exocytosis releases these proteins adjacent to the target cells. One NK cell granule protein, called **perforin**, facilitates the entry of other granule proteins, called **granzymes**, into the cytoplasm of target cells. The granzymes are enzymes that initiate a sequence of signaling events that cause death of the target cells by apoptosis. The signaling pathways that cause apoptosis are discussed in Chapter 14. By killing cells infected by viruses and intracellular bacteria, NK cells eliminate reservoirs of

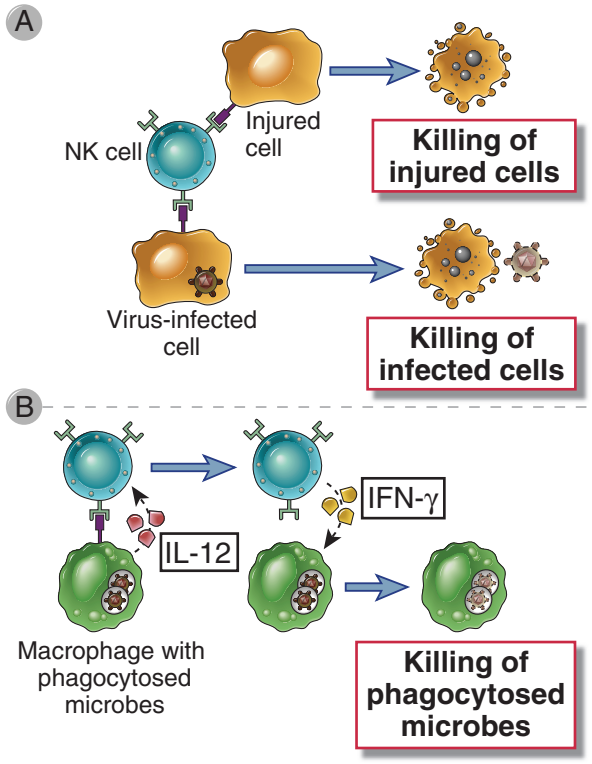


FIGURE 4–8 Functions of NK cells. A, NK cells recognize ligands on infected cells or cells undergoing other types of stress and kill the host cells. In this way, NK cells eliminate reservoirs of infection as well as dysfunctional cells. **B,** NK cells respond to IL-12 produced by macrophages and secrete IFN-γ, which activates the macrophages to kill phagocytosed microbes.

infection. Some tumors, especially those of hematopoietic origin, are targets of NK cells, perhaps because the tumor cells do not express normal levels or types of class I MHC molecules.

NK cell–derived IFN-γ serves to activate macrophages, like IFN-γ produced by T cells, and increases the capacity of macrophages to kill phagocytosed bacteria (see Chapter 10). IFN-γ produced by NK cells in lymph nodes can also direct the differentiation of naive T cells into T_H1 cells (see Chapter 9).

NK cells play several important roles in defense against intracellular microbes. They kill virally infected cells before antigen-specific CTLs can become fully active, that is, during the first few days after viral infection. Early in the course of a viral infection, NK cells are expanded and activated by IL-12 and IL-15, and they kill infected cells, especially those that display reduced levels of class I MHC molecules. In addition, IFN-γ secreted by NK cells activates macrophages to destroy phagocytosed microbes. This IFN-γ–dependent NK cell–macrophage reaction can control an infection with intracellular bacteria such as *Listeria monocytogenes* for several days or weeks and thus allow time for T cell–mediated immunity to develop and eradicate the infection. Depletion of NK cells leads to increased susceptibility to infection by some viruses and intracellular bacteria. In mice lacking T cells, the NK cell response may be adequate to keep infection with such microbes in check for some time, but the animals eventually succumb in the absence of T cell–mediated immunity. NK cells may also be important later in the body's response to infection by killing infected cells that have escaped CTL-mediated immune attack by reducing expression of class I MHC molecules. Because NK cells can kill certain tumor cells in vitro, it has also been proposed that NK cells serve to kill malignant clones in vivo.

T and B Lymphocytes with Limited Antigen Receptor Specificities

As we will discuss in greater detail in later chapters, most T and B lymphocytes are components of the adaptive immune system and are characterized by a highly diverse repertoire of specificities for different antigens. The diversity of antigen receptors is generated by random somatic recombination of a large set of germline DNA segments as well as modification of nucleotide sequences at the junctions between the recombined segments, yielding unique antigen receptor genes in each lymphocyte clone (see Chapter 8). However, certain subsets of T and B lymphocytes have very little diversity because the same antigen receptor gene DNA segments are recombined in each clone and there is little or no modification of junctional sequences. It appears that these T and B cell subsets recognize structures expressed by many different or commonly encountered microbial species; in other words, they recognize PAMPs. T cell subsets with limited antigen receptor diversity include invariant natural killer T cells (iNKT), γδ T cells, and intraepithelial T cells with αβ TCRs (mentioned earlier). B cell subsets that produce antibodies with a limited set of specificities include B-1 B cells and marginal zone B cells. Although these T and B cells perform similar effector functions as do their more clonally diverse counterparts, the nature of their specificities places them in a special category of lymphocytes that is akin more to effector cells of innate immunity than to cells of adaptive immunity. These special T and B cell subsets are described in Chapters 10 and 11, respectively.

Mast Cells

Mast cells are present in the skin and mucosal epithelium and rapidly secrete proinflammatory cytokines and lipid mediators in response to infections and other stimuli. We introduced mast cells in Chapter 2. Recall that these cells contain abundant cytoplasmic granules containing various inflammatory mediators that are released when the cells are activated, either by microbial products or by a special antibody-dependent mechanism. The granule contents include vasoactive amines (such as histamine) that cause vasodilation and increased capillary permeability, and proteolytic enzymes that can kill bacteria or inactivate microbial toxins. Mast cells also synthesize and secrete lipid mediators (such as prostaglandins) and cytokines (such as TNF). Because mast cells are usually located adjacent to blood vessels (see Fig. 2-1), their released granule contents rapidly induce changes in the blood vessels that promote acute inflammation. Mast cells express TLRs, and TLR ligands can induce mast cell degranulation. Mast cell–deficient mice are impaired in controlling bacterial infections, probably because of impaired innate immune responses. Mast cell products also provide defense against helminths and are responsible for symptoms of allergic diseases. We will return to a detailed discussion of mast cells in relation to allergic diseases in Chapter 19.

SOLUBLE RECOGNITION AND EFFECTOR MOLECULES OF INNATE IMMUNITY

Several different kinds of molecules that recognize microbes and promote innate responses exist in soluble form in the blood and extracellular fluids. These molecules provide early defense against pathogens that are present outside host cells at some part of their life cycle. The soluble effector molecules function in two major ways.

- By binding to microbes, they act as **opsonins** and enhance the ability of macrophages, neutrophils, and dendritic cells to phagocytose the microbes. This is because the phagocytic cells express membrane receptors specific for the opsonins, and these receptors can efficiently mediate the internalization of the complex of opsonin and bound microbe.
- After binding to microbes, soluble mediators of innate immunity promote inflammatory responses that bring more phagocytes to sites of infections, and they may also directly kill microbes.

The soluble effector molecules are sometimes called the humoral branch of innate immunity, analogous to

the humoral branch of adaptive immunity mediated by antibodies. The major components of the humoral innate immune system are natural antibodies, the complement system, collectins, pentraxins, and ficolins. We will next describe the major features and functions of these components of innate immunity.

Natural Antibodies

Many antibodies with millions of different fine specificities are produced in humoral immune responses by B lymphocytes and their progeny, as part of the adaptive immune system, and we will describe antibodies and B cell responses in detail in later chapters. However, there are subsets of B cells that produce antibodies with only a limited number of specificities without overt exposure to foreign antigens, and these are called **natural antibodies**. As is typical for other components of innate immunity, natural antibodies are already present before infections, and they recognize common molecular patterns on microbes or stressed and dying cells. Natural antibodies are usually specific for carbohydrate or lipid molecules but not proteins, and most are IgM antibodies, one of several structural classes of Ig molecules (see Chapter 5). A remarkably large proportion of the natural antibodies in humans and mice are specific for oxidized lipids, including phospholipid head groups such as lysophosphatidylcholine and phosphorylcholine, which are found on bacterial membranes and on apoptotic cells but are not exposed on the surface of healthy host cells. Some experimental evidence indicates that the natural antibodies specific for these phospholipids provide protection against bacterial infections and facilitate the phagocytosis of apoptotic cells. The anti-ABO blood group antibodies, another example of natural antibodies, recognize certain glycolipids (blood group antigens) expressed on the surface of many cell types, including blood cells. Blood group antigens and antibodies are important for transplantation but not for host defense and are discussed in Chapter 16.

The Complement System

The complement system consists of several plasma proteins that work together to opsonize microbes, to promote the recruitment of phagocytes to the site of infection, and in some cases to directly kill the microbes (Fig. 4-9). Complement activation involves proteolytic cascades, in which an inactive precursor enzyme, called a zymogen, is altered to become an active protease that cleaves and thereby induces the proteolytic activity of the next complement protein in the cascade. As the cascade proceeds, the enzymatic activities result in tremendous amplification of the amount of proteolytic products that are generated. These products perform the effector functions of the complement system. Other proteolytic cascades include the blood coagulation pathways and the kinin-kallikrein system that regulates vascular permeability.

The first step in activation of the complement system is recognition of molecules on microbial surfaces but not host cells, and this occurs in three ways, each referred to as a distinct pathway of complement activation.

- The **classical pathway**, so called because it was discovered first, uses a plasma protein called C1q to detect antibodies bound to the surface of a microbe or other structure (Fig. 4-10). Once C1q binds to the Fc portion

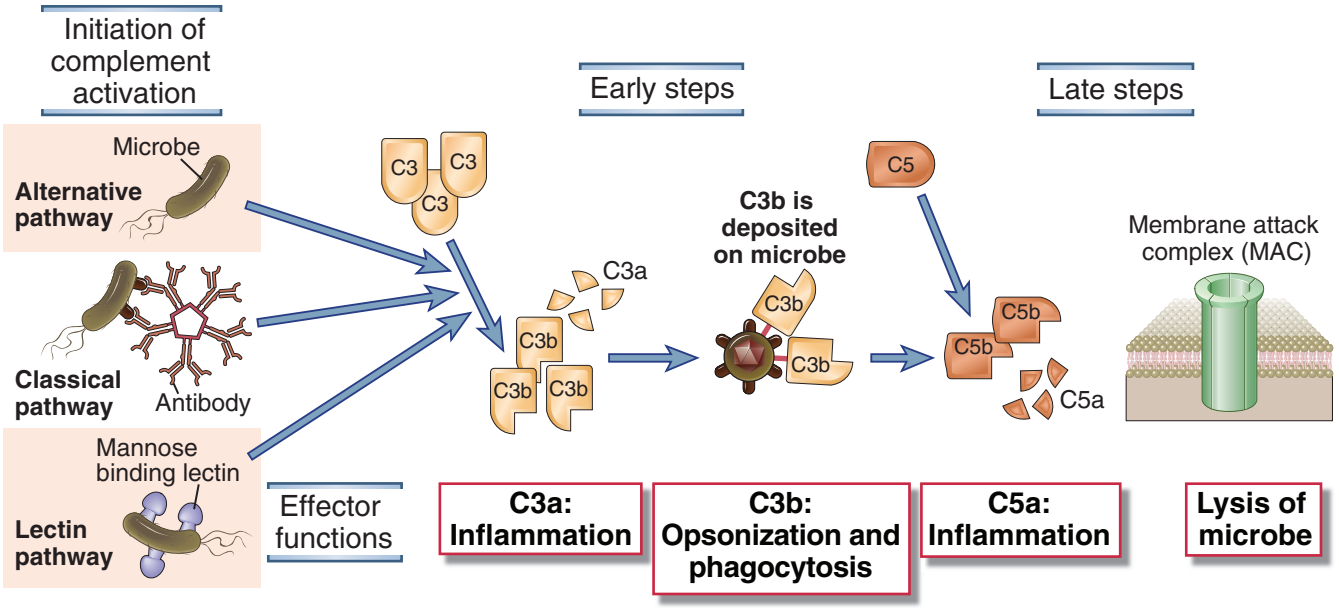


FIGURE 4–9 Pathways of complement activation. The activation of the complement system may be initiated by three distinct pathways, all of which lead to the production of C3b (the early steps). C3b initiates the late steps of complement activation, culminating in the production of peptides that stimulate inflammation (C5a) and polymerized C9, which forms the membrane attack complex, so called because it creates holes in plasma membranes. The principal functions of major proteins produced at different steps are shown. The activation, functions, and regulation of the complement system are discussed in much more detail in Chapter 12.

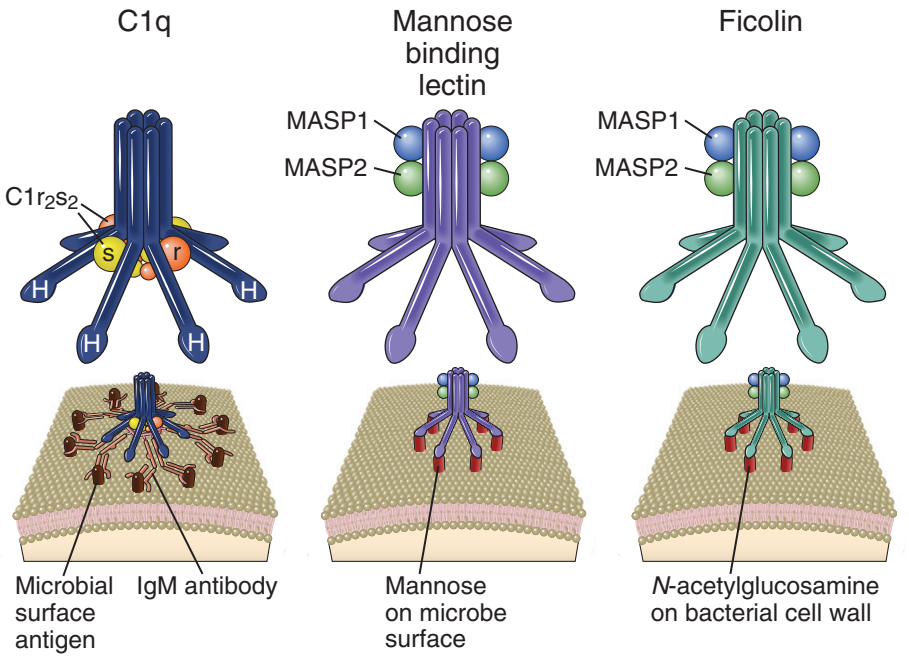


FIGURE 4–10 C1, mannose-binding lectin, and ficolin. These three homologous pentameric proteins can all initiate complement activation on binding to their ligands on cell surfaces. C-type lectin–like globular heads at the end of collagenous-like stalks in the C1q and mannose-binding lectin proteins bind the Fc regions of IgM or mannose on the surface of microbes, respectively. Fibrinogen-like globular heads on ficolin bind *N*-acetylglucosamine on the surface of microbes. Binding results in conformational changes that activate the serine protease activity of C1r and C1s, associated with C1q, or MASP1 and MASP2, associated with mannose-binding lectin and ficolin.

of the antibodies, two associated serine proteases, called C1r and C1s, become active and initiate a proteolytic cascade involving other complement proteins. The classical pathway is one of the major effector mechanisms of the humoral arm of adaptive immune responses (see Chapter 12). Because IgM natural antibodies are very efficient at binding C1q, the classical pathway also participates in innate immunity. In addition, other innate immune system soluble proteins called pentraxins, discussed later, can also bind C1q and initiate the classical pathway.

- The **alternative pathway**, which was discovered later but is phylogenetically older than the classical pathway, is triggered when a complement protein called C3 directly recognizes certain microbial surface structures, such as bacterial LPS. C3 is also constitutively activated in solution at a low level and binds to cell surfaces, but it is then inhibited by regulatory molecules present on mammalian cells. Because microbes lack these regulatory proteins, the spontaneous activation can be amplified on microbial surfaces. Thus, this pathway can distinguish normal self from foreign microbes on the basis of the presence or absence of the regulatory proteins.
- The **lectin pathway** is triggered by a plasma protein called mannose-binding lectin (MBL), which recognizes terminal mannose residues on microbial glycoproteins and glycolipids, similar to the mannose receptor on phagocyte membranes described earlier (see Fig. 4-10). MBL is a member of the collectin family (discussed later) with a hexameric structure similar to the C1q component of the complement system. After MBL binds to microbes, two zymogens called MASP1 (mannan-binding lectin-associated serine protease) and MASP2, with similar functions to C1r and C1s, associate with MBL and initiate downstream proteolytic steps identical to the classical pathway.

Recognition of microbes by any of the three complement pathways results in sequential recruitment and assembly of additional complement proteins into protease complexes (see Fig. 4-9). One of these complexes, called **C3 convertase**, cleaves the central protein of the complement system, **C3**, producing C3a and C3b. The larger C3b fragment becomes covalently attached to the microbial surface where the complement pathway was activated. C3b serves as an opsonin to promote phagocytosis of the microbes. A smaller fragment, C3a, is released and stimulates inflammation by acting as a chemoattractant for neutrophils. C3b binds other complement proteins to form a protease called **C5 convertase** that cleaves C5, generating a secreted peptide (C5a) and a larger fragment (C5b) that remains attached to the microbial cell membranes. C5a is also a chemoattractant; in addition, it induces changes in blood vessels that make them leak plasma proteins and fluid into sites of infections. C5b initiates the formation of a complex of the complement proteins C6, C7, C8, and C9, which are assembled into a membrane pore, called the **membrane attack complex** (MAC), that causes lysis of the cells where complement is activated.

The complement system is an essential component of innate immunity, and patients with deficiencies in C3 are highly susceptible to recurrent, often lethal, bacterial infections. However, genetic deficiencies in MAC formation (the terminal product of the classical pathway) increase susceptibility to only a limited number of microbes, notably *Neisseria* bacteria, which have thin cell walls that make them especially susceptible to the lytic action of the MAC. The complement system will be discussed in more detail in Chapter 12.

Pentraxins

Several plasma proteins that recognize microbial structures and participate in innate immunity belong to the pentraxin family, which is a phylogenetically old group

of structurally homologous pentameric proteins. Prominent members of this family include the short pentraxins C-reactive protein (CRP) and serum amyloid P (SAP) and the long pentraxin PTX3. Both CRP and SAP bind to several different species of bacteria and fungi. The molecular ligands recognized by CRP and SAP include phosphorylcholine and phosphatidylethanolamine, respectively, which are found on bacterial membranes and on apoptotic cells, as discussed earlier. PTX3 recognizes various molecules on fungi, selected gram-positive and gram-negative bacteria, and viruses. CRP, SAP, and PTX3 all activate complement by binding C1q and initiating the classical pathway.

Plasma concentrations of CRP are very low in healthy individuals but can increase up to 1000-fold during infections and in response to other inflammatory stimuli. The increased levels of CRP are a result of increased synthesis by the liver induced by the cytokines IL-6 and IL-1, which are produced by phagocytes as part of the innate immune response. Liver synthesis and plasma levels of several other proteins, including SAP and others unrelated to the pentraxins, also increase in response to IL-1 and IL-6, and as a group these plasma proteins are called **acute-phase reactants**.

PTX3 is produced by several cell types, including dendritic cells, macrophages, and endothelial cells, in response to TLR ligands and inflammatory cytokines, such as TNF, but it is not an acute-phase reactant. PTX3 is also stored in neutrophil granules and released as neutrophils die. PTX3 recognizes apoptotic cells and certain microorganisms. Studies with knockout mice reveal that PTX3 provides protection against some microbes, including the fungus *Aspergillus fumigatus*.

Collectins and Ficolins

The collectins are a family of trimeric or hexameric proteins, each subunit of which contains a collagen-like tail connected by a neck region to a calcium-dependent (C-type) lectin head. Three members of this family serve as soluble effector molecules in the innate immune system; these are mannose-binding lectin (MBL) and pulmonary surfactant proteins SP-A and SP-D.

MBL, which is a soluble pattern recognition receptor that binds carbohydrates with terminal mannose and fucose, was discussed earlier in relation to the lectin pathway of complement activation (see Fig. 4-10). MBL can also function as an opsonin by binding to and enhancing phagocytosis of microbes. Recall that opsonins simultaneously bind microbes and a surface receptor on phagocyte membranes, and in the case of MBL, the surface receptor is called the C1q receptor because it also binds C1q. This receptor mediates the internalization of microbes that are opsonized by MBL. The gene encoding MBL is polymorphic, and certain alleles are associated with impaired hexamer formation and reduced blood levels. Low MBL levels are associated with increased susceptibility to a variety of infections, especially in combination with other immunodeficiency states.

Surfactant protein A (SP-A) and surfactant protein D (SP-D) are collectins with lipophilic surfactant properties shared by other surfactants. They are found in the alveoli of the lungs, and their major functions appear to be as mediators of innate immune responses in the lung. They bind to various microorganisms and act as opsonins, facilitating ingestion by alveolar macrophages. SP-A and SP-D can also directly inhibit bacterial growth, and they may activate macrophages. SP-A– and SP-D–deficient mice have impaired abilities to resist a variety of pulmonary infections.

Ficolins are plasma proteins that are structurally similar to collectins, possessing a collagen-like domain, but instead of a C-type lectin domain, they have a fibrinogen-type carbohydrate recognition domain (see Fig. 4-10). Ficolins have been shown to bind several species of bacteria, opsonizing them and activating complement in a manner similar to that of MBL. The molecular ligands of the ficolins include *N*-acetylglucosamine and the lipoteichoic acid component of the cell walls of gram-positive bacteria.

Now that we have discussed the general properties and various components of the innate immune system, including the cells, cellular pathogen recognition receptors, and soluble recognition and effector molecules, we can consider how these various components work to protect against pathogens. The three major ways in which the innate immune system protects against infections is by inducing inflammation, inducing antiviral defense, and stimulating adaptive immunity. Many of these reactions are mediated by cytokines, which serve diverse and important roles in innate immunity (Table 4-4). As we discuss below, these cytokines act mainly close to their site of production (paracrine actions), but some of them can also have distant effects (endocrine actions).

THE INFLAMMATORY RESPONSE

The major way by which the innate immune system deals with infections and tissue injury is to stimulate acute inflammation, which is the accumulation of leukocytes, plasma proteins, and fluid derived from the blood at an extravascular tissue site of infection or injury. The leukocytes and plasma proteins normally circulate in the blood and are recruited to sites of infection and injury, where they perform various effector functions that serve to kill microbes and begin to repair tissue damage. Typically, the most abundant leukocyte that is recruited from the blood into acute inflammatory sites is the neutrophil, but blood monocytes, which become macrophages in the tissue, become increasingly prominent over time and may be the dominant population in some reactions. Among the important plasma proteins that enter inflammatory sites are complement proteins, antibodies, and acute-phase reactants. The delivery of these blood-derived components to the inflammatory site is dependent on reversible changes in blood vessels in the infected or damaged tissue. These changes include increased blood flow into the tissue due to arteriolar dilation, increased adhesiveness of circulating leukocytes to the endothelial lining of venules, and increased permeability of the capillaries and venules to plasma proteins and fluid. All these changes are induced by cytokines and

TABLE 4–4 Cytokines of Innate Immunity

Cytokine	Size	Principal Cell Source	Principal Cellular Targets and Biologic Effects
Tumor necrosis factor (TNF)	17 kD; 51-kD homotrimer	Macrophages, T cells	Endothelial cells: activation (inflammation, coagulation) Neutrophils: activation Hypothalamus: fever Liver: synthesis of acute-phase proteins Muscle, fat: catabolism (cachexia) Many cell types: apoptosis
Interleukin-1 (IL-1)	17-kD mature form; 33-kD precursors	Macrophages, endothelial cells, some epithelial cells	Endothelial cells: activation (inflammation, coagulation) Hypothalamus: fever Liver: synthesis of acute-phase proteins
Chemokines (see Table 3-2)	8-12 kD	Macrophages, endothelial cells, T cells, fibroblasts, platelets	Leukocytes: chemotaxis, activation; migration into tissues
Interleukin-12 (IL-12)	Heterodimer of 35-kD and 40-kD subunits	Macrophages, dendritic cells	T cells: T_H1 differentiation NK cells and T cells: IFN-γ synthesis, increased cytotoxic activity
Type I interferons (IFN-α, IFN-β)	IFN-α: 15-21 kD IFN-β: 20-25 kD	IFN-α: macrophages IFN-β: fibroblasts	All cells: antiviral state, increased class I MHC expression NK cells: activation
Interleukin-10 (IL-10)	Homodimer of 34-40 kD; 18-kD subunits	Macrophages, T cells (mainly regulatory T cells)	Macrophages, dendritic cells: inhibition of IL-12 production and expression of costimulators and class II MHC molecules
Interleukin-6 (IL-6)	19-26 kD	Macrophages, endothelial cells, T cells	Liver: synthesis of acute-phase proteins B cells: proliferation of antibody-producing cells
Interleukin-15 (IL-15)	13 kD	Macrophages, others	NK cells: proliferation T cells: proliferation (memory $CD8^+$ cells)
Interleukin-18 (IL-18)	17 kD	Macrophages	NK cells and T cells: IFN-γ synthesis
Interleukin-23 (IL-23)	Heterodimer of unique 19-kD subunit and 40-kD subunit of IL-12	Macrophages and dendritic cells	T cells: maintenance of IL-17–producing T cells
Interleukin-27 (IL-27)	Heterodimer of 28-kD and 13-kD subunits	Macrophages and dendritic cells	T cells: T_H1 differentiation; inhibition of T_H1 cells NK cells: IFN-γ synthesis

small-molecule mediators initially derived from resident cells in the tissue, such as mast cells, macrophages, and endothelial cells, in response to PAMP or DAMP stimulation. As the inflammatory process develops, the mediators may be derived from newly arrived and activated leukocytes and complement proteins.

Acute inflammation can develop in minutes to hours and last for days. Chronic inflammation is a process that takes over from acute inflammation if the infection is not eliminated or the tissue injury is prolonged. It usually involves recruitment and activation of monocytes and lymphocytes. Chronic inflammatory sites also often undergo tissue remodeling, with angiogenesis and fibrosis. Although innate immune stimuli may contribute to chronic inflammation, the adaptive immune system may also be involved because cytokines produced by T cells are powerful inducers of inflammation (see Chapter 10). Detailed descriptions of the various mediators and pathologic manifestations of acute and chronic inflammation can be found in pathology textbooks. We will focus our discussion on particular aspects of the acute inflammatory process that have broad relevance to both innate and adaptive immunity and immune-mediated inflammatory diseases.

The Major Proinflammatory Cytokines TNF, IL-1, and IL-6

One of the earliest responses of the innate immune system to infection and tissue damage is the secretion of cytokines by tissue cells, which is critical for the acute inflammatory response. Three of the most important proinflammatory cytokines of the innate immune system are TNF, IL-1 (which we have mentioned already several times), and IL-6 (see Table 4-4). Tissue macrophages and mast cells are the major source of these cytokines, although other cell types, including endothelial and epithelial cells, can also produce IL-1 and IL-6. We will discuss the major features of these cytokines, focusing mainly on TNF and IL-1, before describing their role in acute inflammation.

Tumor Necrosis Factor

Tumor necrosis factor (TNF) is a mediator of the acute inflammatory response to bacteria and other infectious microbes. The name of this cytokine derives from its original identification as a serum substance (factor) that caused necrosis of tumors, now known to be the result of local inflammation and thrombosis of tumor blood vessels. TNF is also called TNF-α to distinguish it from the closely related TNF-β, also called lymphotoxin. TNF is produced by macrophages, dendritic cells, and other cell types. In macrophages, it is synthesized as a nonglycosylated type II membrane protein and is expressed as a homotrimer, which is able to bind to one form of TNF receptor. The membrane form of TNF is cleaved by a membrane-associated metalloproteinase, releasing a polypeptide fragment, and three of these polypeptide chains polymerize to form a triangular pyramid-shaped circulating TNF protein (Fig. 4-11). The receptor-binding sites are at the base of the pyramid, allowing simultaneous binding of the cytokine to three receptor molecules.

There are two distinct TNF receptors called type I (TNF-RI) and type II (TNF-RII). The affinities of TNF for its receptors are unusually low for a cytokine, the K_d being only ~1×10^{-9} M for binding to TNF-RI and approximately 5×10^{-10} M for binding to TNF-RII. Both TNF receptors are present on most cell types. The TNF receptors are members of a large family of proteins called the TNF receptor superfamily, many of which are involved in immune and inflammatory responses. These receptors exist as trimers in the plasma membrane. Cytokine binding to some TNF receptor family members, such as TNF-RI, TNF-RII, and CD40, leads to the recruitment of proteins, called TNF receptor–associated factors (TRAFs), to the cytoplasmic domains of the receptors. The TRAFs activate transcription factors, notably NF-κB and AP-1. Cytokine binding to other family members, such as TNF-RI, leads to recruitment of an adaptor protein that activates caspases and triggers apoptosis. Thus, different members of the TNF receptor family can induce gene expression or cell death, and some can do both (see Chapter 7).

TNF production by macrophages is stimulated by PAMPs and DAMPs. TLRs, NLRs, and RLRs can all induce TNF gene expression, in part by activation of the NF-κB transcription factor. Many different microbial products can therefore induce TNF production. Large amounts of this cytokine may be produced during infections with gram-negative and gram-positive bacteria, which release the TLR ligands LPS and lipoteichoic acid, respectively, from their cell walls. Septic shock, a life-threatening condition caused when bacteria enter the blood stream, is mediated in large part by TNF. We will discuss septic shock later in this chapter.

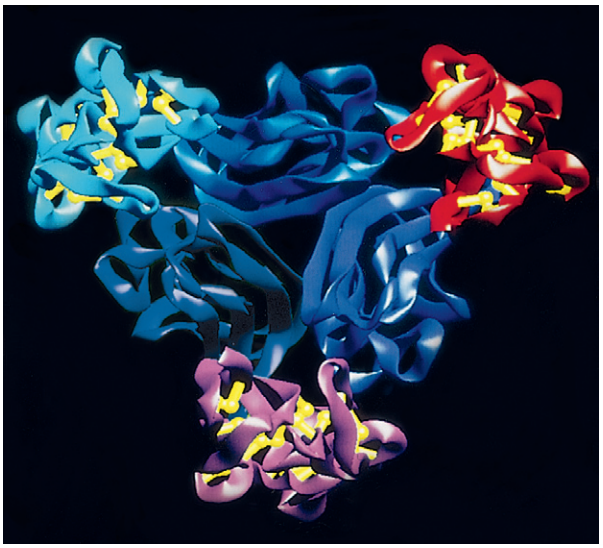


FIGURE 4–11 Structure of the TNF receptor with bound lymphotoxin. The ribbon structure depicts a top view of a complex of three TNF receptors (TNF-RI) and one molecule of the bound cytokine, revealed by x-ray crystallography. Lymphotoxin is a homotrimer in which the three subunits are colored dark blue. The lymphotoxin homotrimer forms an inverted three-sided pyramid with its base at the top and its apex at the bottom. Three TNF-RI molecules, colored magenta, cyan, and red, bind one homotrimer of lymphotoxin, with each receptor molecule interacting with two different lymphotoxin monomers in the homotrimer complex. Disulfide bonds in the receptor are colored yellow. TNF is homologous to lymphotoxin and presumably binds to its receptors in the same way. *(From Banner DW, et al., Cell: Crystal structure of the soluble human 55 kd TNF receptor–human TNFβ complex: 73:431-445. © Cell Press, 1993).*

Interleukin-1

Interleukin-1 (IL-1) is also a mediator of the acute inflammatory response and has many similar actions as TNF. The major cellular source of IL-1, like that of TNF, is activated mononuclear phagocytes. Unlike TNF, IL-1 is also produced by many cell types other than macrophages, such as neutrophils, epithelial cells (e.g., keratinocytes), and endothelial cells. There are two forms of IL-1, called IL-1α and IL-1β, that are less than 30% homologous to each other, but they bind to the same cell surface receptors and have the same biologic activities. The main biologically active secreted form is IL-1β.

IL-1 production usually requires two distinct signals, one that activates new gene transcription and production of a 33-kD precursor pro–IL-1β polypeptide, and a second signal that activates the inflammasome to proteolytically cleave the precursor to generate the 17-kD mature IL-1β protein (see Fig. 4-4). As discussed earlier in the chapter, IL-1β gene transcription is induced by TLR and NOD signaling pathways that activate NF-κB, whereas pro–IL-1β cleavage is mediated by the NLRP3 inflammasome. IL-1 is secreted by a nonclassical pathway because, unlike most secreted proteins, neither IL-1α nor IL-1β has hydrophobic signal sequences to target the nascent polypeptide to the endoplasmic reticulum membrane. One possibility is that mature IL-1 is released mainly when infected cells or activated macrophages die. Some pathogenic bacteria induce both inflammasome-mediated processing of IL-1β and IL-18 in macrophages and caspase-1–dependent cell death, which leads to the release of the inflammatory cytokines. TNF can also stimulate phagocytes and other cell types to produce IL-1. This is an example of a cascade of cytokines that have similar biologic activities.

IL-1 mediates its biologic effects through a membrane receptor called the type I IL-1 receptor, which is expressed

on many cell types, including endothelial cells, epithelial cells, and leukocytes. This receptor is an integral membrane protein that contains an extracellular ligand-binding Ig domain and a Toll/IL-1 receptor (TIR) signaling domain in the cytoplasmic region, described earlier in reference to TLRs. The signaling events that occur when IL-1 binds to the type I IL-1 receptor are similar to those triggered by TLRs and result in the activation of NF-κB and AP-1 transcription factors (see Chapter 7). The type II IL-1 receptor appears incapable of activating downstream signals.

Interleukin-6

IL-6 is another important cytokine in acute inflammatory responses that has both local and systemic effects, including the induction of liver synthesis of a variety of other inflammatory mediators, the stimulation of neutrophil production in the bone marrow, and the differentiation of IL-17–producing helper T cells. IL-6 is synthesized by mononuclear phagocytes, vascular endothelial cells, fibroblasts, and other cells in response to PAMPs and in response to IL-1 and TNF. IL-6 is a homodimer of type I cytokine family polypeptides. The receptor for IL-6 consists of a cytokine-binding polypeptide chain and a signal-transducing subunit (called gp130) that is also the signaling component of receptors for other cytokines. The IL-6 receptor engages a signaling pathway that activates the transcription factor STAT3.

Recruitment of Leukocytes to Sites of Infection

Recruitment of large numbers of neutrophils, followed by monocytes, from blood into tissues typically occurs as part of the acute inflammatory response to infections and tissue injury. The cytokines TNF, IL-1, and IL-6 and chemokines, which are secreted in the local sites of infection or tissue injury, have multiple effects on vascular endothelial cells, leukocytes, and bone marrow, which together increase the local delivery of cells that can fight infections and repair tissues (see Fig. 3-3, Chapter 3). Leukocyte recruitment was described in Chapter 3 and will be only briefly considered here.

Both TNF and IL-1 induce postcapillary venule endothelial cells to express E-selectin and to increase their expression of ICAM-1 and VCAM-1, the ligands for leukocyte integrins. These changes in endothelial adhesion molecule expression are the result of TNF and IL-1 activation of transcription factors, including NF-κB, leading to new adhesion molecule gene transcription. P-selectin expression is also induced on venular endothelial cells at sites of infection and tissue injury, but in large part, this is due to the effects of histamine and thrombin, which stimulate the rapid mobilization of P-selectin stored in granules in the endothelial cell to the cell surface.

TNF and IL-1 also stimulate various cells to secrete chemokines, such as CXCL1 and CCL2, that bind to receptors on neutrophils and monocytes, respectively, increase the affinity of leukocyte integrins for their ligands, and stimulate directional movement of leukocytes. The result of increased selectin, integrin, and chemokine expression is an increase in neutrophil and monocyte adhesion to endothelial cells and transmigration through the vessel wall. The leukocytes that accumulate in the tissues compose an inflammatory infiltrate. The actions of TNF on endothelium and leukocytes are critical for local inflammatory responses to microbes. If inadequate quantities of TNF are present (e.g., in patients treated with drugs that block TNF or in TNF gene knockout mice), a consequence may be failure to contain infections.

In addition, TNF, IL-1, and IL-6 produced at inflammatory sites may enter the blood and be delivered to the bone marrow, where they enhance the production of neutrophils from bone marrow progenitors, usually acting in concert with colony-stimulating factors. In this way, these cytokines increase the supply of cells that can be recruited to the sites of infection.

Phagocytosis and Killing of Microbes by Activated Phagocytes

Neutrophils and macrophages that are recruited into sites of infections ingest microbes into vesicles by the process of phagocytosis and destroy these microbes (Fig. 4-12). Phagocytosis is an active, energy-dependent process of engulfment of large particles (>0.5 μm in diameter) into vesicles. Phagocytic vesicles fuse with lysosomes, where the ingested particles are destroyed, and in this way, the mechanisms of killing, which could potentially injure the phagocyte, are isolated from the rest of the cell.

Neutrophils and macrophages express receptors that specifically recognize microbes, and binding of microbes to these receptors is the first step in phagocytosis. Some of these receptors are pattern recognition receptors, including C-type lectins and scavenger receptors, which we discussed previously. Pattern recognition receptors can contribute to phagocytosis only of organisms that express particular molecular patterns, such as mannose for the mannose receptor. Phagocytes also have high-affinity receptors for certain opsonins, including antibody molecules, complement proteins, and plasma lectins; these receptors are critical for phagocytosis of many different microbes that are coated with the opsonins. One of the most efficient systems for opsonizing microbes is coating them with antibodies. Recall that antibody molecules have antigen-binding sites at one end, and the other end, the Fc region, interacts with effector cells and molecules of the innate immune system. There are several types of antibodies, which we will discuss in detail in Chapters 5 and 12. Phagocytes express high-affinity Fc receptors called FcγRI specific for one type of antibody called IgG (see Chapter 12). Thus, if an individual responds to an infection by making IgG antibodies against microbial antigens, the IgG molecules bind to these antigens, the Fc ends of the bound antibodies can interact with FcγRI on phagocytes, and the end result is efficient phagocytosis of the microbes. Because many different antibodies may be produced that bind to many different microbial products, antibody-mediated opsonization contributes to the phagocytosis of a broader range of microbes than do pattern recognition receptors. Antibody-dependent phagocytosis illustrates a link between innate and adaptive immunity—antibodies are a product of the adaptive immune system (B lymphocytes) that engage

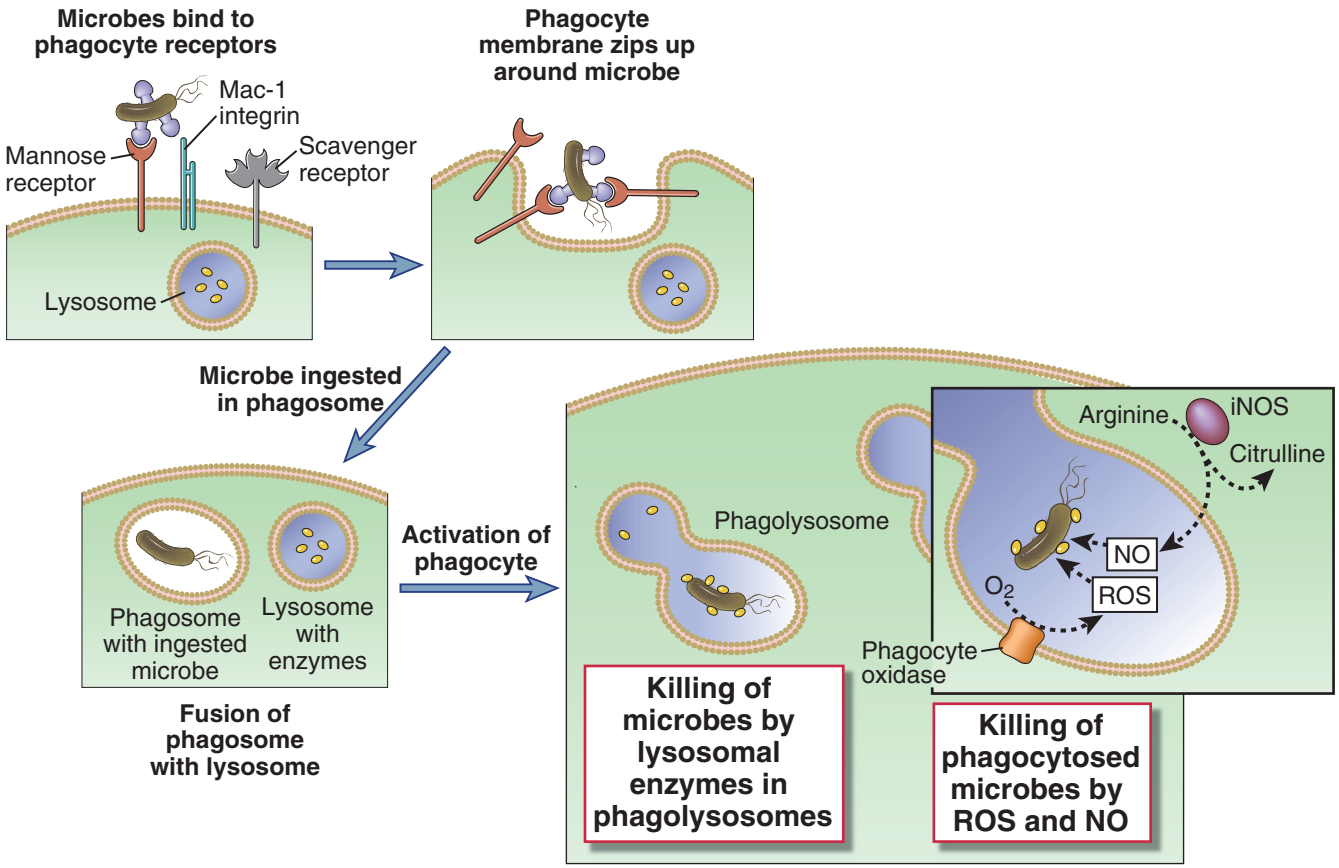


FIGURE 4–12 Phagocytosis and intracellular destruction of microbes. Microbes may be ingested by different membrane receptors of phagocytes; some directly bind microbes, and others bind opsonized microbes. (Note that the Mac-1 integrin binds microbes opsonized with complement proteins, not shown.) The microbes are internalized into phagosomes, which fuse with lysosomes to form phagolysosomes, where the microbes are killed by reactive oxygen and nitrogen species and proteolytic enzymes. iNOS, inducible nitric oxide synthase; NO, nitric oxide; ROS, reactive oxygen species.

innate immune system effector cells (phagocytes) to perform their protective functions.

Once a microbe or particle binds to receptors on a phagocyte, the plasma membrane in the region of the receptors begins to redistribute and extends a cup-shaped projection around the microbe. When the protruding membrane cup extends beyond the diameter of the particle, the top of the cup closes over, or "zips up," and pinches off the interior of the cup to form an inside-out intracellular vesicle (see Fig. 4-12). This vesicle, called a phagosome, contains the ingested foreign particle, and it breaks away from the plasma membrane. The cell surface receptors also deliver activating signals that stimulate the microbicidal activities of phagocytes. Phagocytosed microbes are destroyed, as described next; at the same time, peptides are generated from microbial proteins and presented to T lymphocytes to initiate adaptive immune responses (see Chapter 6).

Activated neutrophils and macrophages kill phagocytosed microbes by the action of microbicidal molecules in phagolysosomes (see Fig. 4-12). Several receptors that recognize microbes, including TLRs, G protein–coupled receptors, antibody Fc and complement C3 receptors, and receptors for cytokines, mainly IFN-γ, function cooperatively to activate phagocytes to kill ingested microbes. Fusion of phagocytic vacuoles (phagosomes) with lysosomes results in the formation of phagolysosomes, where most of the microbicidal mechanisms are concentrated. Three types of microbicidal mechanisms are known to be the most important.

- ***Reactive oxygen species.*** Activated macrophages and neutrophils convert molecular oxygen into reactive oxygen species (ROS), which are highly reactive oxidizing agents that destroy microbes (and other cells). The primary free radical–generating system is the phagocyte oxidase system. Phagocyte oxidase is a multisubunit enzyme that is assembled in activated phagocytes mainly in the phagolysosomal membrane. Phagocyte oxidase is induced and activated by many stimuli, including IFN-γ and signals from TLRs. The function of this enzyme is to reduce molecular oxygen into ROS such as superoxide radicals, with the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) acting as a cofactor. Superoxide is enzymatically dismutated into hydrogen peroxide, which is used by the enzyme myeloperoxidase to convert normally unreactive halide ions into reactive hypohalous acids that are toxic for bacteria. The process by which ROS are produced is called the **respiratory burst** because it occurs during oxygen consumption (cellular respiration). Although the generation of toxic ROS is

commonly viewed as the major function of phagocyte oxidase, another function of the enzyme is to produce conditions within phagocytic vacuoles that are necessary for the activity of the proteolytic enzymes discussed earlier. The oxidase acts as an electron pump, generating an electrochemical gradient across the vacuole membrane, which is compensated for by movement of ions into the vacuole. The result is an increase in pH and osmolarity inside the vacuole, which is necessary for elastase and cathepsin G activity. A disease called chronic granulomatous disease is caused by an inherited deficiency of one of the components of phagocyte oxidase; this deficiency compromises the capacity of neutrophils to kill certain species of gram-positive bacteria (see Chapter 20).

- ***Nitric oxide.*** In addition to ROS, macrophages produce reactive nitrogen species, mainly nitric oxide, by the action of an enzyme called inducible nitric oxide synthase (iNOS). iNOS is a cytosolic enzyme that is absent in resting macrophages but can be induced in response to microbial products that activate TLRs, especially in combination with IFN-γ. iNOS catalyzes the conversion of arginine to citrulline, and freely diffusible nitric oxide gas is released. Within phagolysosomes, nitric oxide may combine with hydrogen peroxide or superoxide, generated by phagocyte oxidase, to produce highly reactive peroxynitrite radicals that can kill microbes. The cooperative and redundant function of ROS and nitric oxide is demonstrated by the finding that knockout mice lacking both iNOS and phagocyte oxidase are more susceptible to bacterial infections than single phagocyte oxidase or iNOS knockout animals are.
- ***Proteolytic enzymes.*** Activated neutrophils and macrophages produce several proteolytic enzymes in the phagolysosomes that function to destroy microbes. One of the important enzymes in neutrophils is elastase, a broad-spectrum serine protease known to be required for killing many types of bacteria. Another important enzyme is cathepsin G. Mouse gene knockout studies have confirmed the essential requirement for these enzymes in phagocyte killing of bacteria.

When neutrophils and macrophages are strongly activated, they can injure normal host tissues by release of lysosomal enzymes, ROS, and nitric oxide. The microbicidal products of these cells do not distinguish between self tissues and microbes. As a result, if these products enter the extracellular environment, they are capable of causing tissue injury.

Other Functions of Activated Macrophages

In addition to killing phagocytosed microbes, macrophages serve many other functions in defense against infections (Fig. 4-13). Several of these functions are mediated by the cytokines the macrophages produce. We have already described how TNF, IL-1, and chemokines made by phagocytes enhance the inflammatory reactions

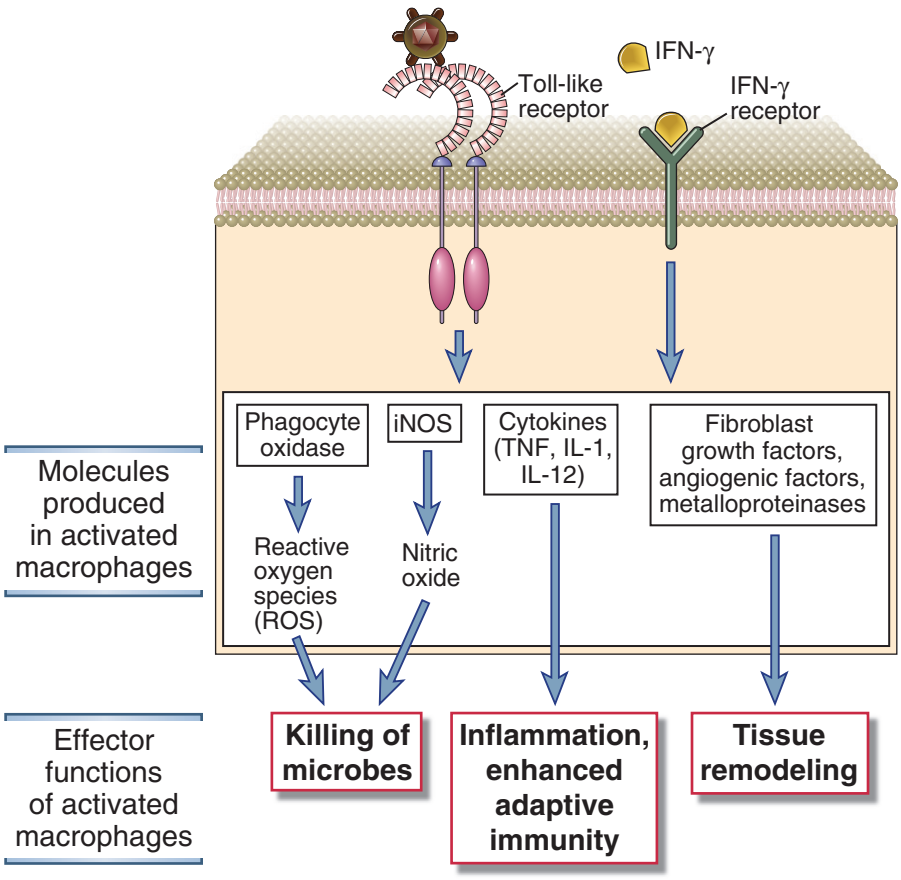


FIGURE 4–13 Effector functions of macrophages. Macrophages are activated by microbial products such as LPS and by NK cell–derived IFN-γ (described earlier in the chapter). The process of macrophage activation leads to the activation of transcription factors, the transcription of various genes, and the synthesis of proteins that mediate the functions of these cells. In adaptive cell-mediated immunity, macrophages are activated by stimuli from T lymphocytes (CD40 ligand and IFN-γ) and respond in essentially the same way (see Chapter 10, Fig. 10-7).

to microbes and bring in more leukocytes and plasma proteins. Activated macrophages also produce growth factors for fibroblasts and endothelial cells that participate in the remodeling of tissues after infections and injury. The role of macrophages in cell-mediated immunity is described in Chapter 10.

Other Cytokines Produced During Innate Immune Responses

In addition to TNF, IL-1, and IL-6, dendritic cells and macrophages activated by PAMPs and DAMPs produce other cytokines that have important roles in innate immune responses (see Table 4-4). Some of the main features of these cytokines and their roles in innate immunity are discussed in this section. These cytokines also have important effects in stimulating adaptive immunity, as we will discuss later in this chapter and in more detail in Chapters 9 and 10.

IL-12 is secreted by dendritic cells and macrophages and stimulates IFN-γ production by NK cells and T cells enhances NK cell and CTL-mediated cytotoxicity, and promotes differentiation of Th1 cells. IL-12 exists as a disulfide-linked heterodimer of 35-kD (p35) and 40-kD (p40) subunits. The p35 subunit is a member of the type I cytokine family. In addition to IL-12, there are other heterodimeric cytokines whose subunits are homologous to either or both of the IL-12 p35 and p40 chains, including IL-23, IL-27, and IL-35. This is of significance because therapeutic antibodies specific for shared subunits are in development for treatment of inflammatory diseases, and some of these antibodies may block the function of more than one cytokine. The principal sources of IL-12 are activated dendritic cells and macrophages. Many cells appear to synthesize the p35 subunit, but macrophages and dendritic cells are the main cell types that produce the p40 component and therefore the biologically active cytokine. During innate immune reactions to microbes, IL-12 is produced in response to TLR and other pattern recognition receptor signaling induced by many microbial stimuli, including bacterial LPS or lipoteichoic acid and virus infections. IFN-γ produced by NK cells or T cells also stimulates IL-12 production, contributing to a positive feedback loop.

The receptor for IL-12 (IL-12R) is a heterodimer composed of β1 and β2 subunits, both of which are members of the type I cytokine receptor family. Both chains are required for high-affinity binding of IL-12 and for signaling, which activates the transcription factor STAT4. Expression of the β2 chain of the IL-12 receptor is itself enhanced by IFN-γ, whose production is stimulated by IL-12, and this is another example of a positive amplification loop in immune responses. Studies with gene knockout mice and the phenotype of rare patients with mutations in the IL-12 receptor support the conclusion that IL-12 is important for IFN-γ production by NK cells and T cells and for host resistance to intracellular bacteria and some viruses. For example, patients with mutations in the IL-12 receptor β1 subunit have been described, and they are highly susceptible to infections with intracellular bacteria, notably *Salmonella* and atypical mycobacteria. IL-12 secreted by DCs during antigen presentation to naive $CD4^+$ T cells promotes their differentiation into the T_H1 subset of helper T cells, which are important for defense against intracellular infections (see Chapter 9). This is a key way in which innate immunity shapes adaptive immune responses.

IL-18 enhances the functions of NK cells, similar to IL-12. Recall that the production of IL-18, like that of IL-1, is dependent on the inflammasome. Also like IL-1, IL-18 binds to a receptor that signals through a TIR domain.

IL-15 is cytokine that serves important growth-stimulating and survival functions for both NK cells and T cells. IL-15 is structurally homologous to the T cell growth factor IL-2, and the heterotrimeric IL-15 receptor shares two subunits with the IL-2 receptor. An interesting feature of IL-15 is that it can be expressed on the cell surface bound to the α chain of its receptor and in this form can be presented to and stimulate nearby cells that express a receptor composed of the other two chains (β and γ). IL-15 presented this way by dendritic cells to NK cells in lymph nodes activates signaling pathways that promote NK cell IFN-γ production. IL-15 also serves as a survival factor for NK and memory $CD8^+$ T cells.

Systemic and Pathologic Consequences of the Acute Inflammatory Responses

TNF, IL-1, and IL-6 produced during the innate immune response to infection or tissue damage have systemic effects that contribute to host defense and are responsible for many of the clinical signs of infection and inflammatory disease (Fig. 4-14).

- TNF, IL-1, and IL-6 all act on the hypothalamus to induce an increase in body temperature (fever), and these cytokines are therefore called **endogenous pyrogens** (i.e., host-derived fever-causing agents, to distinguish them from LPS, which was considered an exogenous [microbe-derived] pyrogen). This distinction is mainly of historical significance because we now know that even LPS induces fever by the production of the cytokines TNF and IL-1. TNF and IL-1 are pyrogenic at much lower concentrations than IL-6. Fever production in response to TNF, IL-1, and IL-6 is mediated by increased synthesis of prostaglandins by cytokine-stimulated hypothalamic cells. Prostaglandin synthesis inhibitors, such as aspirin, reduce fever by blocking this action of the cytokines. The advantages of fever are not well understood but may relate to enhanced metabolic functions of immune cells, impaired metabolic functions of microbes, and changes in the behavior of the febrile host that reduce risk of worsening infections and injury.
- ***IL-1, TNF, and IL-6 induce hepatocytes to express acute-phase reactants, including CRP, SAP, and fibrinogen, which are secreted into the blood.*** Elevated levels of acute-phase reactants are commonly used clinically as signs of infection or other inflammatory processes. The pentraxins CRP and SAP play protective roles in infections, as we discussed earlier in the chapter, and fibrinogen, the precursor of fibrin, contributes to homeostasis and tissue repair.

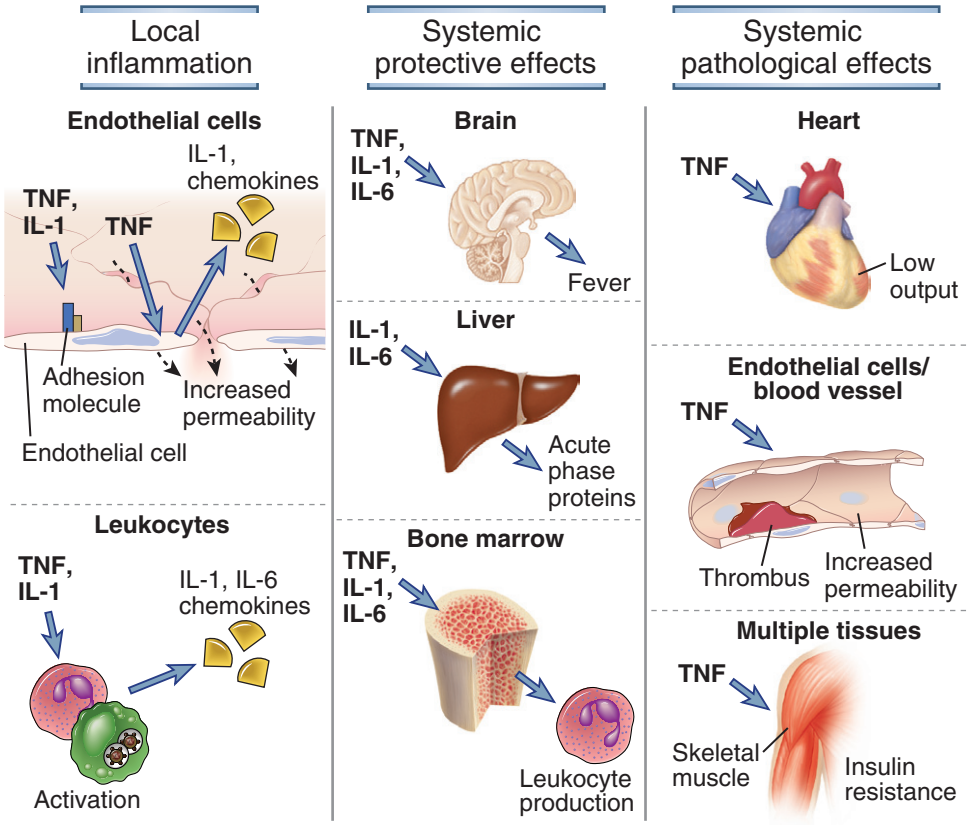


FIGURE 4–14 Local and systemic actions of cytokines in inflammation. TNF, IL-1, and IL-6 have multiple local and systemic inflammatory effects. TNF and IL-1 act on leukocytes and endothelium to induce acute inflammation, and both cytokines induce the expression of IL-6 from leukocytes and other cell types. TNF, IL-1, and IL-6 mediate protective systemic effects of inflammation, including induction of fever, acute-phase protein synthesis by the liver, and increased production of leukocytes by the bone marrow. Systemic TNF can cause the pathologic abnormalities that lead to septic shock, including decreased cardiac function, thrombosis, capillary leak, and metabolic abnormalities due to insulin resistance.

In severe infections, TNF may be produced in large amounts and causes systemic clinical and pathologic abnormalities. If the stimulus for cytokine production is sufficiently strong, the quantity of TNF may be so large that it enters the blood stream and acts at distant sites as an endocrine hormone (see Fig. 4-14). The principal systemic actions of TNF are the following:

- TNF inhibits myocardial contractility and vascular smooth muscle tone, resulting in a marked fall in blood pressure, or shock.
- TNF causes intravascular thrombosis, mainly as a result of loss of the normal anticoagulant properties of the endothelium. TNF stimulates endothelial cell expression of tissue factor, a potent activator of coagulation, and inhibits expression of thrombomodulin, an inhibitor of coagulation. The endothelial alterations are exacerbated by activation of neutrophils, leading to vascular plugging by these cells. The ability of this cytokine to cause necrosis of tumors, which is the basis of its name, is mainly a result of thrombosis of tumor blood vessels.
- Prolonged production of TNF causes wasting of muscle and fat cells, called cachexia. This wasting results from TNF-induced appetite suppression and reduced synthesis of lipoprotein lipase, an enzyme needed to release fatty acids from circulating lipoproteins so that they can be used by the tissues.

A complication of severe bacterial sepsis is a syndrome called **septic shock**, which may be caused by LPS released from gram-negative bacteria (in which case it is called endotoxin shock) or lipoteichoic acid from gram-positive bacteria. Septic shock is characterized by vascular collapse, disseminated intravascular coagulation, and metabolic disturbances. This syndrome is due to LPS- or lipoteichoic acid–induced TLR signaling leading to the production of TNF and other cytokines, including IL-12, IFN-γ, and IL-1. The concentration of serum TNF may be predictive of the outcome of severe bacterial infections. Septic shock can be reproduced in experimental animals by administration of LPS, lipoteichoic acid, or TNF. Antagonists of TNF can prevent mortality in the experimental models, but clinical trials with anti-TNF antibodies or with soluble TNF receptors have not shown benefit in patients with sepsis. The cause of this therapeutic failure is not known, but it may be because other cytokines elicit the same responses as TNF, an example of redundancy.

Acute inflammation may cause tissue injury because the effector mechanisms that phagocytes use to kill microbes are also highly toxic to host tissues. The

proteolytic enzymes and reactive oxygen species produced by phagocytes that accumulate at a site of infection can injure host cells and degrade extracellular matrix if they are generated in large quantities, especially if the microbes resist being killed and continue to stimulate the innate immune responses. In fact, much of the pathology associated with infections is due to the inflammatory responses and not direct toxic effects of the microbes. Acute inflammation also causes tissue damage in the setting of autoimmune diseases, in which case neutrophils and macrophages accumulate and become activated secondarily to stimulation of the adaptive immune system by self antigens (see Chapter 14). As in inflammation induced by infections, TNF, IL-1, IL-6, and IL-12 are the key inducers of inflammation in autoimmune disease. Antagonists against TNF, IL-1, and IL-12 and antibodies against IL-6 receptors are in clinical use or in trials to reduce inflammation in patients with some of these diseases, such as rheumatoid arthritis, inflammatory bowel disease, and psoriasis.

THE ANTIVIRAL RESPONSE

The major way by which the innate immune system deals with viral infections is to induce the expression of type I interferons, whose most important action is to inhibit viral replication. Earlier in the chapter, we have discussed how several of the pattern recognition receptors, including some TLRs, NLRs, and RLRs, generate signals that stimulate IFN-α and IFN-β gene expression in many different cell types. The type I interferons are secreted from these cells and act on other cells to prevent spread of viral infection. In this section, we will describe the major properties of type I interferons and the antiviral effects of these cytokines.

Type I interferons are a large family of structurally related cytokines that mediate the early innate immune response to viral infections. The term *interferon* derives from the ability of these cytokines to interfere with viral infection. There are many type I interferons, all of which have considerable structural homology and are encoded by genes in a single cluster on chromosome 9. The most important type I interferons in viral defense are IFN-α (which actually includes 13 different closely related proteins) and IFN-β, which is a single protein. Plasmacytoid dendritic cells are the major sources of IFN-α but it may also be produced by mononuclear phagocytes. IFN-β is produced by many cells. The most potent stimuli for type I interferon synthesis are viral nucleic acids. Recall that RIG-like receptors in the cytosol and TLRs 3, 7, 8, and 9, all in endosomal vesicles, recognize viral nucleic acids and initiate signaling pathways that activate the interferon regulatory factor (IRF) family of transcription factors, which induce type I interferon gene expression. In adaptive immunity, antigen-activated T cells stimulate mononuclear phagocytes to synthesize type I interferons. The receptor for type I interferons, which binds both IFN-α and IFN-β, is a heterodimer of two structurally related polypeptides, IFNAR1 and IFNAR2, expressed on all nucleated cells. This receptor signals to activate STAT1, STAT2, and IRF9 transcription factors, which induce expression of several different genes that have the following effects in antiviral defense (Fig. 4-15):

- ***Type I interferons, signaling through the type I interferon receptor, activate transcription of several genes that confer on the cells a resistance to viral infection, called an antiviral state.*** The type I interferon–induced genes include double-stranded RNA–activated serine/threonine protein kinase (PKR), which blocks viral transcriptional and translational events, and 2′,5′ oligoadenylate synthetase and RNase L18, 19, which promote viral RNA degradation. The antiviral action of type I interferon is primarily a paracrine action in that a virally infected cell secretes interferon to act on and protect neighboring cells that are not yet infected. Interferon secreted by an infected cell may also act in an autocrine fashion to inhibit viral replication in that cell.
- ***Type I interferons cause sequestration of lymphocytes in lymph nodes, thus maximizing the opportunity for encounter with microbial antigens.*** The mechanism for this effect of type I interferons is the induction of a molecule on the lymphocytes, called CD69, that forms a complex with and reduces surface expression of the sphingosine 1-phosphate (S1P) receptor S1PR1. Recall from Chapter 3 that lymphocyte egress from lymphoid tissues depends on S1P binding to S1PR1. Therefore, reduced S1PR1 inhibits this egress and keeps lymphocytes in lymphoid organs.
- ***Type I interferons increase the cytotoxicity of NK cells and $CD8^+$ CTLs and promote the differentiation of naive T cells to the T_H1 subset of helper T cells.*** These effects of type I interferons enhance both innate and adaptive immunity against intracellular infections, including viruses and some bacteria.
- ***Type I interferons upregulate expression of class I MHC molecules and thereby increase the probability that virally infected cells will be recognized and killed by $CD8^+$ CTLs.*** Virus-specific $CD8^+$ CTLs recognize peptides derived from viral proteins bound to class I MHC molecules on the surface of infected cells. (We will discuss the details of T cell recognition of peptide-MHC and CTL killing of cells in Chapters 6 and 10.) Therefore, by increasing the amount of class I MHC synthesized by a virally infected cell, type I interferons will increase the number of viral peptide–class I MHC complexes on the cell surface that the CTLs can see and respond to. The end result is the killing of cells that support viral replication, which is needed to eradicate viral infections.

Thus, the principal activities of type I interferon work in concert to combat viral infections. Knockout mice lacking the receptor for type I interferons are susceptible to viral infections. IFN-α is in clinical use as an antiviral agent in certain forms of viral hepatitis. IFN-α is also used for the treatment of some tumors, perhaps because it boosts CTL activity or interferes with cell growth. IFN-β is used as a therapy for multiple sclerosis, but the mechanism of its beneficial effect in this disease is not known.

Protection against viruses is due, in part, to the activation of intrinsic apoptotic death pathways in infected cells

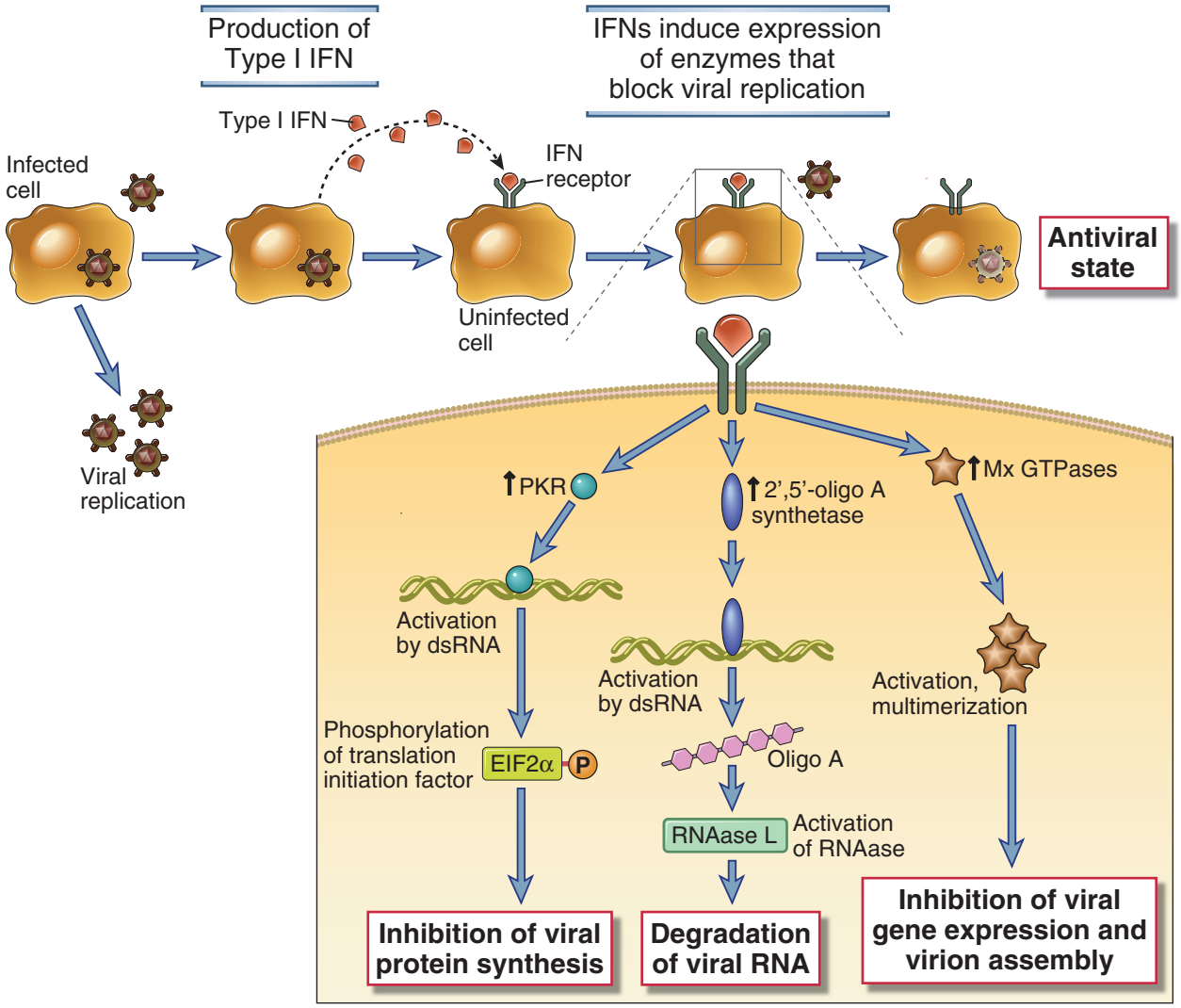


FIGURE 4–15 Biologic actions of type I interferons. Type I interferons (IFN-α, IFN-β) are produced by virus-infected cells in response to intracellular TLR signaling and other sensors of viral RNA. Type I interferons bind to receptors on neighboring uninfected cells and activate JAK-STAT signaling pathways, which induce expression of genes whose products interfere with viral replication. Type I interferons also bind to receptors on infected cells and induce expression of genes whose products enhance the cell's susceptibility to CTL-mediated killing.

and enhanced sensitivity to extrinsic inducers of apoptosis. For example, virally infected cells can sense abnormal DNA replication and abnormal glycoprotein synthesis, leading to initiation of p53-dependent or endoplasmic reticulum–dependent apoptotic pathways, respectively. In addition, virally infected cells are sensitized to TNF-induced apoptosis. Abundant TNF is made by plasmacytoid dendritic cells and macrophages in response to viral infections, in addition to type I interferons. The type I TNF receptor engages both proinflammatory and pro-apoptosis death pathways (see Chapter 7). The dominant pathway that is activated upon TNF binding depends on the state of protein synthesis in the responding cells, and viral infection can shift this balance toward apoptosis.

STIMULATION OF ADAPTIVE IMMUNITY

The innate immune response provides signals that function in concert with antigen to stimulate the proliferation and differentiation of antigen-specific T and B lymphocytes. As the innate immune response is providing the initial defense against microbes, it also sets in motion the adaptive immune response. The activation of lymphocytes requires two distinct signals, the first being antigen and the second being molecules that are produced during innate immune responses to microbes or injured cells (Fig. 4-16). This idea is called the **two-signal hypothesis** for lymphocyte activation. The requirement for antigen (so-called signal 1) ensures that the ensuing immune response is specific. The requirement for additional stimuli triggered by innate immune reactions to microbes (signal 2) ensures that adaptive immune responses are induced when there is a dangerous infection and not when lymphocytes recognize harmless antigens, including self antigens. The molecules produced during innate immune reactions that function as second signals for lymphocyte activation include costimulators (for T cells), cytokines (for both T and B cells), and complement breakdown products (for B cells). We will

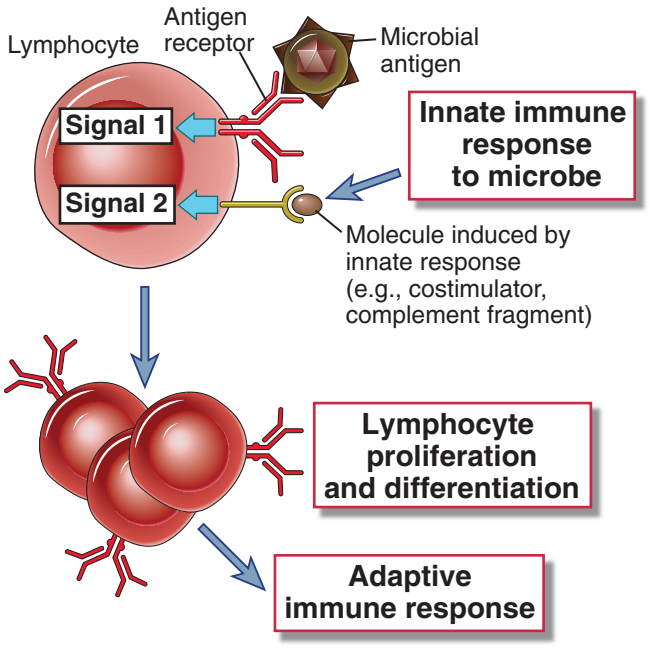


FIGURE 4–16 Stimulation of adaptive immunity by innate immune responses. Antigen recognition by lymphocytes provides signal 1 for the activation of the lymphocytes, and molecules induced on host cells during innate immune responses to microbes provide signal 2. In this illustration, the lymphocytes are B cells, but the same principles apply to T lymphocytes. The nature of second signals differs for B and T cells and is described in later chapters.

return to the nature of second signals for lymphocyte activation in Chapters 9 and 11.

The second signals generated during innate immune responses to different microbes not only enhance the magnitude of the subsequent adaptive immune response but also influence the nature of the adaptive response. A major function of T cell–mediated immunity is to activate macrophages to kill intracellular microbes and to induce robust acute inflammatory responses, beyond those directly induced by the innate immune system, so that a sufficiently large army of phagocytes is called into a site of infection. Infectious agents that engage TLRs and other pattern recognition receptors will tend to stimulate T cell–mediated immune responses. This is because the signaling from these pattern recognition receptors enhances the ability of antigen-presenting cells to induce the differentiation of naive $CD4^+$ T cells into effector cells called T_H1 and T_H17 cells. T_H1 cells produce the cytokine IFN-γ, which can activate macrophages to kill microbes that might otherwise survive within phagocytic vesicles. T_H17 cells produce the cytokine IL-17, which can induce neutrophil-rich inflammation. T_H1 and T_H17 cell-mediated immunity is discussed in detail in Chapters 9 and 10. Many extracellular microbes that enter the blood activate the alternative complement pathway, which in turn enhances the production of antibodies by B lymphocytes. Some of these antibodies opsonize the bacteria and thereby promote their phagocytosis by neutrophils and macrophages. Therefore, humoral immune response serves to eliminate extracellular microbes. The role of complement in enhancing B cell activation is discussed in Chapter 11.

Cytokines produced by cells during innate immune responses to microbes stimulate the proliferation and differentiation of lymphocytes in adaptive immune responses. Examples of cytokines secreted by PAMP-stimulated cells acting on B cells, $CD4^+$ T cells, and $CD8^+$ T cells are given here. The details of the lymphocyte responses to these cytokines will be discussed in more detail in later chapters.

- IL-6 promotes the production of antibodies by activated B cells (see Chapter 11).
- IL-1, IL-6, and IL-23 stimulate the differentiation of naive $CD4^+$ T cells to the T_H17 subset of effector cells (see Chapter 9).
- IL-12 stimulates the differentiation of naive $CD4^+$ T cells to the T_H1 subset of effector cells (see Chapter 9).
- IL-15 promotes the survival of memory $CD8^+$ T cells.

Adjuvants, which are substances that need to be administered together with purified protein antigens to elicit maximal T cell–dependent immune responses (see Chapter 6), work by stimulating innate immune responses at the site of antigen exposure. Adjuvants are useful in experimental immunology and in clinical vaccines. Many adjuvants in experimental use are microbial products, such as killed mycobacteria and LPS, that engage TLRs. The only routinely used adjuvant in human vaccines is alum, composed of either aluminum hydroxide or aluminum phosphate. Among their important effects, adjuvants activate dendritic cells to express more major histocompatibility molecules that are part of the antigen (signal 1) that T cells recognize, increase the expression of costimulators (signal 2) and cytokines needed for T cell activation, and stimulate migration of the dendritic cells to lymph nodes where T cells are located.

FEEDBACK MECHANISMS THAT REGULATE INNATE IMMUNITY

The magnitude and duration of innate immune responses are regulated by a variety of feedback inhibition mechanisms that limit potential damage to tissues. Whereas the inflammatory response is critically important for protection against microbes, it has the potential to cause tissue injury and disease. Several mechanisms have evolved to provide a break on inflammation, and these mechanisms come into play at the same time as or shortly after the initiation of inflammation. Furthermore, the stimuli for the initiation of many of these control mechanisms include the same PAMPs and DAMPs that induce inflammation. We will describe a selected group of these regulatory mechanisms.

IL-10 is a cytokine that is produced by and inhibits activation of macrophages and dendritic cells. IL-10 inhibits the production of various inflammatory cytokines by activated macrophages and dendritic cells, including IL-1, TNF, and IL-12. Because it is both produced by macrophages and inhibits macrophage

functions, IL-10 is an excellent example of a negative feedback regulator. It is not clear whether different stimuli may act on macrophages to induce the production of a regulatory cytokine like IL-10 or effector cytokines like TNF and IL-12, or whether the same stimuli elicit production of all these cytokines but with different kinetics. IL-10 is also produced by some nonlymphoid cell types (e.g., keratinocytes). The Epstein-Barr virus contains a gene homologous to human IL-10, and viral IL-10 has the same activities as the natural cytokine. This raises the intriguing possibility that acquisition of the IL-10 gene during the evolution of the virus has given the virus the ability to inhibit host immunity and thus a survival advantage in the infected host. IL-10 is also produced by regulatory T cells, and we discuss IL-10 in more detail in Chapter 14 in this context.

Mononuclear phagocytes produce a natural antagonist of IL-1 that is structurally homologous to the cytokine and binds to the same receptors but is biologically inactive, so that it functions as a competitive inhibitor of IL-1. It is therefore called **IL-1 receptor antagonist (IL-1RA)**. Synthesis of IL-1RA is induced by many of the same stimuli that induce IL-1 production, and some studies in IL-1RA–deficient mice suggest that this inhibitory cytokine is required to prevent inflammatory diseases of joints and other tissues. Recombinant IL-1RA has been developed as a drug that is effective in the treatment of systemic juvenile rheumatoid arthritis and familial fever syndromes in which IL-1 production is dysregulated. Regulation of IL-1–mediated inflammation may also occur by expression of the type II receptor, which binds IL-1 but does not transduce an activating signal. The major function of this receptor may be to act as a "decoy" that competitively inhibits IL-1 binding to the type I signaling receptor.

Secretion of inflammatory cytokines from a variety of cell types appears to be regulated by the products of autophagy genes. Autophagy is a mechanism by which cells degrade their own organelles, such as mitochondria, by sequestering them within membrane-bound vesicles and fusing the vesicles with lysosomes. This process requires the coordinated actions of many different proteins that are encoded by autophagy (Atg) genes. Targeted mutations in different Atg genes result in enhanced secretion of type I interferons, IL-1, and IL-18 by various cell types and the development of inflammatory bowel disease. The mechanisms by which Atg proteins impair cytokine synthesis are not well understood, but evidence exists for their binding to and inhibition of RLRs and regulation of inflammasome formation. A role for Atg proteins in regulating innate immune responses is further supported by the discovery that polymorphisms in a human Atg are associated with inflammatory bowel disease.

There are numerous negative regulatory signaling pathways that block the activating signals generated by pattern recognition receptors and inflammatory cytokines. Suppressors of cytokine signaling (SOCS) proteins are inhibitors of JAK-STAT signaling pathways linked to cytokine receptors. TLR signaling in macrophages and dendritic cells induces the expression of SOCS proteins, which limit responses of these cells to exogenous cytokines such as type I interferons. Proinflammatory responses of cells to TLR signaling are negatively regulated by SHP-1, an intracellular protein phosphatase that negatively regulates numerous tyrosine kinase–dependent signaling pathways in lymphocytes. There are many other examples of kinases and phosphatases that inhibit TLR, NLR, and RLR signaling.

SUMMARY

- The innate immune system provides the first line of host defense against microbes. The mechanisms of innate immunity exist before exposure to microbes. The cellular components of the innate immune system include epithelial barriers, leukocytes (neutrophils, macrophages, NK cells, lymphocytes with invariant antigen receptors, and mast cells).
- The innate immune system uses cell-associated pattern recognition receptors, present on plasma and endosomal membranes and in the cytoplasm, to recognize structures called pathogen-associated molecular patterns (PAMPs), which are shared by microbes, are not present on mammalian cells, and are often essential for survival of the microbes, thus limiting the capacity of microbes to evade detection by mutating or losing expression of these molecules. In addition, these receptors recognize molecules made by the host but whose expression or location indicates cellular damage; these are called damage-associated molecular patterns (DAMPs).
- TLRs, present on the cell surface and in endosomes, are the most important family of pattern recognition receptors, recognizing a wide variety of ligands, including bacterial cell wall components and microbial nucleic acids. Cytoplasmic pattern recognition receptors exist that recognize microbial molecules. These receptors include the RIG-like receptors (RLRs), which recognize viral RNA, and the NOD-like receptors (NLRs), which recognize bacterial cell wall constituents and also sense sodium urate and other crystals.
- Pattern recognition receptors, including TLRs and RLRs, signal to activate the transcription factors NF-κB and AP-1, which promote inflammatory gene expression, and the IRF transcription factors that promote expression of the antiviral type I interferon genes. The inflammasome, a specialized complex that forms in response to PAMPs and DAMPs, is composed of a NOD-like receptor, an adaptor, and the enzyme caspase-1, the main function of which is to produce active forms of the inflammatory cytokines IL-1 and IL-18.
- Soluble pattern recognition and effector molecules are found in the plasma, including pentraxins (e.g., CRP), collectins (e.g., MBL), and ficolins. These molecules bind microbial ligands and enhance clearance by complement-dependent and complement-independent mechanisms.

- NK cells are lymphocytes that defend against intracellular microbes by killing infected cells and providing a source of the macrophage-activating cytokine IFN-γ. NK cell recognition of infected cells is regulated by a combination of activating and inhibitory receptors. Inhibitory receptors recognize class I MHC molecules, because of which NK cells do not kill normal host cells but do kill cells in which class I MHC expression is reduced, such as virus-infected cells.
- The complement system includes several plasma proteins that become activated in sequence by proteolytic cleavage to generate fragments of the C3 and C5 proteins, which promote inflammation, or opsonize and promote phagocytosis of microbes. Complement activation also generates membrane pores that kill some types of bacteria. The complement system is activated on microbial surfaces and not on normal host cells because microbes lack regulatory proteins that inhibit complement. In innate immune responses, complement is activated mainly spontaneously on microbial cell surfaces and by mannose-binding lectin to initiate the alternative and lectin pathways, respectively.
- The two major effector functions of innate immunity are to induce inflammation, which involves the delivery of microbe-killing leukocytes and soluble effector molecules from blood into tissues, and to block viral infection of cells by the antiviral actions of type 1 interferons. Both types of effector mechanism are induced by the PAMPs and DAMPs, which initiate signaling pathways in tissue cells and leukocytes that activate transcription factors and lead to the expression of cytokines and other inflammatory mediators.
- Several cytokines produced mainly by activated macrophages mediate inflammation. TNF and IL-1 activate endothelial cells, stimulate chemokine production, and increase neutrophil production by the bone marrow. IL-1 and TNF both induce IL-6 production, and all three cytokines mediate systemic effects, including fever and acute-phase protein synthesis by the liver. IL-12 and IL-18 stimulate production of the macrophage-activating cytokine IFN-γ by NK cells and T cells. These cytokines function in innate immune responses to different classes of microbes, and some (IL-1, IL-6, IL-12, IL-18) modify adaptive immune responses that follow the innate immune response.
- Neutrophils and monocytes (the precursors of tissue macrophages) migrate from blood into inflammatory sites during innate immune responses because of the effects of cytokines and chemokines produced by PAMP- and DAMP-stimulated tissue cells.
- Neutrophils and macrophages phagocytose microbes and kill them by producing ROS, nitric oxide, and enzymes in phagolysosomes. Macrophages also produce cytokines that stimulate inflammation and promote tissue remodeling at sites of infection. Phagocytes recognize and respond to microbial products by several different types of receptors, including TLRs, C-type lectins, scavenger receptors, and *N*-formyl met-leu-phe receptors.
- Molecules produced during innate immune responses stimulate adaptive immunity and influence the nature of adaptive immune responses. Dendritic cells activated by microbes produce cytokines and costimulators that enhance T cell activation and differentiation into effector T cells. Complement fragments generated by the alternative pathway provide second signals for B cell activation and antibody production.
- Innate immune responses are regulated negative feedback mechanisms that limit potential damage to tissues. IL-10 is a cytokine that is produced by and inhibits activation of macrophages and dendritic cells. Inflammatory cytokine secretion is regulated by autophagy gene products. Negative signaling pathways block the activating signals generated by pattern recognition receptors and inflammatory cytokines.

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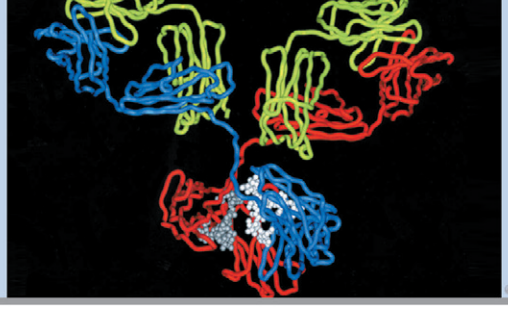
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CHAPTER

5

Antibodies and Antigens

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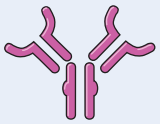
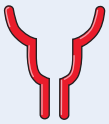
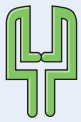
SUMMARY, 106

Antibodies are circulating proteins that are produced in vertebrates in response to exposure to foreign structures known as antigens. Antibodies are incredibly diverse and specific in their ability to recognize foreign molecular structures and are the primary mediators of humoral immunity against all classes of microbes. Emil von Behring and Shibasaburo Kitasato's successful treatment of diphtheria in 1890 with serum from animals immunized with an attenuated form of the diphtheria toxin established the protective role of circulating proteins and led to the birth of modern immunology. The family of circulating proteins that mediate these protective responses was initially called antitoxins. When it was appreciated that similar proteins could be generated against many substances, not just microbial toxins, these proteins were given the general name **antibodies**. The substances that generated or were recognized by antibodies were then called **antigens**. Antibodies, major histocompatibility complex (MHC) molecules (see Chapter 6), and T cell antigen receptors (see Chapter 7) are the three classes of molecules used by the adaptive immune system to bind antigens (Table 5-1). Of these three, antibodies recognize the widest range of antigenic structures, show the greatest ability to discriminate between different antigens, and bind antigens with the greatest strength. Antibodies represent the first of the three types of antigen-binding molecules to be discovered and characterized. Therefore, we begin our discussion of how the immune system specifically recognizes antigens by describing the structure and the antigen-binding properties of antibodies.

Antibodies can exist in two forms: membrane-bound antibodies on the surface of B lymphocytes function as receptors for antigen, and secreted antibodies that reside in the circulation, tissues, and mucosal sites neutralize toxins, prevent the entry and spread of pathogens, and eliminate microbes. The recognition of antigen by membrane-bound antibodies on naive B cells activates these lymphocytes and initiates a humoral immune response. Antibodies are also produced in a secreted form by antigen-stimulated B cells. In the effector phase of humoral immunity, these secreted antibodies bind to antigens and trigger several effector mechanisms that eliminate the antigens. The elimination of antigen often requires interaction of antibody with other components of the immune system, including molecules such as complement proteins and cells that include phagocytes and eosinophils. Antibody-mediated effector functions include neutralization of microbes or toxic microbial products; activation of the complement system; opsonization of pathogens for enhanced phagocytosis; antibody-dependent cell-mediated cytotoxicity, by which antibodies target infected cells for lysis by cells of the innate immune system; and antibody-mediated mast cell activation to expel parasitic worms. These functions of antibodies are described in detail in Chapter 12. In this chapter, we discuss the structural features of antibodies that underlie their antigen recognition and effector functions.

B lymphocytes are the only cells that synthesize antibody molecules. These cells express an integral membrane form of the antibody molecule on the cell surface, where it functions as the B cell antigen receptor. After exposure

TABLE 5–1 Features of Antigen Binding by the Antigen-Recognizing Molecules of the Immune System

Feature	Antigen-Binding Molecule		
	Immunoglobulin (Ig) 	T cell receptor (TCR)* 	MHC molecules* 
Antigen-binding site	Made up of three CDRs in V_H and three CDRs in V_L domains	Made up of three CDRs in V_α and three CDRs in V_β domains	Peptide-binding cleft made of α1 and α2 (class I) and α1 and β1 (class II) domains
Nature of antigen that may be bound	Macromolecules (proteins, lipids, polysaccharides) and small chemicals	Peptide-MHC complexes	Peptides
Nature of antigenic determinants recognized	Linear and conformational determinants of various macromolecules and chemicals	Linear determinants of peptides; only 2 or 3 amino acid residues of a peptide bound to an MHC molecule	Linear determinants of peptides; only some amino acid residues of a peptide
Affinity of antigen binding	K_d 10^{-7}-10^{-11} M; average affinity of Igs increases during immune response	K_d 10^{-5}-10^{-7} M	K_d 10^{-6}-10^{-9} M; extremely stable binding
On-rate and off-rate	Rapid on-rate, variable off-rate	Slow on-rate, slow off-rate	Slow on-rate, very slow off-rate

*The structures and functions of MHC and TCR molecules are discussed in Chapters 6 and 7, respectively.

CDR, complementarity-determining region; K_d, dissociation constant; MHC, major histocompatibility complex; (only class II molecules depicted); V_H, variable domain of heavy chain Ig; V_L, variable domain of light chain Ig.

to an antigen, B cells differentiate into plasma cells that secrete antibodies. Secreted forms of antibodies accumulate in the plasma (the fluid portion of the blood), in mucosal secretions, and in the interstitial fluid of tissues.

When blood or plasma forms a clot, antibodies remain in the residual fluid called **serum**. Serum lacks coagulation factors but otherwise contains all the proteins found in plasma. Any serum sample that contains detectable antibody molecules that bind to a particular antigen is commonly called an **antiserum**. The study of antibodies and their reactions with antigens is therefore classically called **serology**. The concentration of antibody molecules in serum specific for a particular antigen is often estimated by determining how many serial dilutions of the serum can be made before binding can no longer be observed; sera with a high concentration of antibody molecules specific for a particular antigen are said to have a high titer.

A healthy 70-kg adult human produces about 2 to 3 g of antibodies every day. Almost two thirds of this is an antibody called IgA, which is produced by activated B cells and plasma cells in the walls of the gastrointestinal and respiratory tracts and is actively transported across epithelial cells into the lumens of these tracts. The large amount of IgA produced reflects the large surface areas of these organs.

ANTIBODY STRUCTURE

An understanding of the structure of antibodies has provided important insights into their function. The analysis of antibody structure also paved the way to the eventual characterization of the genetic organization of antigen receptor genes in both B and T cells and the elucidation of the mechanisms of immune diversity, issues that will be considered in depth in Chapter 8.

Early studies of antibody structure relied on antibodies purified from the blood of individuals immunized with various antigens. It was not possible, using this approach, to define antibody structure precisely because serum contains a mixture of different antibodies produced by many clones of B lymphocytes that may each respond to different portions (epitopes) of an antigen (so-called polyclonal antibodies). A major breakthrough in obtaining antibodies whose structures could be elucidated was the discovery that patients with multiple myeloma, a monoclonal tumor of antibody-producing plasma cells, often have large amounts of biochemically identical antibody molecules (produced by the neoplastic clone) in their blood and urine. Immunologists found that these antibodies could be purified to homogeneity and analyzed. The recognition that myeloma cells make monoclonal immunoglobulins led to an extremely powerful technique for producing monoclonal antibodies, described later in the chapter. The availability of homogeneous populations of antibodies and monoclonal antibody–producing plasma cells permitted the detailed structural analysis and molecular cloning of the genes for individual antibody molecules, which remain some of the major advances in our understanding of the immune system.

General Features of Antibody Structure

Plasma or serum proteins are traditionally separated by solubility characteristics into albumins and globulins and

may be more extensively separated by migration in an electric field, a process called electrophoresis. Most antibodies are found in the third fastest migrating group of globulins, named **gamma globulins** for the third letter of the Greek alphabet. Another common name for antibody is **immunoglobulin (Ig)**, referring to the immunity-conferring portion of the gamma globulin fraction. The terms *immunoglobulin* and *antibody* are used interchangeably throughout this book.

All antibody molecules share the same basic structural characteristics but display remarkable variability in the regions that bind antigens. This variability of the antigen-binding regions accounts for the capacity of different antibodies to bind a tremendous number of structurally diverse antigens. There are believed to be a million or more different antibody molecules in every individual (theoretically, the antibody repertoire may include more than 10^{11} different antibodies), each with unique amino acid sequences in their antigen-combining sites. The effector functions and common physicochemical properties of antibodies are associated with the non–antigen-binding portions, which exhibit relatively few variations among different antibodies.

An antibody molecule has a symmetric core structure composed of two identical light chains and two identical heavy chains (Fig. 5-1). Both the light chains and the heavy chains contain a series of repeating, homologous units, each about 110 amino acid residues in length, that fold independently in a globular motif that is called an **Ig domain**. An Ig domain contains two layers of β-pleated sheet, each layer composed of three to five strands of antiparallel polypeptide chain (Fig. 5-2). The two layers are held together by a disulfide bridge, and adjacent strands of each β sheet are connected by short loops. It is the amino acids in some of these loops that are the most variable and critical for antigen recognition, as discussed later.

Both heavy chains and light chains consist of amino-terminal variable (V) regions that participate in antigen recognition and carboxyl-terminal constant (C) regions; the C regions of the heavy chains mediate effector functions. In the heavy chains, the V region is composed of

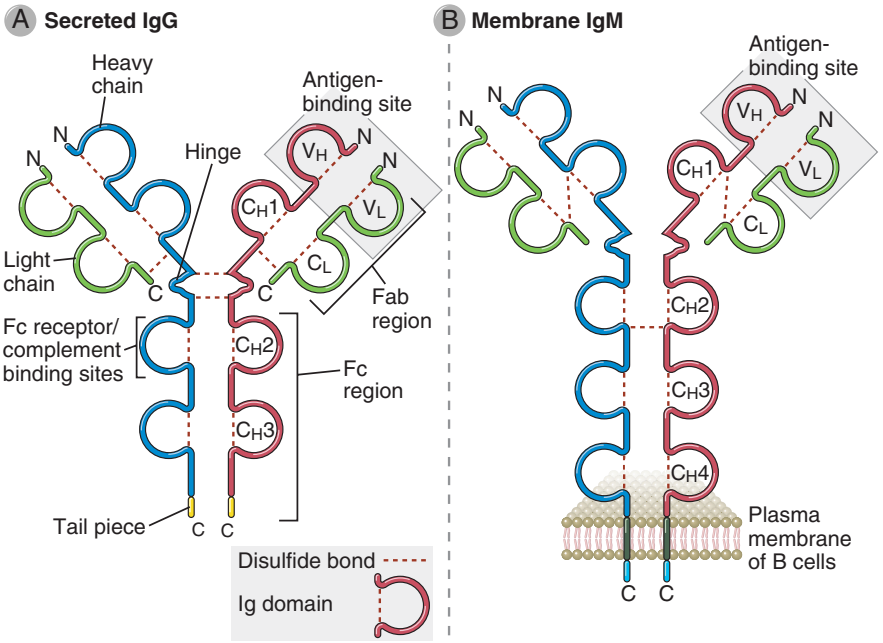


FIGURE 5–1 Structure of an antibody molecule. A, Schematic diagram of a secreted IgG molecule. The antigen-binding sites are formed by the juxtaposition of V_L and V_H domains. The heavy chain C regions end in tail pieces. The locations of complement- and Fc receptor–binding sites within the heavy chain constant regions are approximations. **B,** Schematic diagram of a membrane-bound IgM molecule on the surface of a B lymphocyte. The IgM molecule has one more C_H domain than IgG does, and the membrane form of the antibody has C-terminal transmembrane and cytoplasmic portions that anchor the molecule in the plasma membrane. **C,** Structure of a human IgG molecule as revealed by x-ray crystallography. In this ribbon diagram of a secreted IgG molecule, the heavy chains are colored blue and red, and the light chains are colored green; carbohydrates are shown in gray. *(Courtesy of Dr. Alex McPherson, University of California, Irvine.)*

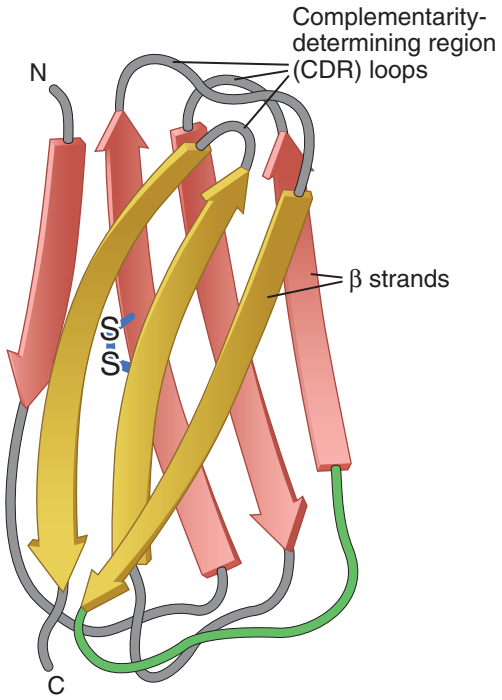


FIGURE 5–2 Structure of an Ig domain. Each domain is composed of two antiparallel arrays of β strands, colored yellow and red, to form two β-pleated sheets held together by a disulfide bond. A C domain is schematically depicted that contains three and four β strands in the two sheets. Note that the loops connect β strands that are sometimes adjacent in the same β-pleated sheet but that loops sometimes represent connections between the two different sheets that make up an Ig domain. Three loops in each variable domain contribute to antigen binding and are called complementarity determining regions (CDRs).

one Ig domain and the C region is composed of three or four Ig domains. Each light chain is made up of one V region Ig domain and one C region Ig domain. Variable regions are so named because they contain regions of variability in amino acid sequence that distinguish the antibodies made by one clone of B cells from the antibodies made by other clones. The V region of one heavy chain (V_H) and the adjoining V region of one light chain (V_L) form an antigen-binding site (see Fig. 5-1). Because the core structural unit of each antibody molecule contains two heavy chains and two light chains, every antibody molecule has at least two antigen-binding sites. The C region domains are separate from the antigen-binding site and do not participate in antigen recognition. The heavy chain C regions interact with other effector molecules and cells of the immune system and therefore mediate most of the biologic functions of antibodies. In addition, heavy chains exist in two forms that differ at their carboxyl-terminal ends: one form of the heavy chain anchors membrane-bound antibodies in the plasma membranes of B lymphocytes, and the other form is secreted when associated with Ig light chains. The C regions of light chains do not participate in effector functions and are not directly attached to cell membranes.

Heavy and light chains are covalently linked by disulfide bonds formed between cysteine residues in the carboxyl terminus of the light chain and the C_H1 domain of the heavy chain. Noncovalent interactions between the V_L and V_H domains and between the C_L and C_H1 domains may also contribute to the association of heavy and light chains. The two heavy chains of each antibody molecule are also covalently linked by disulfide bonds. In IgG antibodies, these bonds are formed between cysteine residues in the C_H2 regions, close to the region known as the hinge (see later). In other isotypes, the disulfide bonds may be in different locations. Noncovalent interactions (e.g., between the third C_H domains [C_H3]) may also contribute to heavy chain pairing.

The associations between the chains of antibody molecules and the functions of different regions of antibodies were first deduced from experiments done by Rodney Porter in which rabbit IgG was cleaved by proteolytic enzymes into fragments with distinct structural and functional properties. In IgG molecules, the unfolded "hinge" region between the C_H1 and C_H2 domains of the heavy chain is the segment most susceptible to proteolytic cleavage. If rabbit IgG is treated with the enzyme papain under conditions of limited proteolysis, the enzyme acts on the hinge region and cleaves the IgG into three separate pieces (Fig. 5-3A). Two of the pieces are identical to each other and consist of the complete light chain (V_L and C_L) associated with a V_H-C_H1 fragment of the heavy chain. These fragments retain the ability to bind antigen because each contains paired V_L and V_H domains, and they are called Fab (fragment, antigen binding). The third piece is composed of two identical, disulfide-linked peptides containing the heavy chain C_H2 and C_H3 domains. This piece of IgG has a propensity to self-associate and to crystallize into a lattice and is therefore called Fc (fragment, crystallizable). When pepsin (instead of papain) is used to cleave rabbit IgG under limiting conditions, proteolysis occurs distal to the hinge region, generating a $F(ab')_2$ antigen-binding fragment of IgG with the hinge and the interchain disulfide bonds intact (see Fig. 5-3B).

The results of limited papain or pepsin proteolysis of other isotypes besides IgG, or of IgGs of species other than the rabbit, do not always recapitulate the studies with rabbit IgG. However, the basic organization of the Ig molecule that Porter deduced from his experiments is common to all Ig molecules of all isotypes and of all species. In fact, these proteolysis experiments provided the first evidence that the antigen recognition functions and the effector functions of Ig molecules are spatially segregated.

Many other proteins in the immune system as well as numerous proteins that have nothing to do with immunity contain domains with an Ig fold structure—that is, two adjacent β-pleated sheets held together by a disulfide bridge. Although such a domain structure evolved long before the development of vertebrates, all molecules that contain this type of domain are said to belong to the **Ig superfamily**, and all the gene segments encoding the Ig domains of these molecules are believed to have evolved from one ancestral gene. Ig domains are classified as V-like or C-like on the basis of closest homology to either Ig V or Ig C domains. V domains are formed from a longer polypeptide than are C domains and contain two extra β

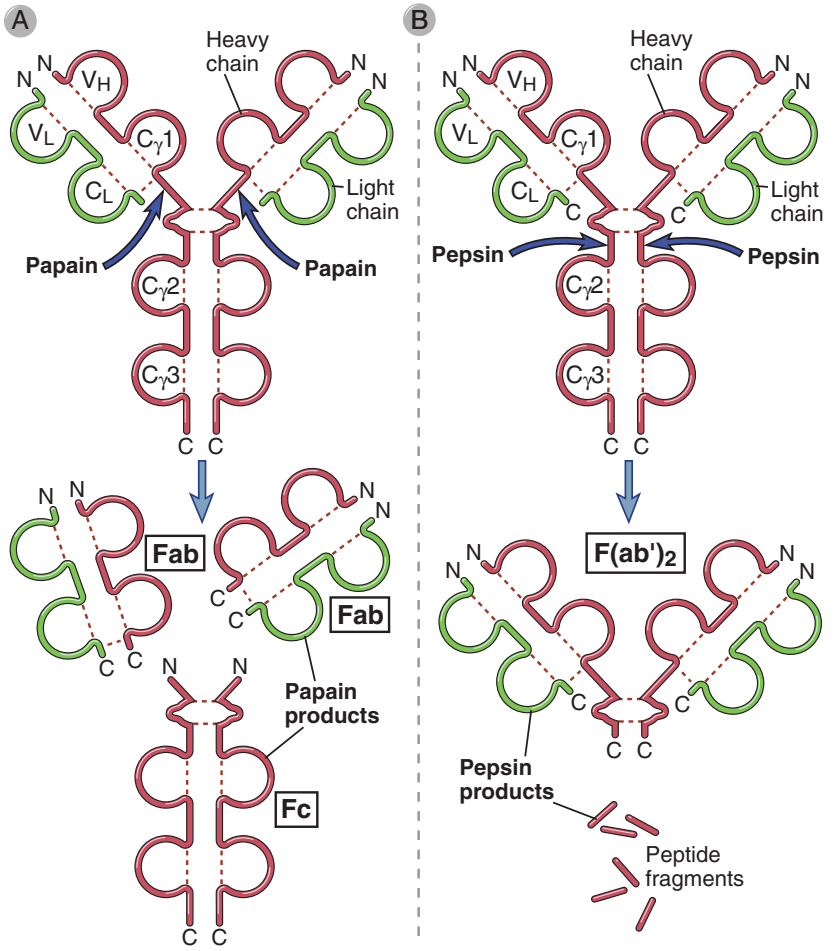


FIGURE 5–3 Proteolytic fragments of an IgG molecule. IgG molecules are cleaved by the enzymes papain **(A)** and pepsin **(B)** at the sites indicated by arrows. Papain digestion allows separation of two antigen-binding regions (the Fab fragments) from the portion of the IgG molecule that binds to complement and Fc receptors (the Fc fragment). Pepsin generates a single bivalent antigen-binding fragment, $F(ab')_2$.

strands within the β sheet sandwich. A third type of Ig domain, called C2 or H, has a length similar to C domains but has sequences typical of both V and C domains. Examples of Ig superfamily members of relevance in the immune system are depicted in Figure 5-4.

Structural Features of Antibody Variable Regions

Most of the sequence differences and variability among different antibodies are confined to three short stretches in the V region of the heavy chain and to three stretches in the V region of the light chain. These diverse stretches are known as **hypervariable segments**, and they correspond to three protruding loops connecting adjacent strands of the β sheets that make up the V domains of Ig heavy and light chain proteins (Fig. 5-5). The hypervariable regions are each about 10 amino acid residues long, and they are held in place by the more conserved framework sequences that make up the Ig domain of the V region. The genetic mechanisms leading to amino acid

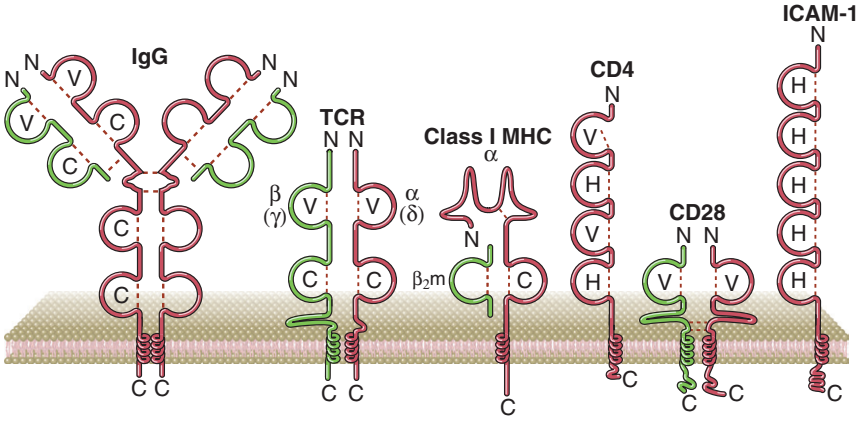


FIGURE 5–4 Examples of Ig superfamily proteins in the immune system. Examples included a membrane-bound IgG molecule, the T cell receptor, an MHC class I molecule, a coreceptor on T cells, the CD4 molecule, CD28, a costimulatory receptor on T cells, and the adhesion molecule ICAM-1.

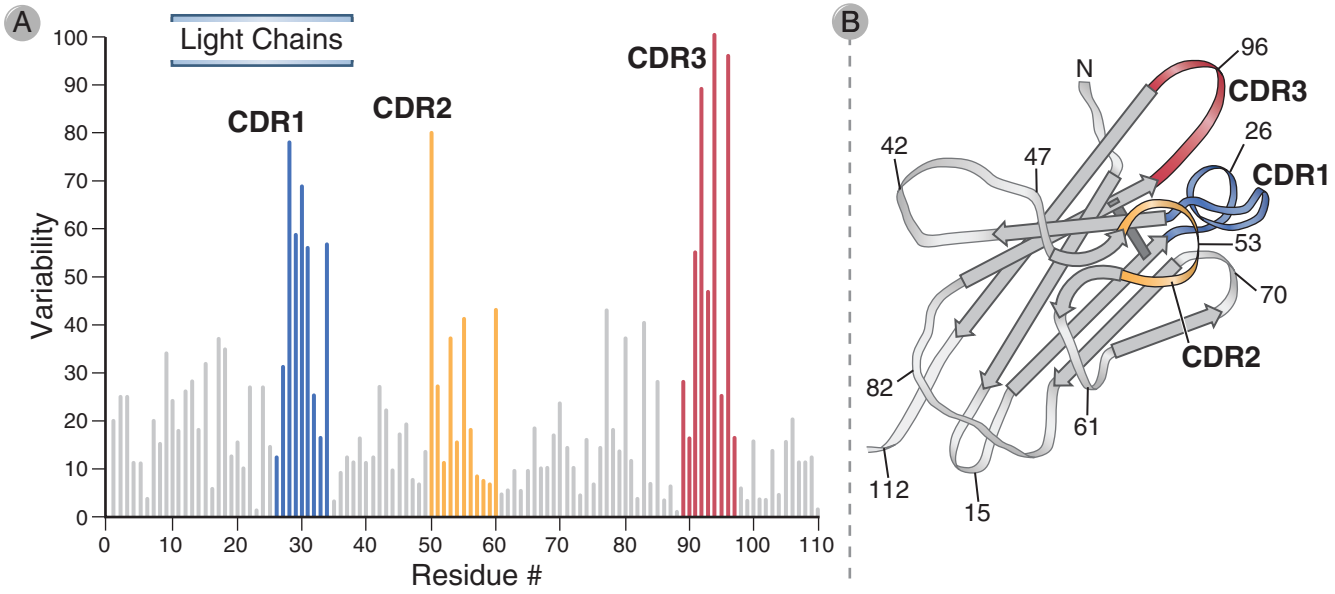


FIGURE 5–5 Hypervariable regions in Ig molecules. A, Kabat-Wu plot of amino acid variability in Ig molecules. The histograms depict the extent of variability, defined as the number of differences in each amino acid residue among various independently sequenced Ig light chains, plotted against amino acid residue number, measured from the amino terminus. This method of analysis, developed by Elvin Kabat and Tai Te Wu, indicates that the most variable residues are clustered in three "hypervariable" regions, colored in blue, yellow, and red, corresponding to CDR1, CDR2, and CDR3, respectively. Three hypervariable regions are also present in heavy chains. *(Courtesy of Dr. E. A. Kabat, Department of Microbiology, Columbia University College of Physicians and Surgeons, New York.)* **B,** Three-dimensional view of the hypervariable CDR loops in a light chain V domain. The V region of a light chain is shown with CDR1, CDR2, and CDR3 loops, colored in blue, yellow, and red, respectively. These loops correspond to the hypervariable regions in the variability plot in **A**. Heavy chain hypervariable regions (not shown) are also located in three loops, and all six loops are juxtaposed in the antibody molecule to form the antigen-binding surface (see Fig. 5-6).

variability are discussed in Chapter 8. In an antibody molecule, the three hypervariable regions of a V_L domain and the three hypervariable regions of a V_H domain are brought together to form an antigen-binding surface. The hypervariable loops can be thought to be like fingers protruding from each variable domain, three fingers from the heavy chain and three fingers from the light chain coming together to form an antigen-binding site (Fig. 5-6). Because these sequences form a surface that is complementary to the three-dimensional structure of the bound antigen, the hypervariable regions are also called **complementarity-determining regions (CDRs)**. Proceeding from either the V_L or the V_H amino terminus, these regions are called CDR1, CDR2, and CDR3. The CDR3s of both the V_H segment and the V_L segment are the most variable of the CDRs. As we will discuss in Chapter 8, there are special mechanisms for generating more sequence diversity in CDR3 than in CDR1 and CDR2. Sequence differences among the CDRs of different antibody molecules contribute to distinct interaction surfaces and therefore to specificities of individual antibodies. The ability of a V region to fold into an Ig domain is mostly determined by the conserved sequences of the framework regions adjacent to the CDRs. Confining the sequence variability to three short stretches allows the basic structure of all antibodies to be maintained despite the variability among different antibodies.

Antigen binding by antibody molecules is primarily a function of the hypervariable regions of V_H and V_L. Crystallographic analyses of antigen-antibody complexes show that the amino acid residues of the hypervariable regions form multiple contacts with bound antigen (see Fig. 5-6). The most extensive contact is with the third hypervariable region (CDR3), which is also the most variable of the three CDRs. However, antigen binding is not solely a function of the CDRs, and framework residues may also contact the antigen. Moreover, in the binding of some antigens, one or more of the CDRs may be outside the region of contact with antigen, thus not participating in antigen binding.

Structural Features of Antibody Constant Regions

Antibody molecules can be divided into distinct classes and subclasses on the basis of differences in the structure of their heavy chain C regions. The classes of antibody molecules are also called **isotypes** and are named IgA, IgD, IgE, IgG, and IgM (Table 5-2). In humans, IgA and IgG isotypes can be further subdivided into closely related subclasses, or subtypes, called IgA1 and IgA2 and IgG1, IgG2, IgG3, and IgG4. (Mice, which are often used in the study of immune responses, differ in that the IgG isotype is divided into the IgG1, IgG2a, IgG2b, and IgG3 subclasses; certain strains of mice, including C57BL/6, lack the gene for IgG2a but synthesize a related isotype called IgG2c). The heavy chain C regions of all antibody molecules of one isotype or subtype have essentially the same amino acid sequence. This sequence is different in antibodies of other isotypes or subtypes. Heavy chains are designated by the letter of the Greek alphabet corresponding to the isotype of the antibody: IgA1 contains α1 heavy chains; IgA2, α2; IgD, δ; IgE, ε; IgG1, γ1;

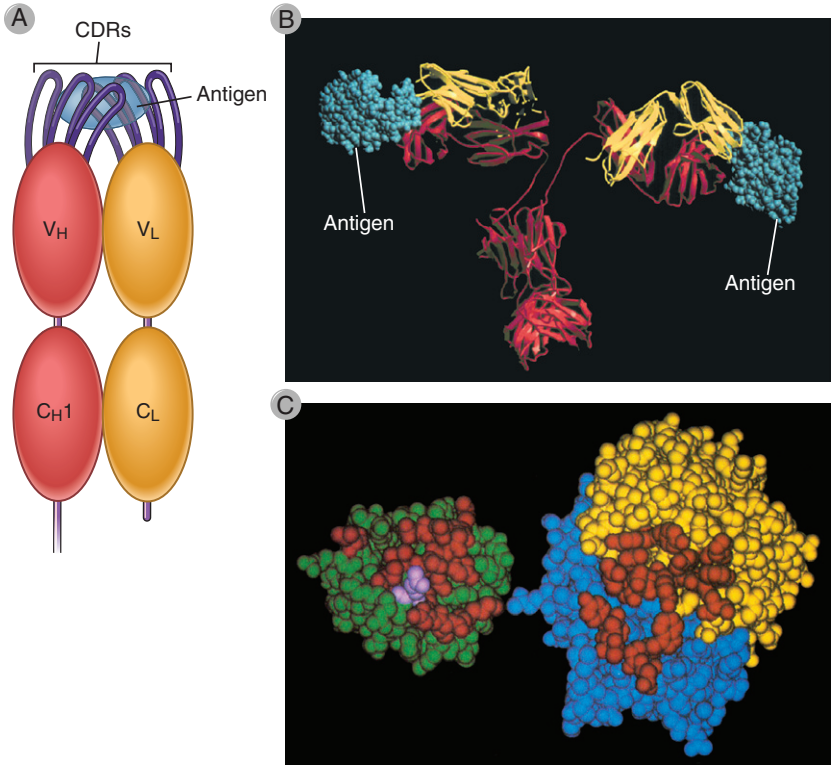


FIGURE 5–6 Binding of an antigen by an antibody. A, A schematic view of complementarity-determining regions (CDRs) generating an antigen-binding site. CDRs from the heavy chain and the light chain are loops that protrude from the surface of the two Ig V domains and in combination create an antigen-binding surface. **B,** This model of a globular protein antigen (hen egg lysozyme) bound to an antibody molecule shows how the antigen-binding site can accommodate soluble macromolecules in their native (folded) conformation. The heavy chains of the antibody are red, the light chains are yellow, and the antigen is blue. *(Courtesy of Dr. Dan Vaughn, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.)* **C,** A view of the interacting surfaces of hen egg lysozyme (in green) and a Fab fragment of a monoclonal anti–hen egg lysozyme antibody (V_H in blue and V_L in yellow) is provided. The residues of hen egg lysozyme and of the Fab fragment that interact with one another are shown in red. A critical glutamine residue on lysozyme (in magenta) fits into a "cleft" in the antibody. *(Reprinted with permission from Amit AG, RA Mariuzza, SE Phillips, and RJ Poljak. Three dimensional structure of an antigen antibody complex at 2.8A resolution. Science 233, 747-753, 1986. Copyright 1986 AAAS.)*

IgG2, γ2; IgG3, γ3; IgG4, γ4; and IgM, μ. In human IgM and IgE antibodies, the C regions contain four tandem Ig domains (see Fig. 5-1). The C regions of IgG, IgA, and IgD contain only three Ig domains. These domains are generically designated C_H domains and are numbered sequentially from amino terminus to carboxyl terminus (e.g., C_H1, C_H2, and so on). In each isotype, these regions may be designated more specifically (e.g., Cγ1, Cγ2 in IgG).

Different isotypes and subtypes of antibodies perform different effector functions. The reason for this is that most of the effector functions of antibodies are mediated by the binding of heavy chain C regions to Fc receptors on different cells, such as phagocytes, NK cells, and mast cells, and to plasma proteins, such as complement proteins. Antibody isotypes and subtypes differ in their C regions and therefore in what they bind to and what effector functions they perform. The effector functions mediated by each antibody isotype are listed in Table 5-2 and are discussed in more detail later in this chapter and in Chapter 12.

Antibody molecules are flexible, permitting them to bind to different arrays of antigens. Every antibody contains at least two antigen-binding sites, each formed by a pair of V_H and V_L domains. Many Ig molecules can orient these binding sites so that two antigen molecules on a planar (e.g., cell) surface may be engaged at once (Fig. 5-7). This flexibility is conferred, in large part, by a **hinge region** located between C_H1 and C_H2 in certain isotypes. The hinge region varies in length from 10 to more than 60 amino acid residues in different isotypes. Portions of this sequence assume an unfolded and flexible conformation, permitting molecular motion between the C_H1 and C_H2 domains. Some of the greatest differences between the constant regions of the IgG subclasses are concentrated in the hinge. This leads to different overall shapes of the IgG subtypes. In addition, some flexibility of antibody molecules is due to the ability of each V_H domain to rotate with respect to the adjacent C_H1 domain.

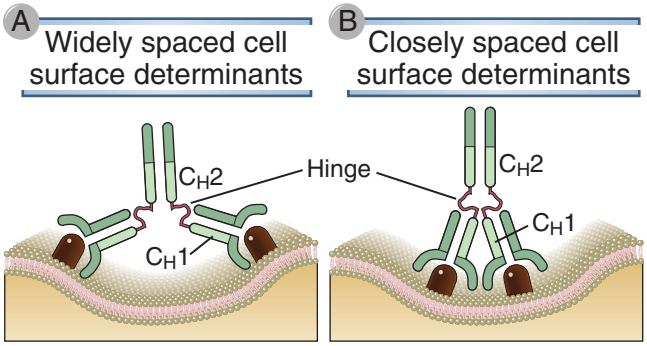
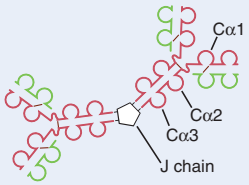
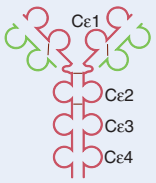
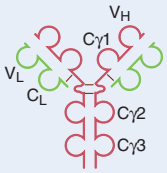
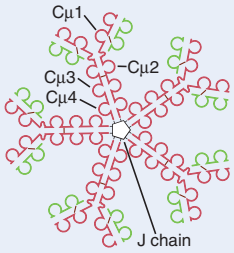


FIGURE 5–7 Flexibility of antibody molecules. The two antigen-binding sites of an Ig monomer can simultaneously bind to two determinants separated by varying distances. In **A,** an Ig molecule is depicted binding to two widely spaced determinants on a cell surface, and in **B,** the same antibody is binding to two determinants that are close together. This flexibility is mainly due to the hinge regions located between the C_H1 and C_H2 domains, which permit independent movement of antigen-binding sites relative to the rest of the molecule.

There are two classes, or isotypes, of light chains, called κ and λ, that are distinguished by their carboxyl-terminal constant (C) regions. An antibody molecule has either two identical κ light chains or two identical λ light chains.

TABLE 5–2 Human Antibody Isotypes

Isotope of Antibody	Subtypes (H Chain)	Serum Concentration (mg/mL)	Serum Half-life (days)	Secreted Form		Functions
IgA	IgA1,2 (α1 or α2)	3.5	6	IgA (dimer) Monomer, dimer, trimer		Mucosal immunity
IgD	None (δ)	Trace	3	None		Naive B cell antigen receptor
IgE	None (ε)	0.05	2	IgE Monomer		Defense against helminthic parasites, immediate hypersensitivity
IgG	IgG1-4 (γ1, γ2, γ3, or γ4)	13.5	23	IgG1 Monomer		Opsonization, complement activation, antibody-dependent cell-mediated cytotoxicity, neonatal immunity, feedback inhibition of B cells
IgM	None (μ)	1.5	5	IgM Pentamer		Naive B cell antigen receptor, complement activation

The effector functions of antibodies are discussed in detail in Chapter 12.

In humans, about 60% of antibody molecules have κ light chains and about 40% have λ light chains. Marked changes in this ratio can occur in patients with B cell tumors because the many neoplastic cells, being derived from one B cell clone, produce a single species of antibody molecules, all with the same light chain. In fact, a skewed ratio of κ-bearing cells to λ-bearing cells is often used clinically in the diagnosis of B cell lymphomas. In mice, κ-containing antibodies are about 10 times more abundant than λ-containing antibodies. Unlike in heavy chain isotypes, there are no known differences in function between κ-containing antibodies and λ-containing antibodies.

Secreted and membrane-associated antibodies differ in the amino acid sequence of the carboxyl-terminal end of the heavy chain C region. In the secreted form, found in blood and other extracellular fluids, the carboxyl-terminal portion is hydrophilic. The membrane-bound form of antibody contains a carboxyl-terminal stretch that includes a hydrophobic α-helical transmembrane anchor region followed by an intracellular juxtamembrane positively charged stretch that helps anchor the protein in the membrane (Fig. 5-8). In membrane IgM and IgD molecules, the cytoplasmic portion of the heavy chain is short, only three amino acid residues in length; in membrane IgG and IgE molecules, it is somewhat longer, up to 30 amino acid residues in length.

Secreted IgG and IgE and all membrane Ig molecules, regardless of isotype, are monomeric with respect to the basic antibody structural unit (i.e., they contain two heavy chains and two light chains). In contrast, the secreted forms of IgM and IgA form multimeric complexes in which two or more of the four-chain core antibody structural units are covalently joined. IgM may be secreted as pentamers and hexamers of the core four-chain structure, whereas IgA is often secreted as a dimer. These complexes are formed by interactions between regions, called tail pieces, that are located at the carboxyl-terminal ends of the secreted forms of μ and α heavy chains (see Table 5-2). Multimeric IgM and IgA molecules also contain an additional 15-kD polypeptide called the joining (J) chain, which is disulfide bonded to the tail pieces and serves to

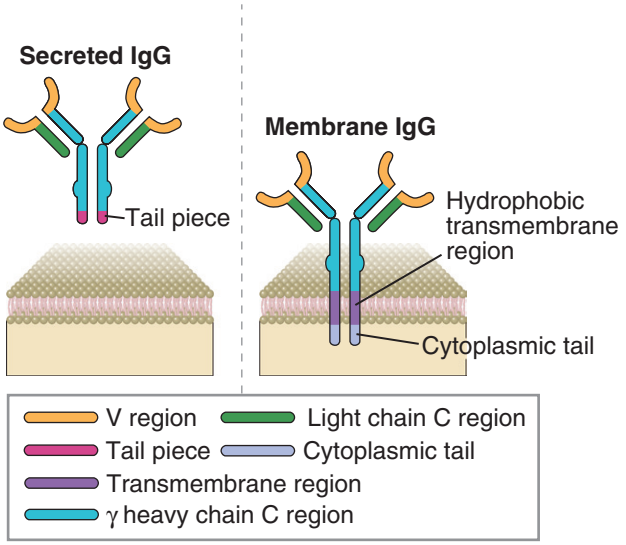


FIGURE 5–8 Membrane and secreted forms of Ig heavy chains. The membrane forms of the Ig heavy chains, but not the secreted forms, contain transmembrane regions made up of hydrophobic amino acid residues and cytoplasmic domains that differ significantly among the different isotypes. The cytoplasmic portion of the membrane form of the μ chain contains only three residues, whereas the cytoplasmic region of IgG heavy chains contains 20 to 30 residues. The secreted forms of the antibodies end in C-terminal tail pieces, which also differ among isotypes: μ has a long tail piece (21 residues) that is involved in pentamer formation, whereas IgGs have a short tail piece (3 residues).

stabilize the multimeric complexes and to transport multimers across epithelia from the basolateral to the luminal end. As we shall see later, multimeric forms of antibodies bind to antigens more avidly than monomeric forms do, even if both types of antibody contain Fab fragments that individually bind the antigen equally well.

Antibodies of different species differ from one another in the C regions and in framework parts of the V regions. Therefore, when Ig molecules from one species are introduced into another (e.g., horse serum antibodies or mouse monoclonal antibodies injected into humans), the recipient mounts an immune response and makes antibodies largely against the C regions of the introduced Ig. The response often creates an illness called serum sickness (see Chapter 18) and thus greatly limits the ability to treat individuals with antibodies produced in other species. Much effort has been devoted to overcoming this problem with monoclonal antibodies, and this issue is discussed in more depth later. Smaller sequence differences are present in antibodies from different individuals even of the same species, reflecting inherited polymorphisms in the genes encoding the C regions of Ig heavy and light chains. When a polymorphic variant found in some individuals of a species can be recognized by an antibody, the variants are referred to as **allotypes**, and the antibody that recognizes an allotypic determinant is called an anti-allotypic antibody. The differences between antibody V regions map to CDRs and constitute the **idiotypes** of antibodies. An antibody that recognizes some aspect of the CDRs of another antibody is therefore called an anti-idiotypic antibody. There have been interesting theories that individuals produce anti-idiotypic antibodies against their own antibodies that control immune responses, but there is little evidence to support the importance of this potential mechanism of immune regulation.

Monoclonal Antibodies

A tumor of plasma cells (myeloma or plasmacytoma) is monoclonal and therefore produces antibodies of a single specificity. In most cases, the specificity of the tumor-derived antibody is not known, so the antibody cannot be used to specifically detect or bind to molecules of interest. However, the discovery of monoclonal antibodies produced by these tumors led to the idea that it may be possible to produce similar monoclonal antibodies of any desired specificity by immortalizing individual antibody-secreting cells from an animal immunized with a known antigen. A technique to accomplish this was described by Georges Kohler and Cesar Milstein in 1975 and has proved to be one of the most valuable advances in all of scientific research and clinical medicine. The method relies on fusing B cells from an immunized animal (typically a mouse) with a myeloma cell line and growing the cells under conditions in which the unfused normal and tumor cells cannot survive (Fig. 5-9). The resultant fused cells that grow out are called hybridomas; each hybridoma makes only one Ig. The antibodies secreted by many hybridoma clones are screened for binding to the antigen of interest, and this single clone with the desired specificity is selected and expanded. The products of these individual clones are **monoclonal antibodies** that are each specific for a single epitope on the antigen or antigen mixture used to identify antibody-secreting clones.

Monoclonal antibodies have many practical applications in research and in medical diagnosis and therapy. Some of their common applications include the following:

- Identification of phenotypic markers unique to particular cell types. The basis for the modern classification of lymphocytes and other leukocytes is the recognition of individual cell populations by specific monoclonal antibodies. These antibodies have been used to define clusters of differentiation (CD) markers for various cell types (see Chapter 2).
- Immunodiagnosis. The diagnosis of many infectious and systemic diseases relies on the detection of particular antigens or antibodies in the circulation or in tissues by use of monoclonal antibodies in immunoassays (see Appendix IV).
- Tumor detection. Tumor-specific monoclonal antibodies are used for detection of tumors by imaging techniques and by staining tissues with labeled antibodies.
- Therapy. Advances in medical research have led to the identification of cells and molecules that are involved in the pathogenesis of many diseases. Monoclonal antibodies, because of their exquisite specificity, provide a means of targeting these cells and molecules. A number of monoclonal antibodies are used therapeutically today (Table 5-3). Some examples include

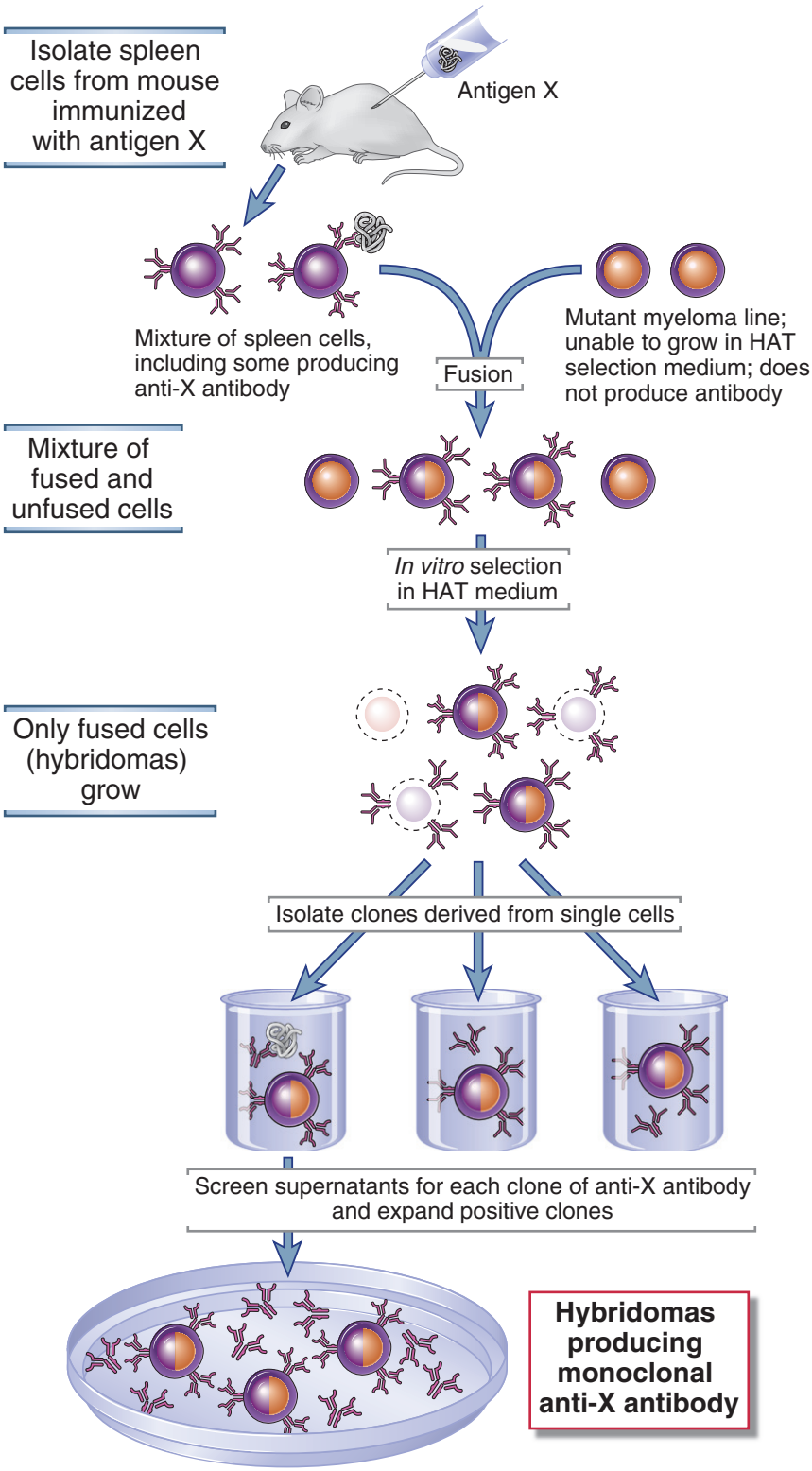


FIGURE 5–9 The generation of monoclonal antibodies. In this procedure, spleen cells from a mouse that has been immunized with a known antigen or mixture of antigens are fused with an enzyme-deficient partner myeloma cell line, with use of chemicals such as polyethylene glycol that can facilitate the fusion of plasma membranes and the formation of hybrid cells that retain many chromosomes from both fusion partners. The myeloma partner used is one that does not secrete its own Igs. These hybrid cells are then placed in a selection medium that permits the survival of only immortalized hybrids; these hybrid cells are then grown as single cell clones and tested for the secretion of the antibody of interest. The selection medium includes hypoxanthine, aminopterin, and thymidine and is therefore called HAT medium. There are two pathways of purine synthesis in most cells, a de novo pathway that needs tetrahydrofolate and a salvage pathway that uses the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRT). Myeloma cells that lack HGPRT are used as fusion partners, and they normally survive using de novo purine synthesis. In the presence of aminopterin, tetrahydrofolate is not made, resulting in a defect in de novo purine synthesis and also a specific defect in pyrimidine biosynthesis, namely, in generating TMP from dUMP. Hybrid cells receive HGPRT from the splenocytes and have the capacity for uncontrolled proliferation from the myeloma partner; if they are given hypoxanthine and thymidine, these cells can make DNA in the absence of tetrahydrofolate. As a result, only hybrid cells survive in HAT medium.

TABLE 5–3 Monoclonal Antibodies of Therapeutic Significance

Target	Effect	Diseases
CD20	B cell depletion	Rheumatoid arthritis, multiple sclerosis, other autoimmune diseases
VEGF	Blocking of tumor angiogenesis	Breast cancer, colon cancer
HER2/Neu	Depletion of tumor cells with HER2 amplification	Breast cancer
TNF	Inhibition of T cell–mediated inflammation	Rheumatoid arthritis, Crohn's disease

antibodies against the cytokine tumor necrosis factor (TNF) used to treat rheumatoid arthritis and other inflammatory diseases, antibodies against CD20 for the treatment of B cell leukemias and for depleting B cells in certain autoimmune disorders, antibodies against the type 2 epidermal growth factor receptor to target breast cancer cells, antibodies against vascular endothelial growth factor (a cytokine that promotes angiogenesis) in patients with colon cancer, and so on.

- Functional analysis of cell surface and secreted molecules. In biologic research, monoclonal antibodies that bind to cell surface molecules and either stimulate or inhibit particular cellular functions are invaluable tools for defining the functions of surface molecules, including receptors for antigens. Monoclonal antibodies are also widely used to purify selected cell populations from complex mixtures to facilitate the study of the properties and functions of these cells.

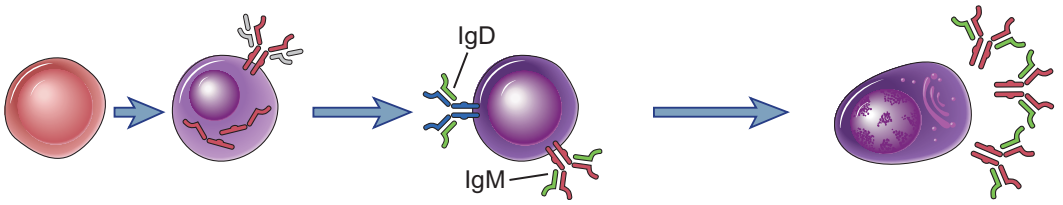
One of the limitations of monoclonal antibodies for therapy is that these antibodies are most easily produced by immunizing mice, but patients treated with mouse monoclonal antibodies may make antibodies against the mouse Ig, called a human anti-mouse antibody (HAMA) response. These anti-Ig antibodies eliminate the injected monoclonal antibody and can also cause serum sickness. Genetic engineering techniques have been used to expand the usefulness of monoclonal antibodies. The complementary DNAs (cDNAs) that encode the polypeptide chains of a monoclonal antibody can be isolated from a hybridoma, and these genes can be manipulated in vitro. As discussed before, only small portions of the antibody molecule are responsible for binding to antigen; the remainder of the antibody molecule can be thought of as a framework. This structural organization allows the DNA segments encoding the antigen-binding sites from a mouse monoclonal antibody to be "stitched" into a cDNA encoding a human myeloma protein, creating a hybrid gene. When it is expressed, the resultant hybrid protein, which retains the antigen specificity of the original mouse monoclonal but has the core structure of a human Ig, is referred to as a humanized antibody. Humanized antibodies are far less likely than mouse monoclonals to appear "foreign" in humans and to induce anti-antibody responses.

SYNTHESIS, ASSEMBLY, AND EXPRESSION OF Ig MOLECULES

Immunoglobulin heavy and light chains, like most secreted and membrane proteins, are synthesized on membrane-bound ribosomes in the rough endoplasmic reticulum. The protein is translocated into the endoplasmic reticulum, and Ig heavy chains are N-glycosylated during the translocation process. The proper folding of Ig heavy chains and their assembly with light chains are regulated by proteins resident in the endoplasmic reticulum called chaperones. These proteins, which include calnexin and a molecule called BiP (binding protein), bind to newly synthesized Ig polypeptides and ensure that they are retained or targeted for degradation unless they fold properly and assemble into complete Ig molecules. The covalent association of heavy and light chains, stabilized by the formation of disulfide bonds, is part of the assembly process and also occurs in the endoplasmic reticulum. After assembly, the Ig molecules are released from chaperones, transported into the cisternae of the Golgi complex where carbohydrates are modified, and then routed to the plasma membrane in vesicles. Antibodies of the membrane form are anchored in the plasma membrane, and the secreted form is transported out of the cell.

The maturation of B cells from bone marrow progenitors is accompanied by specific changes in Ig gene expression, resulting in the production of Ig molecules in different forms (Fig. 5-10). The earliest cell in the B lymphocyte lineage that produces Ig polypeptides, called the pre-B cell, synthesizes the membrane form of the μ heavy chain. These μ chains associate with proteins called surrogate light chains to form the pre-B cell receptor, and a small proportion of the synthesized pre-B cell receptor is expressed on the cell surface. Immature and mature B cells produce κ or λ light chains, which associate with μ proteins to form IgM molecules. Mature B cells express membrane forms of IgM and IgD (the μ and δ heavy chains associated with κ or λ light chains). These membrane Ig receptors serve as cell surface receptors that recognize antigens and initiate the process of B cell activation. The pre-B cell receptor and the B cell antigen receptor are noncovalently associated with two other integral membrane proteins, Igα and Igβ, which serve signaling functions and are essential for surface expression of IgM and IgD. The molecular and cellular events in B cell maturation underlying these changes in antibody expression are discussed in detail in Chapter 8.

When mature B lymphocytes are activated by antigens and other stimuli, the cells differentiate into antibody-secreting cells. This process is also accompanied by changes in the pattern of Ig production. One such change is the increased production of the secreted form of Ig relative to the membrane form. This alteration occurs at the level of post-transcriptional processing and will be discussed in Chapter 11. The second change is the expression of Ig heavy chain isotypes other than IgM and IgD. This process, called heavy chain isotype (or class) switching, is described later in this chapter and in more detail in Chapter 11, when we discuss B cell activation.



Stage of maturation	Stem cell	Pre-B cell	Immature B cell	Mature B cell	Activated B cell	Antibody-secreting cell
Pattern of immunoglobulin production	None	Cytoplasmic μ heavy chain and pre-B receptor	Membrane IgM	Membrane IgM, IgD	Low rate Ig secretion; heavy chain isotype switching; affinity maturation	High rate Ig secretion; reduced membrane Ig

FIGURE 5–10 Ig expression during B lymphocyte maturation. Stages in B lymphocyte maturation are shown with associated changes in the production of Ig heavy and light chains. IgM heavy chains are shown in red, IgD heavy chains in blue, and light chains in green. The molecular events accompanying these changes are discussed in Chapters 8 and 11.

Half-life of Antibodies

Different antibody isotypes have very different half-lives in circulation. IgE has a very short half-life of about 2 days in the circulation (although cell-bound IgE associated with the high-affinity IgE receptor on mast cells has a very long half-life; see Chapter 19). Circulating IgA has a half-life of about 3 days, and circulating IgM has a half-life of about 4 days. In contrast, circulating IgG molecules have a half-life of about 21 to 28 days.

The long half-life of IgG is attributed to its ability to bind to a specific Fc receptor called the **neonatal Fc receptor** (FcRn), which is also involved in the transport of IgG from the maternal circulation across the placental barrier as well as the transfer of maternal IgG across the intestine in neonates. FcRn structurally resembles MHC class I molecules but lacks a peptide-binding groove, and in specific cell types, such as the placenta and the neonatal intestine, it transports IgG molecules across cells without targeting them to lysosomes. In adult vertebrates, FcRn is found on the surface of endothelial cells (and other cell types) and binds to micropinocytosed IgG in acidic endosomes. FcRn does not target bound IgG to lysosomes but sequesters the IgG for a while and then returns it to the circulation, when it recycles to the cell surface and releases the IgG at neutral pH (Fig. 5-11). This intracellular sequestration of IgG for significant periods prevents it from being targeted for degradation

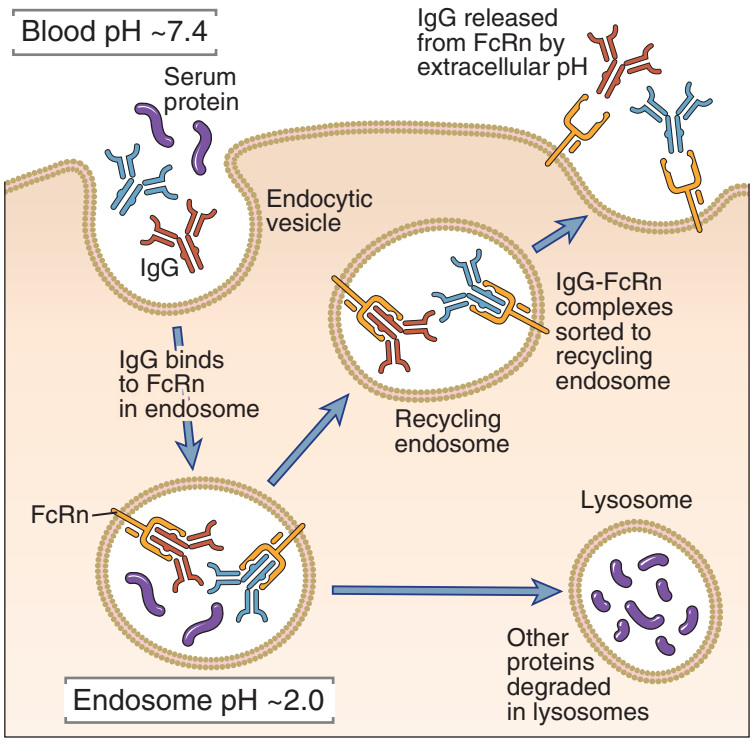


FIGURE 5–11 FcRn contributes to the long half-life of IgG molecules. Micropinocytosed IgG molecules in endothelial cells bind the FcRn, an IgG-binding receptor in the acidic environment of endosomes. In endothelial cells, FcRn sequesters IgG molecules and releases them when vesicles fuse with the cell surface, exposing FcRn-IgG complexes to neutral pH.

as rapidly as most other serum proteins, including other antibody isotypes, and as a result, IgG has a relatively long half-life. This long half-life of IgG has been used to provide a therapeutic advantage for certain infused proteins by producing fusion proteins containing the biologically active part of the protein and the Fc portion of IgG. One therapeutically useful fusion protein is TNFR-Ig, which consists of the extracellular domain of the type II TNF receptor fused to an IgG Fc domain; it is used to treat certain autoimmune disorders, such as rheumatoid arthritis and psoriasis, in which it blocks the inflammatory actions of TNF. Another therapeutically useful fusion protein is CTLA4-Ig, which contains the extracellular domain of the CTLA-4 inhibitory receptor fused to the Fc portion of human IgG; it has also been used in the treatment of rheumatoid arthritis and may serve more broadly as an immunosuppressive therapeutic (see Fig. 9-7, Chapter 9).

ANTIBODY BINDING OF ANTIGENS

All the functions of antibodies are dependent on their ability to specifically bind antigens. We next consider the nature of antigens and how they are recognized by antibodies.

Features of Biologic Antigens

An antigen is any substance that may be specifically bound by an antibody molecule or T cell receptor. Antibodies can recognize as antigens almost every kind of biologic molecule, including simple intermediary metabolites, sugars, lipids, autacoids, and hormones, as well as macromolecules such as complex carbohydrates, phospholipids, nucleic acids, and proteins. This is in contrast to T cells, which mainly recognize peptides (see Chapter 6).

Although all antigens are recognized by specific lymphocytes or by antibodies, only some antigens are capable of activating lymphocytes. Molecules that stimulate immune responses are called **immunogens**. Only macromolecules are capable of stimulating B lymphocytes to initiate humoral immune responses because B cell activation requires either the bringing together (cross-linking) of multiple antigen receptors or protein antigens to elicit T cell help. Small chemicals, such as dinitrophenol, may bind to antibodies and are therefore antigens but cannot activate B cells on their own (i.e., they are not immunogenic). To generate antibodies specific for such small chemicals, immunologists commonly attach multiple copies of the small molecules to a protein or polysaccharide before immunization. In these cases, the small chemical is called a **hapten**, and the large molecule to which it is conjugated is called a **carrier**. The hapten-carrier complex, unlike free hapten, can act as an immunogen (see Chapter 11).

Macromolecules, such as proteins, polysaccharides, and nucleic acids, are usually much bigger than the antigen-binding region of an antibody molecule (see Fig. 5-6). Therefore, any antibody binds to only a portion of the macromolecule, which is called a **determinant** or an **epitope**. These two words are synonymous and are used interchangeably throughout this book. Macromolecules typically contain multiple determinants, some of which may be repeated and each of which, by definition, can be bound by an antibody. The presence of multiple identical determinants in an antigen is referred to as **polyvalency** or **multivalency**. Most globular proteins do not contain multiple identical epitopes and are not polyvalent, unless they are in aggregates. In the case of polysaccharides and nucleic acids, many identical epitopes may be regularly spaced, and the molecules are said to be polyvalent. Cell surfaces, including microbes, often display polyvalent arrays of protein or carbohydrate antigenic determinants. Polyvalent antigens can induce clustering of the B cell receptor and thus initiate the process of B cell activation (see Chapter 7).

The spatial arrangement of different epitopes on a single protein molecule may influence the binding of antibodies in several ways. When determinants are well separated, two or more antibody molecules can be bound to the same protein antigen without influencing each other; such determinants are said to be nonoverlapping. When two determinants are close to one another, the binding of antibody to the first determinant may cause steric interference with the binding of antibody to the second; such determinants are said to be overlapping. In rarer cases, binding of one antibody may cause a conformational change in the structure of the antigen, positively or negatively influencing the binding of a second antibody at another site on the protein by means other than steric hindrance. Such interactions are called allosteric effects.

Any available shape or surface on a molecule that may be recognized by an antibody constitutes an antigenic determinant or epitope. Antigenic determinants may be delineated on any type of compound, including but not restricted to carbohydrates, proteins, lipids, and nucleic acids. In the case of proteins, the formation of some determinants depends only on the primary structure, and the formation of other determinants reflects tertiary structure, or conformation (shape) (Fig. 5-12). Epitopes formed by several adjacent amino acid residues are called **linear determinants**. The antigen-binding site of an antibody can usually accommodate a linear determinant made up of about six amino acids. If linear determinants appear on the external surface or in a region of extended conformation in the native folded protein, they may be accessible to antibodies. More often, linear determinants may be inaccessible in the native conformation and appear only when the protein is denatured. In contrast, **conformational determinants** are formed by amino acid residues that are not in a sequence but become spatially juxtaposed in the folded protein. Antibodies specific for certain linear determinants and antibodies specific for conformational determinants can be used to ascertain whether a protein is denatured or in its native conformation, respectively. Proteins may be subjected to modifications such as glycosylation, phosphorylation, ubiquitination, acetylation, and proteolysis. These modifications, by altering the structure of the protein, can produce new epitopes. Such epitopes are called **neoantigenic determinants**, and they too may be recognized by specific antibodies.

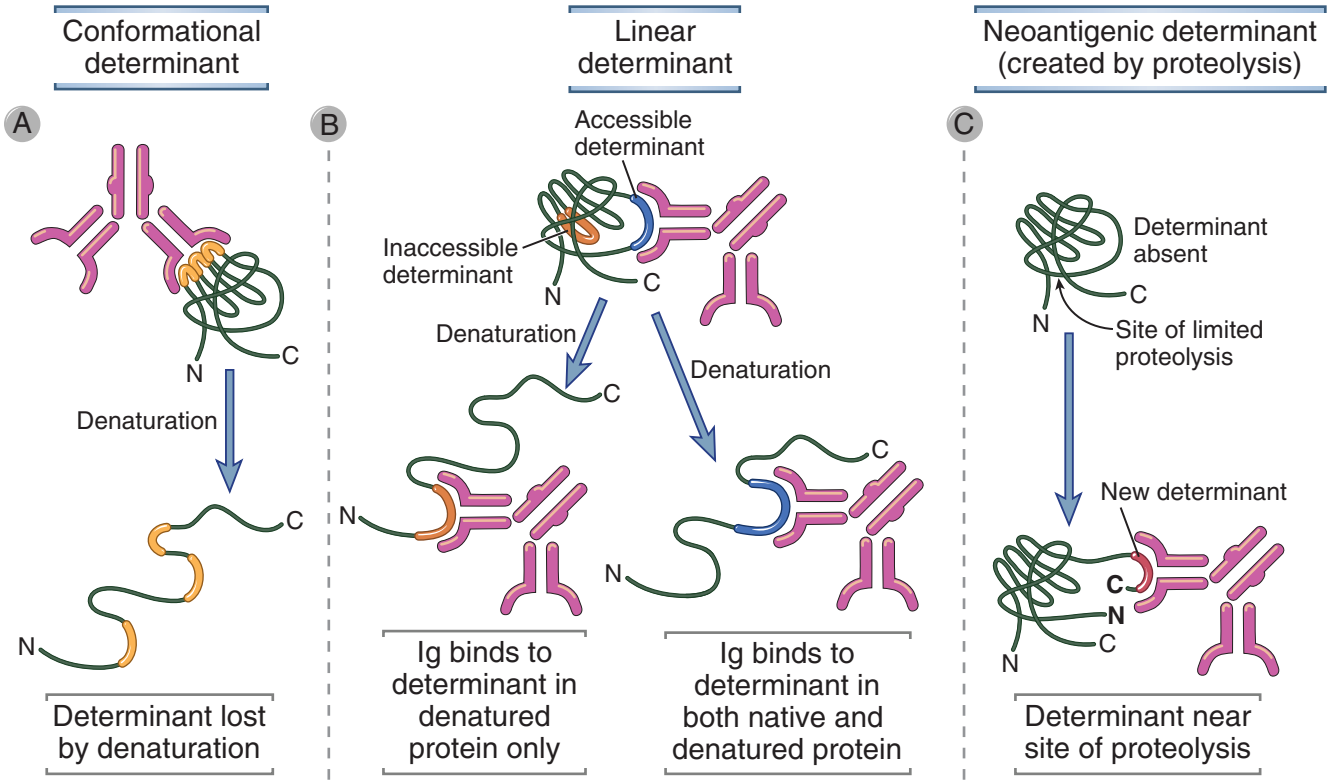


FIGURE 5–12 The nature of antigenic determinants. Antigenic determinants (shown in orange, red, and blue) may depend on protein folding (conformation) as well as on primary structure. Some determinants are accessible in native proteins and are lost on denaturation **(A),** whereas others are exposed only on protein unfolding **(B)**. Neodeterminants arise from postsynthetic modifications such as peptide bond cleavage **(C)**.

Structural and Chemical Basis of Antigen Binding

The antigen-binding sites of many antibodies are planar surfaces that can accommodate conformational epitopes of macromolecules, allowing the antibodies to bind large macromolecules (see Fig. 5-6). The six CDRs, three from the heavy chain and three from the light chain, spread out to form a broad surface. Similar broad binding surfaces are characteristic of the binding sites of T cell receptors. In contrast, MHC molecules contain antigen-binding clefts that bind small peptides. In a number of antibodies specific for small molecules, such as monosaccharides and drugs, the antigen is bound in a cleft generated by the close apposition of CDRs from the V_L and V_H domains.

The recognition of antigen by antibody involves noncovalent, reversible binding. Various types of noncovalent interactions may contribute to antibody binding of antigen, including electrostatic forces, hydrogen bonds, van der Waals forces, and hydrophobic interactions. The relative importance of each of these depends on the structures of the binding site of the individual antibody and of the antigenic determinant. The strength of the binding between a single combining site of an antibody and an epitope of an antigen is called the **affinity** of the antibody. The affinity is commonly represented by a dissociation constant (K_d), which indicates how easy it is to separate an antigen-antibody complex into its constituents. A smaller K_d indicates a stronger or higher affinity interaction because a lower concentration of antigen and of antibody is required for complex formation. The K_d of antibodies produced in typical humoral immune responses usually varies from about 10^{-7} M to 10^{-11} M. Serum from an immunized individual will contain a mixture of antibodies with different affinities for the antigen, depending primarily on the amino acid sequences of the CDRs.

Because the hinge region of antibodies gives them flexibility, a single antibody may attach to a single multivalent antigen by more than one binding site. For IgG or IgE, this attachment can involve, at most, two binding sites, one on each Fab. For pentameric IgM, however, a single antibody may bind at up to 10 different sites (Fig. 5-13). Polyvalent antigens will have more than one copy of a particular determinant. Although the affinity of any one antigen-binding site will be the same for each epitope of a polyvalent antigen, the strength of attachment of the antibody to the antigen must take into account binding of all the sites to all the available epitopes. This overall strength of attachment is called the **avidity** and is much greater than the affinity of any one antigen-binding site. Thus, a low-affinity IgM molecule can still bind tightly to a polyvalent antigen because many low-affinity interactions (up to 10 per IgM molecule) can produce a high-avidity interaction. This is because an antibody with multiple binding sites will have at least one binding site physically bound to the antigen for a longer time than an antibody with just two binding sites; the latter is more likely to "fall off" the antigen and therefore has less avidity for the antigen, even though each Fab fragment

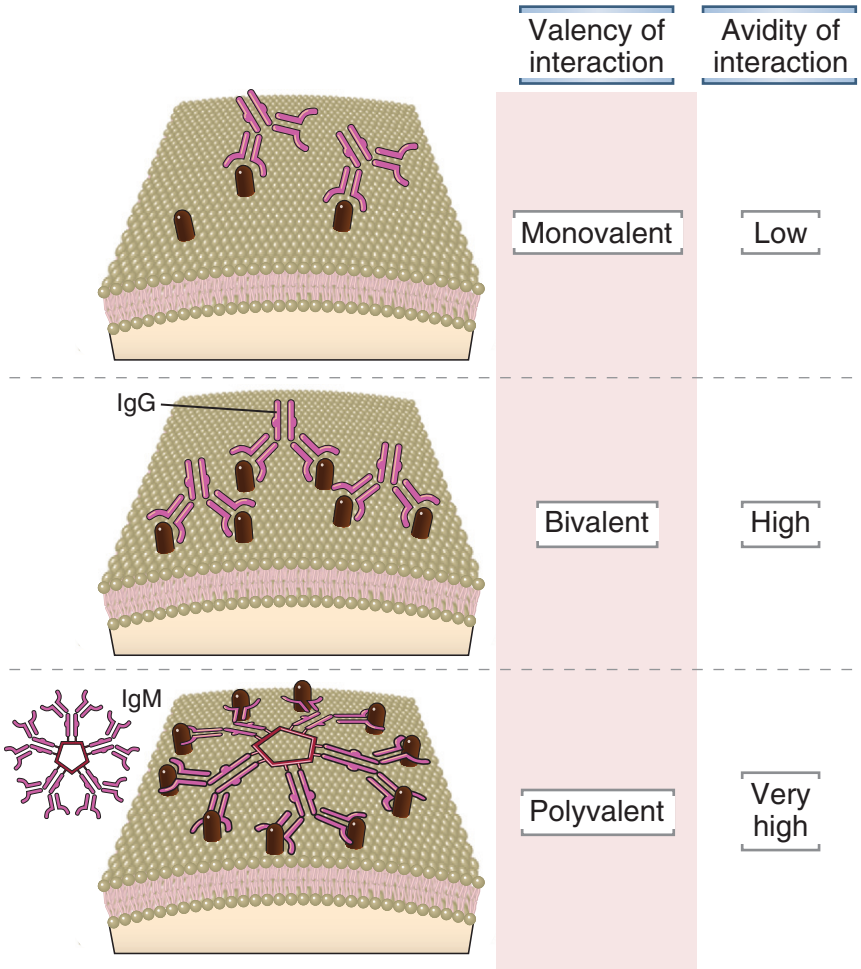


FIGURE 5–13 Valency and avidity of antibody-antigen interactions. Monovalent antigens, or epitopes spaced far apart on cell surfaces, will interact with a single binding site of one antibody molecule. Although the affinity of this interaction may be high, the overall avidity may be relatively low. When repeated determinants on a cell surface are close enough, both the antigen-binding sites of a single IgG molecule can bind, leading to a higher avidity bivalent interaction. The hinge region of the IgG molecule accommodates the shape change needed for simultaneous engagement of both binding sites. IgM molecules have 10 identical antigen-binding sites that can theoretically bind simultaneously with 10 repeating determinants on a cell surface, resulting in a polyvalent, high-avidity interaction.

on both forms may possess an equivalent affinity for the antigen.

Polyvalent antigens are important from the viewpoint of B cell activation, as discussed earlier. Polyvalent interactions between antigen and antibody are also of biologic significance because many effector functions of antibodies are triggered optimally when two or more antibody molecules are brought close together by binding to a polyvalent antigen. If a polyvalent antigen is mixed with a specific antibody in a test tube, the two interact to form **immune complexes** (Fig. 5-14). As discussed in Chapters 12 and 18, immune complexes can also contain complement fragments. At the correct concentration, called a zone of equivalence, antibody and antigen form an extensively cross-linked network of attached molecules such that most or all of the antigen and antibody molecules are complexed into large masses. Immune complexes may be dissociated into smaller aggregates either by increasing the concentration of antigen so that free antigen molecules will displace antigen bound to the antibody (zone of antigen excess) or by increasing antibody so that free antibody molecules will displace bound antibody from antigen determinants (zone of antibody excess). If a zone of equivalence is reached in vivo, large immune complexes can form in the circulation. Immune complexes that are trapped or formed in tissues can initiate an inflammatory reaction, resulting in immune complex diseases (see Chapter 18).

STRUCTURE-FUNCTION RELATIONSHIPS IN ANTIBODY MOLECULES

Many structural features of antibodies are critical for their ability to recognize antigens and for their effector functions. In the following section, we summarize how the structure of antibodies contributes to their functions.

Features Related to Antigen Recognition

Antibodies are able to specifically recognize a wide variety of antigens with varying affinities. All the features of antigen recognition reflect the properties of antibody V regions.

Specificity

Antibodies can be remarkably specific for antigens, distinguishing between small differences in chemical structure. Classic experiments performed by Karl Landsteiner in the late 1920s and early 1930s demonstrated that antibodies made in response to an aminobenzene hapten

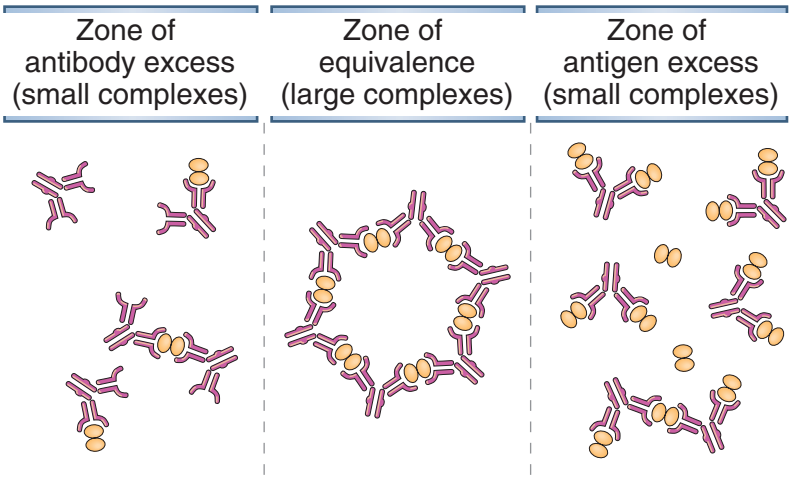


FIGURE 5–14 Antigen-antibody complexes. The sizes of antigen-antibody (immune) complexes are a function of the relative concentrations of antigen and antibody. Large complexes are formed at concentrations of multivalent antigens and antibodies that are termed the zone of equivalence; the complexes are smaller in relative antigen or antibody excess.

with a meta-substituted sulfonate group would bind strongly to this hapten but weakly or not at all to ortho- or para-substituted isomers. These antigens are structurally similar and differ only in the location of the sulfonate group on the benzene ring.

The fine specificity of antibodies applies to the recognition of all classes of molecules. For example, antibodies can distinguish between two linear protein determinants differing by only a single conservative amino acid substitution that has little effect on secondary structure. Because the biochemical constituents of all living organisms are fundamentally similar, this high degree of specificity is necessary so that antibodies generated in response to the antigens of one microbe usually do not react with structurally similar self molecules or with the antigens of other microbes. However, some antibodies produced against one antigen may bind to a different but structurally related antigen. This is referred to as a **cross-reaction**. Antibodies that are produced in response to a microbial antigen sometimes cross-react with self antigens, and this may be the basis of certain immunologic diseases (see Chapter 18).

Diversity

As we discussed earlier in this chapter, an individual is capable of making a tremendous number of structurally distinct antibodies, perhaps more than 10^{11}, each with a distinct specificity. The ability of antibodies in any individual to specifically bind a large number of different antigens is a reflection of antibody **diversity**, and the total collection of antibodies with different specificities represents the **antibody repertoire**. The genetic mechanisms that generate such a large antibody repertoire occur exclusively in lymphocytes. This diversity is generated by random recombination of a limited set of inherited germline DNA sequences to form functional genes that encode the V regions of heavy and light chains as well as by the addition of nucleotide sequences during the recombination process. These mechanisms are discussed in detail in Chapter 8. The millions of resulting variations in structure are concentrated in the hypervariable regions of both heavy and light chains and thereby determine specificity for antigens.

Affinity Maturation

The ability of antibodies to neutralize toxins and infectious microbes is dependent on tight binding of the antibodies. As we have discussed, tight binding is achieved by high-affinity and high-avidity interactions. A mechanism for the generation of high-affinity antibodies involves subtle changes in the structure of the V regions of antibodies during T cell–dependent humoral immune responses to protein antigens. These changes come about by a process of somatic mutation in antigen-stimulated B lymphocytes that generates new V domain structures, some of which bind the antigen with greater affinity than did the original V domains (Fig. 5-15). Those B cells producing higher affinity antibodies preferentially bind to the antigen and, as a result of selection, become the dominant B cells with each subsequent exposure to the antigen. This process, called **affinity maturation**, results in an increase in the average binding affinity of antibodies for an antigen as a humoral immune response evolves. Thus, an antibody produced during a primary immune response to a protein antigen often has a K_d in the range of 10^{-7} to 10^{-9} M; in secondary responses, the affinity increases, with a K_d of 10^{-11} M or even less. The mechanisms of somatic mutation and affinity maturation are discussed in Chapter 11.

Features Related to Effector Functions

Many of the effector functions of immunoglobulins are mediated by the Fc portions of the molecules, and antibody isotypes that differ in these Fc regions perform distinct functions. We have mentioned previously that the effector functions of antibodies require the binding of heavy chain C regions, which make up the Fc portions, to other cells and plasma proteins. For example, IgG coats microbes and targets them for phagocytosis by neutrophils and macrophages. This occurs because the antigen-complexed IgG molecule is able to bind, through its Fc region, to γ heavy chain–specific Fc receptors (FcRs) that are expressed on neutrophils and macrophages. In contrast, IgE binds to mast cells and triggers their degranulation because mast cells express IgE-specific FcRs. Another Fc-dependent effector mechanism of humoral immunity

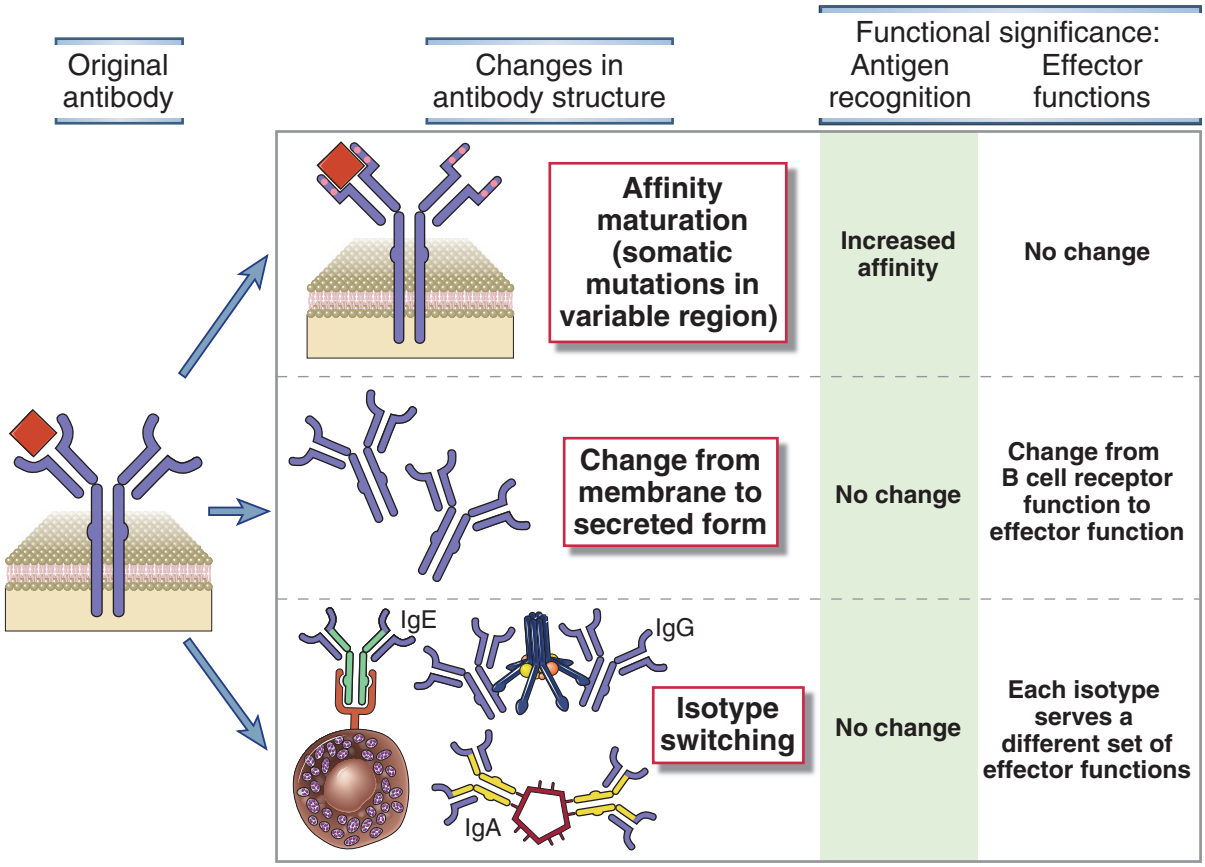


FIGURE 5–15 Changes in antibody structure during humoral immune responses. The illustration depicts the changes in the structure of antibodies that may be produced by the progeny of activated B cells (one clone) and the related changes in function. During affinity maturation, mutations in the V region (indicated by red dots) lead to changes in fine specificity without changes in C region–dependent effector functions. Activated B cells may shift production from largely membrane-bound antibodies containing transmembrane and cytoplasmic regions to secreted antibodies. Secreted antibodies may or may not show V gene mutations (i.e., secretion of antibodies occurs before and after affinity maturation). In isotype switching, the C regions change (indicated by color change from purple to green or yellow) without changes in the antigen-binding V region. Isotype switching is seen in membrane-bound and secreted antibodies. The molecular basis for these changes is discussed in Chapter 11.

is activation of the classical pathway of the complement system. The system generates inflammatory mediators and promotes microbial phagocytosis and lysis. It is initiated by the binding of a complement protein called C1q to the Fc portions of antigen-complexed IgG or IgM. The FcR- and complement-binding sites of antibodies are found within the heavy chain C domains of the different isotypes (see Fig. 5-1). The structure and functions of FcRs and complement proteins are discussed in detail in Chapter 12.

The effector functions of antibodies are initiated only by molecules that have bound antigens and not by free Ig. The reason that only antibodies with bound antigens activate effector mechanisms is that two or more adjacent antibody Fc portions are needed to bind to and trigger various effector systems, such as complement proteins and FcRs of phagocytes (see Chapter 12). This requirement for adjacent antibody molecules ensures that the effector functions are targeted specifically toward eliminating antigens that are recognized by the antibody and that circulating free antibodies do not wastefully, and inappropriately, trigger effector responses.

Changes in the isotypes of antibodies during humoral immune responses influence how and where the responses work to eradicate antigen. After stimulation by an antigen, a single clone of B cells may produce antibodies with different isotypes that nevertheless possess identical V domains and therefore identical antigen specificity. Naive B cells, for example, simultaneously produce IgM and IgD that function as membrane receptors for antigens. When these B cells are activated by foreign antigens, typically of microbial origin, they may undergo a process called **isotype** (or **class**) **switching** in which the type of C_H region, and therefore the antibody isotype, produced by the B cell changes, but the V regions and the specificity do not (see Fig. 5-15). As a result of isotype switching, different progeny of the original IgM- and IgD-expressing B cell may produce isotypes and subtypes that are best able to eliminate the antigen. For example, the antibody response to many bacteria and viruses is dominated by IgG antibodies, which promote phagocytosis of the microbes, and the response to helminths consists mainly of IgE, which aids in the destruction of the parasites. Switching to the IgG isotype also prolongs the effectiveness of humoral immune responses because of the long half-life of IgG antibodies. The mechanisms and functional significance of isotype switching are discussed in Chapter 11.

The heavy chain C regions of antibodies also determine the tissue distribution of antibody molecules. As we mentioned earlier, after B cells are activated, they gradually lose expression of the membrane-bound antibody and express more of it as a secreted protein (see Fig. 5-15). IgA can be secreted efficiently through mucosal epithelia and is the major class of antibody in mucosal secretions and milk (see Chapter 13). Neonates are protected from infections by IgG antibodies they acquire from their mothers through the placenta during gestation and through the intestine early after birth. This transfer of maternal IgG is mediated by the neonatal Fc receptor, which we described earlier as the receptor responsible for the long half-life of IgG antibody.

SUMMARY

- Antibodies, or immunoglobulins, are a family of structurally related glycoproteins produced in membrane-bound or secreted form by B lymphocytes.
- Membrane-bound antibodies serve as receptors that mediate the antigen-triggered activation of B cells.
- Secreted antibodies function as mediators of specific humoral immunity by engaging various effector mechanisms that serve to eliminate the bound antigens.
- The antigen-binding regions of antibody molecules are highly variable, and any one individual has the potential to produce more than 10^{11} different antibodies, each with distinct antigen specificity.
- All antibodies have a common symmetric core structure of two identical covalently linked heavy chains and two identical light chains, each linked to one of the heavy chains. Each chain consists of two or more independently folded Ig domains of about 110 amino acids containing conserved sequences and intrachain disulfide bonds.
- The N-terminal domains of heavy and light chains form the V regions of antibody molecules, which differ among antibodies of different specificities. The V regions of heavy and light chains each contain three separate hypervariable regions of about 10 amino acids that are spatially assembled to form the antigen-combining site of the antibody molecule.
- Antibodies are classified into different isotypes and subtypes on the basis of differences in the heavy chain C regions, which consist of three or four Ig C domains, and these classes and subclasses have different functional properties. The antibody classes are called IgM, IgD, IgG, IgE, and IgA. Both light chains of a single Ig molecule are of the same light chain isotype, either κ or λ, which differ in their single C domains.
- Most of the effector functions of antibodies are mediated by the C regions of the heavy chains, but these functions are triggered by binding of antigens to the spatially distant combining site in the V region.
- Monoclonal antibodies are produced from a single clone of B cells and recognize a single antigenic determinant. Monoclonal antibodies can be generated in the laboratory and are widely used in research, diagnosis, and therapy.
- Antigens are substances specifically bound by antibodies or T lymphocyte antigen receptors. Antigens that bind to antibodies represent a wide variety of biologic molecules, including sugars, lipids, carbohydrates, proteins, and nucleic acids. This is in contrast to T cell antigen receptors, which recognize only peptide antigens.
- Macromolecular antigens contain multiple epitopes, or determinants, each of which may be recognized by an antibody. Linear epitopes of protein antigens consist of a sequence of adjacent amino acids, and conformational determinants are formed by folding of a polypeptide chain.
- The affinity of the interaction between the combining site of a single antibody molecule and a single epitope is generally represented by the dissociation constant (K_d) calculated from binding data. Polyvalent antigens contain multiple identical epitopes to which identical antibody molecules can bind. Antibodies can bind to two or, in the case of IgM, up to 10 identical epitopes simultaneously, leading to enhanced avidity of the antibody-antigen interaction.
- The relative concentrations of polyvalent antigens and antibodies may favor the formation of immune complexes that may deposit in tissues and cause damage.
- Antibody binding to antigen can be highly specific, distinguishing small differences in chemical structures, but cross-reactions may also occur in which two or more antigens may be bound by the same antibody.
- Several changes in the structure of antibodies made by one clone of B cells may occur in the course of an immune response. B cells initially produce only membrane-bound Ig, but in activated B cells and plasma cells, synthesis is induced of soluble Ig with the same antigen-binding specificity as the original membrane-bound Ig receptor. Changes in the use of C region gene segments without changes in V regions are the basis of isotype switching, which leads to changes in effector function without a change in specificity. Point mutations in the V regions of an antibody specific for an antigen lead to increased affinity for that antigen (affinity maturation).

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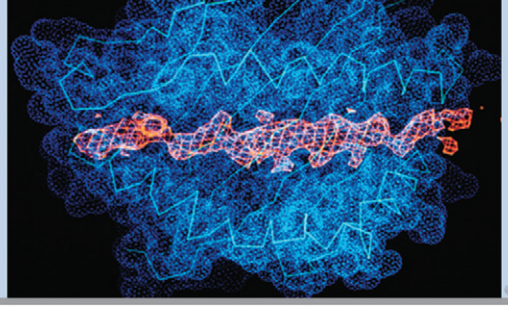
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CHAPTER

6

Major Histocompatibility Complex Molecules and Antigen Presentation to T Lymphocytes

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The principal functions of T lymphocytes are to eradicate infections by intracellular microbes and to activate other cells, such as macrophages and B lymphocytes. To serve these functions, T cells have to overcome several challenges.

- There are very few naive T cells specific for any one antigen, and this small number has to be able to locate the foreign antigen, react against it, and eliminate it. Solving this problem requires a specialized system for capturing antigen and bringing it to the organs where T cell responses can be initiated. The specialized cells that capture and display antigens and activate T lymphocytes are called **antigen-presenting cells (APCs)**. Of these, dendritic cells are especially important for activating naive T cells, the critical event in initiating cell-mediated immune responses.
- Lymphocytes have to be able to combat pathogens at any site in the body regardless of where the pathogens enter. T cells find antigens from all regions of the body by visiting every secondary lymphoid organ in the body as a part of their recirculation (see Chapter 2). To facilitate immune responses, dendritic cells, which are present in all tissue sites, capture antigens and migrate to the same regions of lymphoid organs where recirculating T cells localize, thus maximizing the chance of T cells of a particular specificity finding the relevant antigen.
- The functions of some types of T lymphocytes require that they interact with other cells of the immune system, which may be dendritic cells, macrophages, and B lymphocytes. Other types of T lymphocytes must be able to interact with any infected host cell. To ensure that T cells interact only with other host cells but not directly with microbes, T cell antigen receptors are designed to see antigens displayed by host cell surface molecules and not antigens on microbes or antigens that are free in the circulation or extracellular fluids. This is in striking contrast to B lymphocytes, whose antigen receptors and secreted products, antibodies, can recognize antigens on microbial surfaces, and soluble antigens as well as cell-associated antigens. The task of displaying host cell–associated antigens for recognition by $CD4^+$ and $CD8^+$ T cells is performed by specialized proteins called **major histocompatibility complex (MHC)** molecules, which are expressed on the surfaces of host cells.
- Different T cells have to be able to respond to microbial antigens in different cellular compartments. For instance, defense against viruses in the circulation has

to be mediated by antibodies, and the production of the most effective antibodies requires the participation of $CD4^+$ helper T cells. But if the same virus infects a tissue cell, it becomes inaccessible to the antibody, and its eradication requires that $CD8^+$ cytotoxic T lymphocytes (CTLs) kill the infected cells and eliminate the reservoir of infection. This separation of optimal responses occurs because APCs differentially handle antigens in different locations (extracellular and intracellular, respectively) and present these antigens to the different classes of T cells. The task of segregating antigens from various anatomic compartments and displaying them to different T cell populations is also performed by MHC molecules.

Thus, antigen capture and display to T cells is a specialized and finely orchestrated process with many important functional implications. Elucidation of the cell biology and molecular basis of this complex process has been a fascinating accomplishment, which encompasses fundamental biology as well as fine structural detail. In this chapter, we will describe how antigens are captured and displayed to T cells. In Chapter 7, we describe the antigen receptors of T cells, and in Chapters 9 and 10, we discuss the activation and effector functions of T lymphocytes.

TABLE 6–1 Features of Antigens Recognized by T Lymphocytes

Features of Antigens Recognized by T Cells	Explanation
Most T cells recognize peptides and no other molecules.	Only peptides bind to MHC molecules.
T cells recognize linear peptides and not conformational determinants of protein antigens.	Linear peptides bind to clefts of MHC molecules, and protein conformation is lost during the generation of these peptides.
T cells recognize cell-associated and not soluble antigens.	T cell receptors recognize only MHC-like shapes, and MHC molecules are membrane proteins that display stably bound peptides on cell surfaces.
$CD4^+$ and $CD8^+$ T cells preferentially recognize antigens sampled from the extracellular and cytosolic pools, respectively.	Pathways of assembly of MHC molecules ensure that class II molecules display peptides that are derived from extracellular proteins and taken up into vesicles in APCs and that class I molecules present peptides from cytosolic proteins; CD4 and CD8 bind to nonpolymorphic regions of class II and class I MHC molecules, respectively.

PROPERTIES OF ANTIGENS RECOGNIZED BY T LYMPHOCYTES

Our current understanding of T cell antigen recognition is the culmination of a vast amount of research that began with studies of the nature of antigens that stimulate cell-mediated immunity. The early studies showed that the physicochemical forms of antigens that are recognized by T cells are different from those recognized by B lymphocytes and antibodies, and this knowledge led to the discovery of the role of the MHC in T cell antigen recognition. Several features of antigen recognition are unique to T lymphocytes (Table 6-1).

Most T lymphocytes recognize only short linear peptides, and in fact, they are specific for the amino acid sequences of peptides, whereas B cells can recognize peptides, proteins, nucleic acids, carbohydrates, lipids, and small chemicals. As a result, T cell–mediated immune responses are usually induced by foreign protein antigens (the natural source of foreign peptides), whereas humoral immune responses are induced by protein and nonprotein antigens. Some T cells are specific for small chemical haptens such as dinitrophenol, urushiol of poison ivy, and β lactams of penicillin antibiotics. In these situations, it is likely that the haptens bind to self proteins and that hapten-conjugated peptides are recognized by T cells. The peptide specificity of T cells is true for $CD4^+$ and $CD8^+$ cells; as we shall discuss at the end of this chapter, there are some small populations of T cells that are capable of recognizing nonprotein antigens.

The reason that T cells recognize only peptides is that ***the antigen receptors of $CD4^+$ and $CD8^+$ T cells are specific for antigens that are displayed by MHC molecules, and these molecules can bind peptides but no other chemical structures*** (Fig. 6-1). Thus, every T cell is specific for a combination of amino acid residues of a peptide antigen plus portions of the MHC molecule. As we shall discuss later, MHC molecules are highly polymorphic, and variations in MHC molecules among individuals influence both peptide binding and T cell recognition. A single T cell can recognize a specific peptide displayed by only one of the large number of different MHC molecules that exist. This phenomenon is called **MHC restriction**, and we will describe its molecular basis later in the chapter.

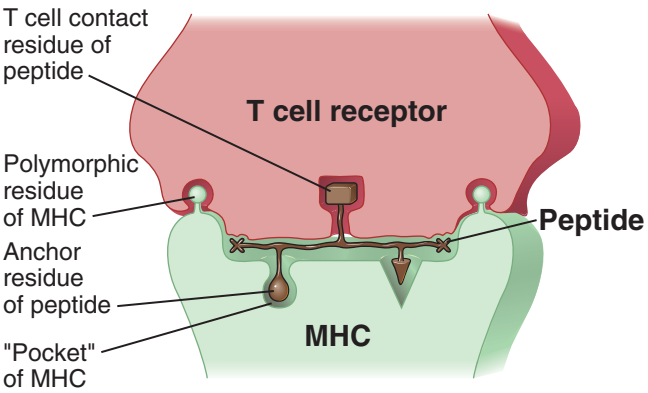


FIGURE 6–1 A model for T cell recognition of a peptide-MHC complex. This schematic illustration shows an MHC molecule binding and displaying a peptide and a T cell receptor recognizing two polymorphic residues of the MHC molecule and one residue of the peptide.

We start our discussion of antigen presentation by describing how APCs capture antigens and transport them to T cells.

ANTIGEN CAPTURE AND THE FUNCTIONS OF ANTIGEN-PRESENTING CELLS

The realization that various cells other than T cells are needed to present antigens to T lymphocytes came first from studies in which protein antigens that were known to elicit T cell responses were labeled and injected into mice, to ask what cells bound (and, by implication, recognized) these antigens. The surprising result was that the injected antigens were associated mainly with non-T cells. This type of experiment was quickly followed by studies showing that protein antigens that were physically associated with macrophages were much more immunogenic, on a molar basis, than the same antigens injected into mice in soluble form. In these early experiments, the macrophage populations studied likely included dendritic cells, since, as discussed below, naive T cells are best activated by dendritic cells. Subsequent cell culture experiments showed that purified $CD4^+$ T cells could not respond to protein antigens, but they responded very well if non-T cells such as dendritic cells or macrophages were added to the cultures. These results led to the concept that a critical step in the induction of a T cell response is the presentation of the antigen to T lymphocytes by other cells, and the name *antigen-presenting cells* was born. The first APCs identified were macrophages, and the responding T cells were $CD4^+$ helper cells. It soon became clear that several cell populations, described later, can function as APCs in different situations. By convention, APC is still the term used to refer to specialized cells that display antigens to $CD4^+$ T lymphocytes; as we shall see later in the chapter, all nucleated cells can display protein antigens to $CD8^+$ T lymphocytes, and they are not called APCs.

We begin with a discussion of some of the general properties of APCs for $CD4^+$ T lymphocytes.

- ***Different cell types function as APCs to activate naive and previously differentiated effector T cells*** (Fig. 6-2 and Table 6-2). Dendritic cells are the most effective APCs for activating naive T cells and therefore for initiating T cell responses. Macrophages and B lymphocytes also function as APCs, but mostly for previously activated $CD4^+$ helper T cells rather than for naive T cells. Their roles as APCs are described later in this chapter and in more detail in Chapters 10 and 11. Dendritic cells, macrophages, and B lymphocytes express class II MHC molecules and other molecules involved in stimulating T cells and are therefore capable of activating $CD4^+$ T lymphocytes. For this reason, these three cell types have been called professional APCs; however, this term is sometimes used to refer only to dendritic cells because this is the only cell type whose dedicated function is to capture and present antigens and the only APC capable of initiating primary T cell responses.

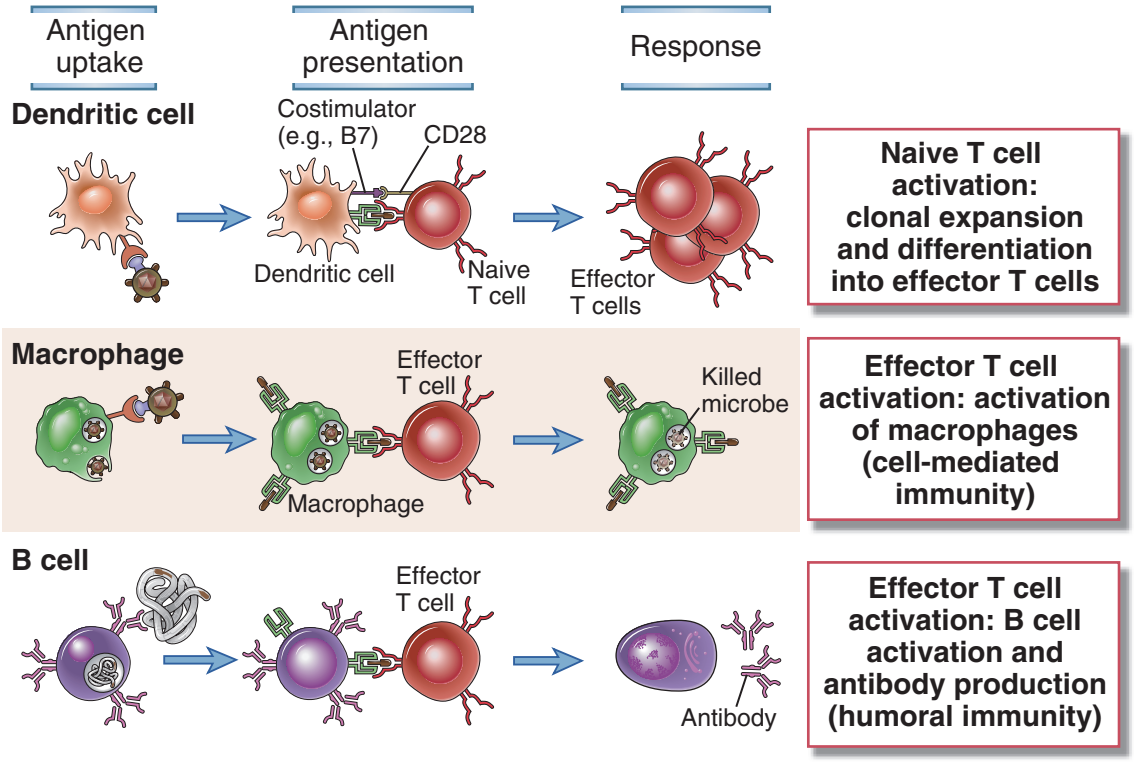


FIGURE 6–2 Functions of different antigen-presenting cells. The three major types of APCs for $CD4^+$ T cells function to display antigens at different stages and in different types of immune responses. Note that effector T cells activate macrophages and B lymphocytes by production of cytokines and by expressing surface molecules; these will be described in later chapters.

TABLE 6–2 Properties and Functions of Antigen-Presenting Cells

Cell Type	Expression of		Principal Function
	Class II MHC	Costimulators	
Dendritic cells	Constitutive; increases with maturation; increased by IFN-γ	Constitutive; increases with maturation; inducible by IFN-γ, CD40-CD40L interactions	Initiation of T cell responses to protein antigens (priming)
Macrophages	Low or negative; inducible by IFN-γ	Inducible by LPS, IFN-γ, CD40-CD40L interactions	Effector phase of cell-mediated immune responses (T cell–enhanced killing of phagocytosed microbes)
B lymphocytes	Constitutive; increased by IL-4	Induced by T cells (CD40-CD40L interactions), antigen receptor cross-linking	Antigen presentation to $CD4^+$ helper T cells in humoral immune responses (cognate T cell–B cell interactions)
Vascular endothelial cells	Inducible by IFN-γ; constitutive in humans	Constitutive (inducible in mice)	May promote activation of antigen-specific T cells at site of antigen exposure
Various epithelial and mesenchymal cells	Inducible by IFN-γ	Probably none	No known physiologic function

IFN-γ, interferon-γ; IL-4, interleukin-4; LPS, lipopolysaccharide.

- ***APCs display peptide-MHC complexes for recognition by T cells and also provide additional stimuli to the T cells that are required for the full responses of the T cells.*** These stimuli, sometimes called "second signals," are more important for activation of naive T cells than for previously activated effector and memory cells. The membrane-bound molecules of APCs that serve to activate T cells are called **costimulators** because they function together with antigen in T cell stimulation. APCs also secrete cytokines that play critical roles in T cell differentiation into effector cells. These costimulators and cytokines are described in Chapter 9.
- ***The antigen-presenting function of APCs is enhanced by exposure to microbial products.*** This is one reason that the immune system responds better to microbes than to harmless, nonmicrobial substances. Dendritic cells and macrophages express Toll-like receptors and other microbial sensors (see Chapter 4) that respond to microbes by increasing the expression of MHC molecules and costimulators, by improving the efficiency of antigen presentation, and by activating the APCs to produce cytokines, all of which stimulate T cell responses. In addition, dendritic cells that are activated by microbes express chemokine receptors that stimulate their migration to sites where T cells are present. The induction of optimal T cell responses to purified protein antigens requires that the antigens be administered with substances called **adjuvants**. Adjuvants either are products of microbes, such as killed mycobacteria (used experimentally), or they mimic microbes and enhance the expression of costimulators and cytokines as well as the antigen-presenting functions of APCs (see Chapter 9).
- ***APCs that present antigens to T cells also receive signals from these lymphocytes that enhance their antigen-presenting function.*** In particular, $CD4^+$ T cells that are activated by antigen recognition and costimulation express surface molecules, notably one called CD40 ligand (CD154), that binds to CD40 on dendritic cells and macrophages, and the T cells secrete cytokines such as interferon-γ (IFN-γ) that bind to their receptors on these APCs. The combination of CD40 signals and cytokines activates the APCs, resulting in increased ability to process and present antigens, increased expression of costimulators, and secretion of cytokines that activate the T cells. This bidirectional interaction between APCs displaying the antigen and T lymphocytes that recognize the antigen functions as a positive feedback loop that plays an important role in maximizing the immune response (see Chapter 9).

Role of Dendritic Cells in Antigen Capture and Display

The primary responses of naive $CD4^+$ T cells are initiated in the peripheral lymphoid organs, to which protein antigens are transported after being collected from their portal of entry (Fig. 6-3). The common routes through which foreign antigens, such as microbes, enter a host are the skin and the epithelia of the gastrointestinal and respiratory systems. In addition, microbial antigens may be produced in any tissue that has been colonized or infected by a microbe. The skin, mucosal epithelia, and parenchymal organs contain numerous lymphatic capillaries that drain lymph from these sites and into the regional lymph nodes. Some antigens are transported in the lymph by APCs, primarily dendritic cells, that capture the antigen and enter lymphatic vessels, and other antigens may be in a free form. Thus, the lymph contains a sampling of all the soluble and cell-associated antigens present in tissues. The antigens become concentrated in lymph nodes, which are interposed along lymphatic vessels and act as filters that sample the lymph before it reaches the blood (see Chapter 2). Antigens that enter the blood stream may be similarly sampled by the spleen.

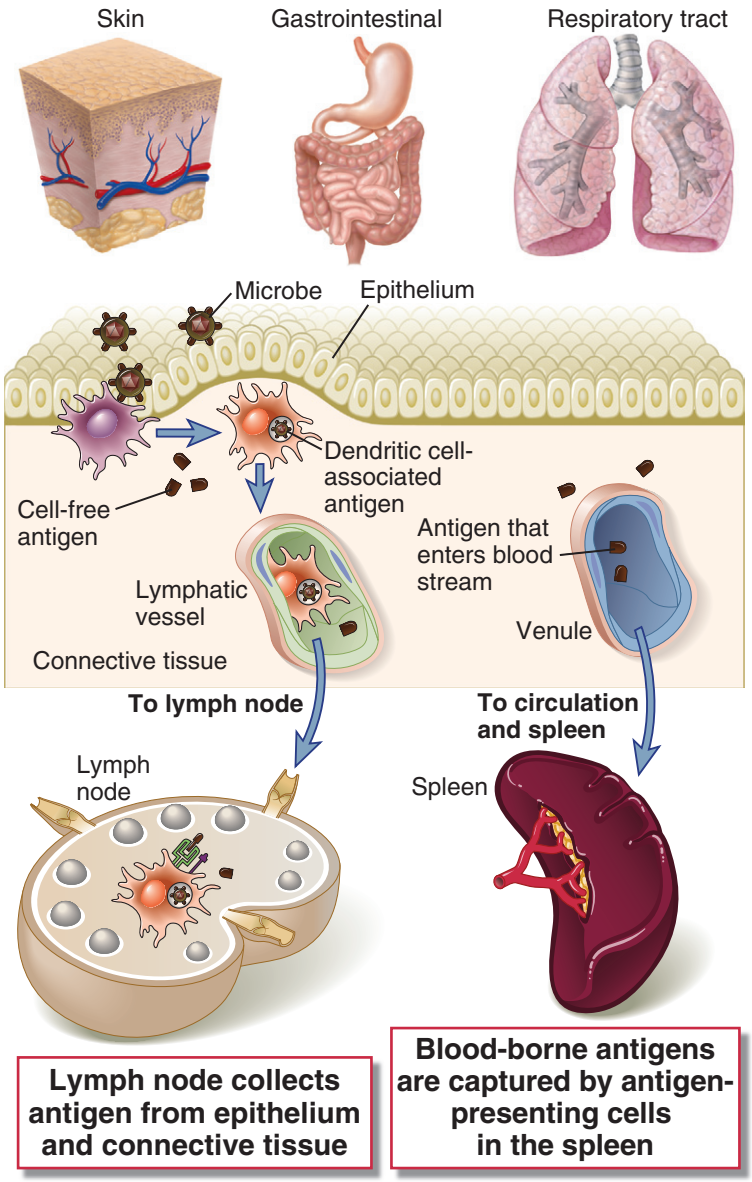


FIGURE 6–3 Routes of antigen entry. Microbial antigens commonly enter through the skin and gastrointestinal and respiratory tracts, where they are captured by dendritic cells and transported to regional lymph nodes. Antigens that enter the blood stream are captured by APCs in the spleen.

The cells that are designed to capture, transport, and present antigens to T cells are the dendritic cells. We next describe their major characteristics and their functions in initiating T cell responses.

Morphology and Populations of Dendritic Cells

Dendritic cells (DCs) are present in lymphoid organs, in the epithelia of the skin and gastrointestinal and respiratory tracts, and in the interstitium of most parenchymal organs. These cells (introduced in Chapter 2) are identified morphologically by their membranous or spine-like projections (Fig. 6-4). All DCs are thought to arise from bone marrow precursors, and most are related in lineage to mononuclear phagocytes (see Fig. 2-2). Several subsets of DCs have been identified that may be distinguished by the expression of various cell surface markers and may play different roles in immune responses. The two main types are called conventional DCs and plasmacytoid DCs (Table 6-3).

- **Conventional DCs,** previously called myeloid DCs, were first identified by their morphology and ability to stimulate strong T cell responses, and are the most numerous DC subset in lymphoid organs. They are derived from bone marrow progenitors, can be cultured from bone marrow or blood cells, including blood monocytes, and give rise to the resident tissue population of DCs. On activation by encounter with microbes or cytokines, DCs in epithelia and tissues mature and migrate into draining lymph nodes, where they initiate T cell responses (see Fig. 6-4). Tissue-derived conventional DCs are also sometimes classified into the Langerhans cell type, representing DCs in epithelia and in skin-draining lymph nodes, and the interstitial/dermal type, representing DCs in most other tissues. The prototypes of epithelial DCs are the Langerhans cells of the epidermis. Because of their long cytoplasmic processes, Langerhans cells occupy as much as 25% of the surface area of the epidermis,

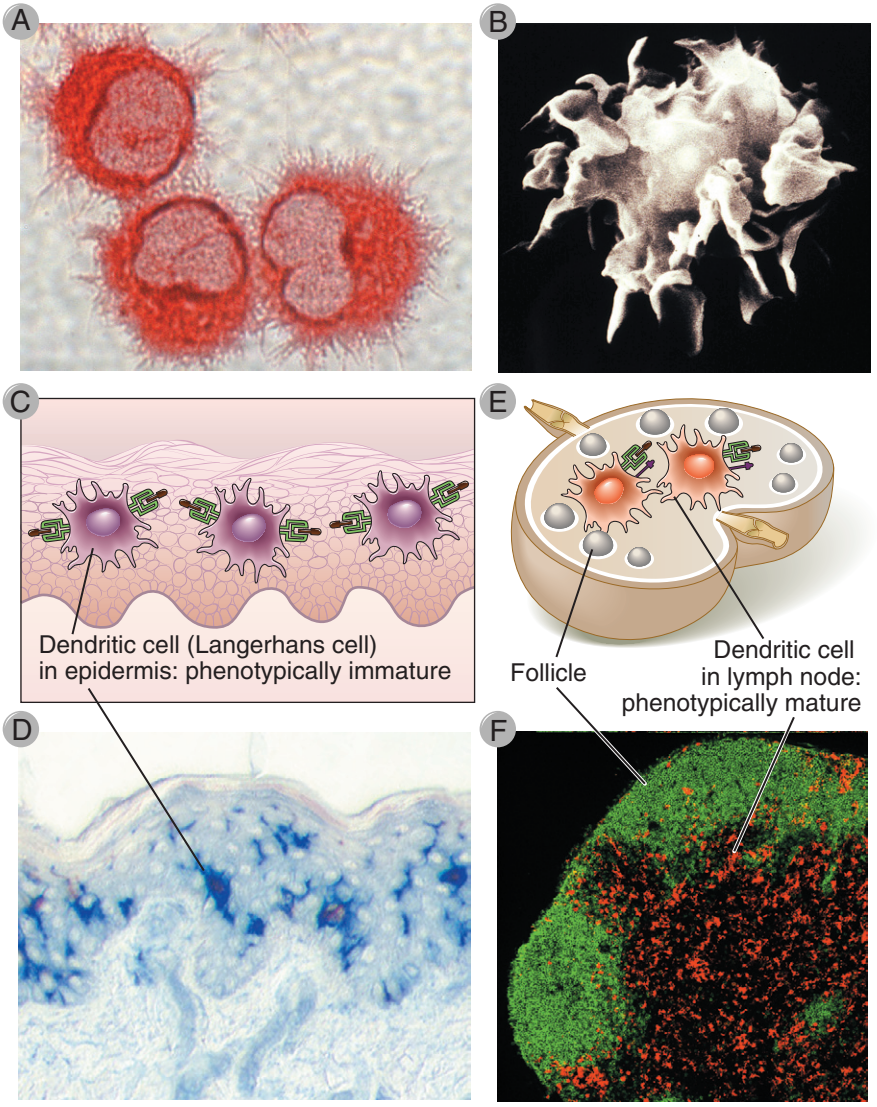


FIGURE 6–4 Dendritic cells. A, Light micrograph of cultured dendritic cells derived from bone marrow precursors. **B,** A scanning electron micrograph of a dendritic cell showing the extensive membrane projections. **C, D,** Dendritic cells in the skin, illustrated schematically **(C)** and in a section of the skin **(D)** stained with an antibody specific for Langerhans cells (which appear blue in this immunoenzyme stain). **E, F,** Dendritic cells in a lymph node, illustrated schematically **(E)** and in a section of a mouse lymph node **(F)** stained with fluorescently labeled antibodies against B cells in follicles (green) and dendritic cells in the T cell zone (red). *(**A, B,** and **D** courtesy of Dr. Y-J Liu, M.D. Anderson Cancer Center, Houston, Texas; **F** courtesy of Drs. Kathryn Pape and Jennifer Walter, University of Minnesota School of Medicine, Minneapolis.)*

TABLE 6–3 The Major Subpopulations of Dendritic Cells

Feature	Conventional (Myeloid) Dendritic Cells	Plasmacytoid Dendritic Cells
Surface markers	CD11c high CD11b high	CD11c low CD11b negative B220 high
Growth factors for in vitro derivation	GM-CSF, Flt3-ligand	Flt3-ligand
Expression of Toll-like receptors (TLRs)	TLRs 4, 5, 8 high	TLRs 7, 9 high
Major cytokines produced	TNF, IL-6	Type I interferons
Postulated major functions	Induction of T cell responses against most antigens	Innate immunity and induction of T cell responses against viruses

Other subsets of dendritic cells have been described on the basis of the expression of various surface markers (such as CD4, CD8, and CD11b) or migration from tissue sites (Langerhans-type dendritic cells from epithelia and interstitial dendritic cells from tissues). Note that all DCs express class II MHC molecules. Some authorities also refer to monocyte-derived dendritic cells, which can be generated from blood monocytes cultured with various cytokines and may develop in vivo during inflammatory reactions.

even though they constitute less than 1% of the cell population (see Fig. 6-4). DCs in intestinal epithelia appear to send out processes that traverse the epithelial cells and project into the lumen, where they may function to capture microbial antigens. A population of conventional DCs that expresses the T cell marker CD8 has been described in mice (but this marker does not demarcate a subset of DCs in humans). $CD8^+$ DCs were also called lymphoid DCs because of the suggestion that they develop from common lymphoid progenitors, but it is now known that they develop from myeloid precursors, and the term lymphoid DC is not generally used. Other subsets of conventional DCs have been described, but the functions of these subsets have not been defined yet.

- **Plasmacytoid DCs** resemble plasma cells morphologically and acquire the morphology and functional properties of DCs only after activation. They develop in the bone marrow from a precursor that also gives rise to conventional DCs and are found in the blood and in small numbers in lymphoid organs, particularly the T cell zones of the spleen and lymph nodes. The major function of plasmacytoid DCs is the secretion of large amounts of type I interferons in response to viral infections (see Chapter 4). These cells also play a role in presenting antigens to T lymphocytes.

DCs that migrate from tissue sites to lymph nodes can also be characterized as immature or mature. Many DCs normally residing in lymphoid organs and in nonlymphoid tissues, including epithelia, in the absence of infection or inflammation, appear to be in an immature state, that is, able to capture antigens but unable to activate T cells. These DCs may function to present self antigens to self-reactive T cells and thereby cause inactivation or death of the T cells or generate regulatory T cells. These mechanisms are important for maintaining self-tolerance and preventing autoimmunity (see Chapter 14). As discussed below, DCs that have encountered microbes undergo maturation and function to present the antigens to T cells and to activate the T cells.

Antigen Capture and Transport by Dendritic Cells

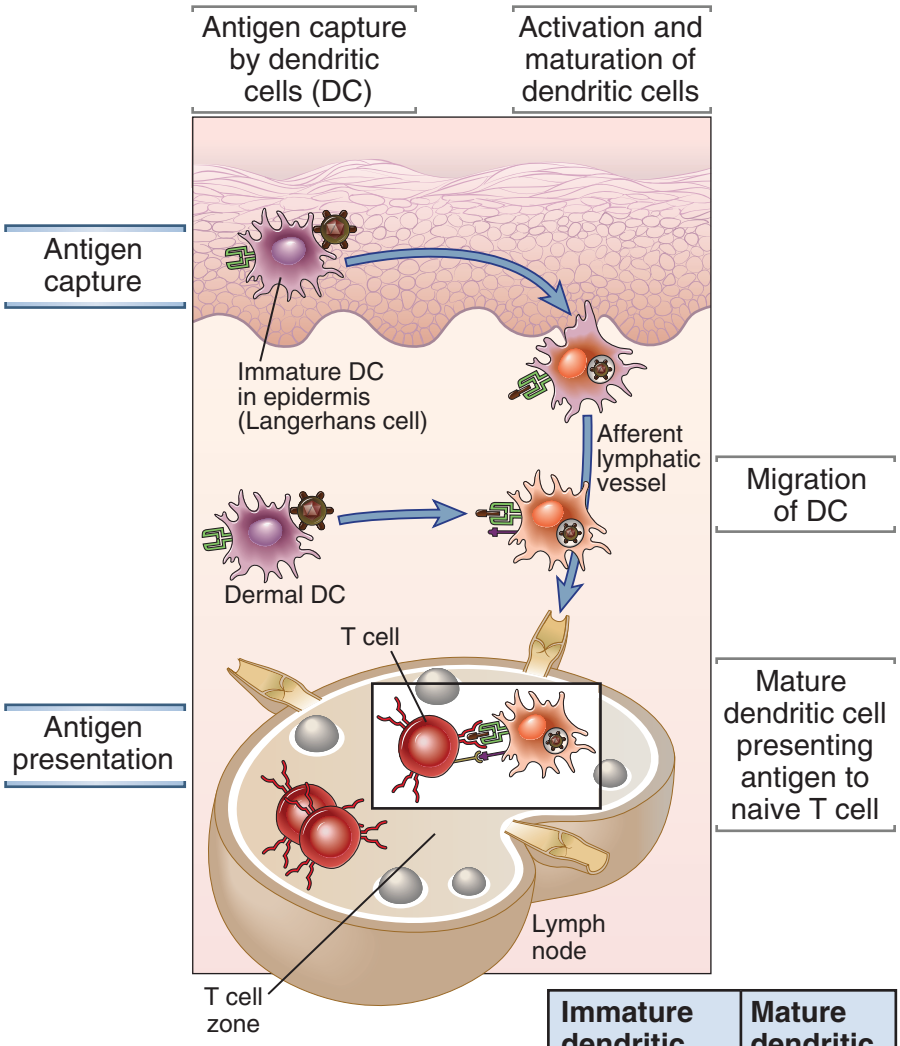
DCs that are resident in epithelia and tissues capture protein antigens and transport the antigens to draining lymph nodes (Fig. 6-5). Resting (immature) DCs express membrane receptors, such as C-type lectins, that bind microbes. DCs use these receptors to capture and endocytose microbes and their antigens and then process the ingested proteins into peptides capable of binding to MHC molecules. Apart from receptor-mediated endocytosis and phagocytosis, DCs can ingest antigens by micropinocytosis and macropinocytosis, processes that do not involve specific recognition receptors but capture whatever might be in the fluid phase in the vicinity of the DCs. At the same time, an innate immune response develops during which microbial products are recognized by Toll-like receptors and other microbial sensors in the DCs and other cells. The DCs are activated by these signals and by cytokines, such as tumor necrosis factor (TNF), produced in response to the microbes. The activated DCs (also called mature DCs) lose their adhesiveness for epithelia or tissues and migrate into lymph nodes. The DCs also begin to express a chemokine receptor called CCR7 that is specific for two chemokines, CCL19 and CCL21, that are produced in the T cell zones of lymph nodes. These chemokines attract the DCs bearing microbial antigens into the T cell zones of the regional lymph nodes. Naive T cells also express CCR7, and this is why naive T cells migrate to the same regions of lymph nodes where antigen-bearing DCs are concentrated (see Chapter 3). The colocalization of antigen-bearing DCs and naive T cells maximizes the chance of T cells with receptors for the antigen finding that antigen. Maturation also converts the DCs from cells whose function is to capture antigen into cells that are able to present antigens to naive T cells and to activate the lymphocytes. Mature DCs express high levels of MHC molecules with bound peptides as well as costimulators required for T cell activation. Thus, by the time these cells become resident in lymph nodes, they have developed into potent APCs with the ability to activate T lymphocytes. Naive T cells that recirculate through lymph nodes encounter these APCs, and the T cells that are specific for the displayed peptide-MHC complexes are activated. This is the initial step in the induction of T cell responses to protein antigens.

Antigens may also be transported to lymphoid organs in soluble form. Resident DCs in the lymph nodes and spleen may capture lymph- and blood-borne antigens, respectively, and also be driven to mature by microbial products. When lymph enters a lymph node through an afferent lymphatic vessel, it drains into the subcapsular sinus, and some of the lymph enters fibroblast reticular cell conduits that originate from the sinus and traverse the cortex (see Chapter 2). Once in the conduits, low-molecular-weight antigens can be extracted by DCs whose processes interdigitate between the reticular cells. Other antigens in the subcapsular sinus are taken up by macrophages and DCs, which carry the antigens into the cortex. B cells in the node may also recognize and internalize soluble antigens. DCs, macrophages, and B cells that have taken up protein antigens can then process and present these antigens to naive T cells and to effector T cells that have been generated by previous antigen stimulation.

The collection and concentration of foreign antigens in lymph nodes are supplemented by two other anatomic adaptations that serve similar functions. First, the mucosal surfaces of the gastrointestinal and respiratory systems, in addition to being drained by lymphatic capillaries, contain specialized collections of secondary lymphoid tissue that can directly sample the luminal contents of these organs for the presence of antigenic material. The best characterized of these mucosal lymphoid organs are Peyer's patches of the ileum and the pharyngeal tonsils (see Chapter 13). Second, the blood stream is monitored by APCs in the spleen for any antigens that reach the circulation. Such antigens may reach the blood either directly from the tissues or by way of the lymph from the thoracic duct.

Antigen-Presenting Function of Dendritic Cells

Many studies done in vitro and in vivo have established that the induction of primary T cell–dependent immune



	Immature dendritic cell	Mature dendritic cell
Principal function	Antigen capture	Antigen presentation to T cells
Expression of Fc receptors, mannose receptors	++	–
Expression of molecules involved in T cell activation: B7, ICAM-1, IL-12	– or low	++
Class II MHC molecules		
Half-life	~10 hr	>100 hr
Number of surface molecules	~10^6	~7×10^6

FIGURE 6–5 Role of dendritic cells in antigen capture and presentation. Immature dendritic cells in the skin (Langerhans cells) or dermis (dermal DCs) capture antigens that enter through the epidermis and transport the antigens to regional lymph nodes. During this migration, the dendritic cells mature and become efficient APCs. The table summarizes some of the changes during dendritic cell maturation that are important in the functions of these cells.

responses to protein antigens requires the presence of DCs to capture and to present the antigens to the T cells. This was first shown for $CD4^+$ T cell responses but is now known to be true for $CD8^+$ T cells as well.

Several properties of DCs make them the most efficient APCs for initiation of primary T cell responses.

- DCs are strategically located at the common sites of entry of microbes and foreign antigens (in epithelia) and in tissues that may be colonized by microbes.
- DCs express receptors that enable them to capture microbes and to respond to microbes.
- These cells migrate from epithelia and tissues preferentially to the T cell zones of lymph nodes, through which naive T lymphocytes circulate, searching for foreign antigens.
- Mature DCs express high levels of peptide-MHC complexes, costimulators, and cytokines, all of which are needed to activate naive T lymphocytes.

DCs can ingest infected cells and present antigens from these cells to $CD8^+$ T lymphocytes. DCs are the best APCs to induce the primary responses of $CD8^+$ T cells, but this poses a special problem because the antigens these lymphocytes recognize may be produced in any cell type infected by a virus, not necessarily DCs. Some specialized DCs have the ability to ingest virus-infected cells or cellular fragments and present antigens from these cells to $CD8^+$ T lymphocytes. This process is called cross-presentation, or cross-priming, and is described later in the chapter.

Functions of Other Antigen-Presenting Cells

Although DCs have a critical role in initiating primary T cell responses, other cell types are also important APCs in different situations (see Fig. 6-2 and Table 6-2).

- ***In cell-mediated immune responses, macrophages present the antigens of phagocytosed microbes to effector T cells, which respond by activating the macrophages to kill the microbes.*** This process is central to cell-mediated immunity and delayed-type hypersensitivity (see Chapter 10). Circulating monocytes are able to migrate to any site of infection and inflammation, where they differentiate into macrophages and phagocytose and destroy microbes. $CD4^+$ T cells recognize microbial antigens being presented by the macrophages and provide signals that enhance the microbicidal activities of these macrophages.
- ***In humoral immune responses, B lymphocytes internalize protein antigens and present peptides derived from these proteins to helper T cells.*** This antigen-presenting function of B cells is essential for helper T cell–dependent antibody production (see Chapter 11).
- ***All nucleated cells can present peptides, derived from cytosolic protein antigens, to $CD8^+$ T lymphocytes.*** All nucleated cells are susceptible to viral infections and cancer-causing mutations. Therefore, it is important that the immune system be able to recognize cytosolic antigens, such as viral antigens and mutated proteins, in any cell type. $CD8^+$ CTLs are the cell population that recognize these antigens and eliminate the cells in which the antigens are produced. Phagocytosed microbes may also be recognized by $CD8^+$ CTLs if these microbes or their antigens escape from phagocytic vesicles into the cytosol.
- Vascular endothelial cells in humans express class II MHC molecules and may present antigens to blood T cells that have become adherent to the vessel wall. This may contribute to the recruitment and activation of effector T cells in cell-mediated immune reactions (see Chapter 10). Endothelial cells in grafts are also targets of T cells reacting against graft antigens (see Chapter 16). Various epithelial and mesenchymal cells may express class II MHC molecules in response to the cytokine IFN-γ. The physiologic significance of antigen presentation by these cell populations is unclear. Because most of them do not express costimulators and are not efficient at processing proteins into MHC-binding peptides, it is unlikely that they contribute significantly to the majority of T cell responses. Thymic epithelial cells constitutively express MHC molecules and play a critical role in presenting peptide-MHC complexes to maturing T cells in the thymus as part of the selection processes that shape the repertoire of T cell specificities (see Chapter 8).

THE MAJOR HISTOCOMPATIBILITY COMPLEX (MHC)

Discovery of the MHC

The discovery of the fundamental role of the MHC in antigen recognition by $CD4^+$ and $CD8^+$ T cells has revolutionized the field of immunology and paved the way for our current understanding of the activation and functions of lymphocytes.

The Mouse MHC (H-2 Complex)

The MHC was discovered from studies of tissue transplantation, well before the structure and function of MHC molecules were elucidated. It was known that tissues, such as skin, exchanged between nonidentical animals are rejected, whereas the same grafts between identical twins are accepted. This result showed that inherited genes must be involved in the process of tissue rejection. In the 1940s, to analyze the genetic basis of graft rejection, George Snell and colleagues produced inbred mouse strains by repetitive mating of siblings. Inbred mice are homozygous at every genetic locus (i.e., they express only one allele of every gene, even the polymorphic genes), and every mouse of an inbred strain is genetically identical (syngeneic) to every other mouse of the same strain (i.e., they all express the same alleles). Different strains may express different alleles and are said to be allogeneic to one another. By breeding congenic strains of mice that rejected grafts from other strains but were identical for all other genes, these investigators showed that a single genetic region is primarily responsible for rapid rejection of tissue grafts, and this region was called the major histocompatibility locus (*histo,* tissue). The particular locus that was identified in mice

by Snell's group was linked to a gene on chromosome 17 encoding a blood group antigen called antigen II, and therefore this region was named histocompatibility-2, or simply H-2. Initially, this locus was thought to contain a single gene that controlled tissue compatibility. However, occasional recombination events occurred within the H-2 locus during interbreeding of different strains, indicating that it actually contained several different but closely linked genes, many of which were involved in graft rejection. The genetic region that controlled graft rejection and contained several linked genes was named the **major histocompatibility complex**. Although not known at the time of Snell's experiments, transplant rejection is in large part a T cell–mediated process (see Chapter 16), and therefore it is not surprising that there is a relationship between MHC genes, which encode the peptide-binding MHC molecules that T cells recognize, and graft rejection.

The Human MHC (HLA)

The human MHC was discovered by searching for cell surface molecules in one individual that would be recognized as foreign by another individual. This task became feasible when Jean Dausset, Jan van Rood, and their colleagues discovered that individuals who had received multiple blood transfusions and patients who had received kidney transplants contained antibodies that recognized cells from the blood or kidney donors and multiparous women had circulating antibodies that recognized paternal cells. The proteins recognized by these antibodies were called **human leukocyte antigens (HLA)** (*leukocyte* because the antibodies were tested by binding to the leukocytes of other individuals, and *antigens* because the molecules were recognized by antibodies). Subsequent analyses have shown that as in mice, the inheritance of particular *HLA* alleles is a major determinant of graft acceptance or rejection (see Chapter 16). Biochemical studies gave the satisfying result that the mouse H-2 proteins and the HLA proteins had essentially identical structures. From these results came the conclusion that genes that determine the fate of grafted tissues are present in all mammalian species and are homologous to the *H-2* genes first identified in mice; these are called MHC genes. Other polymorphic genes that contribute to graft rejection to a lesser degree are called minor histocompatibility genes; we will return to these in Chapter 16, when we discuss transplantation immunology.

Immune Response Genes

For almost 20 years after the MHC was discovered, its only documented role was in graft rejection. This was a puzzle to immunologists because transplantation is not a natural phenomenon, and there was no reason that a set of genes should be preserved through evolution if the only function of the genes was to control the rejection of foreign tissue grafts. In the 1960s and 1970s, it was discovered that MHC genes are of fundamental importance for all immune responses to protein antigens. Baruj Benacerraf, Hugh McDevitt, and their colleagues found that inbred strains of guinea pigs and mice differed in their ability to make antibodies against some simple synthetic polypeptides, and responsiveness was inherited as a dominant mendelian trait. The relevant genes were called *immune response (Ir) genes,* and they were all found to map to the MHC. We now know that Ir genes are, in fact, MHC genes that encode MHC molecules that differ in their ability to bind and display peptides derived from various protein antigens. Responder strains, which can mount immune responses to a particular polypeptide antigen, inherit MHC alleles whose products can bind peptides derived from these antigens, forming peptide-MHC complexes that can be recognized by helper T cells. These T cells then help B cells to produce antibodies. Nonresponder strains express MHC molecules that are not capable of binding peptides derived from the polypeptide antigen, and therefore these strains cannot generate helper T cells or antibodies specific for the antigen. It was also later found that many autoimmune diseases were associated with the inheritance of particular MHC alleles, firmly placing these genes at the center of the mechanisms that control immune responses. Such studies provided the impetus for more detailed analyses of MHC genes and proteins.

The Phenomenon of MHC Restriction

The formal proof that the MHC is involved in antigen recognition by T cells came from the experimental demonstration of MHC restriction by Rolf Zinkernagel and Peter Doherty. In their classic study, reported in 1974, these investigators examined the recognition of virus-infected cells by virus-specific CTLs in inbred mice. If a mouse is infected with a virus, $CD8^+$ CTLs specific for the virus develop in the animal. These CTLs recognize and kill virus-infected cells only if the infected cells express alleles of MHC molecules that are expressed in the animal in which the CTLs were generated (Fig. 6-6). By use of MHC congenic strains of mice (mice that were identical at every genetic locus except the MHC), it was shown that the CTLs and the infected target cell must be derived from mice that share a class I MHC allele. Thus, the recognition of antigens by $CD8^+$ CTLs is restricted by self class I MHC alleles. Subsequent experiments demonstrated that responses of $CD4^+$ helper T lymphocytes to antigens are self class II MHC restricted.

We continue our discussion of the MHC by describing the properties of the genes and then the proteins, and we conclude by describing how these proteins bind and display foreign antigens.

MHC Genes

The MHC locus contains two types of polymorphic MHC genes, the class I and class II MHC genes, which encode two groups of structurally distinct but homologous proteins, and other nonpolymorphic genes whose products are involved in antigen presentation (Fig. 6-7). Class I MHC molecules display peptides to and are recognized by $CD8^+$ T cells, and class II MHC molecules display peptides to $CD4^+$ T cells; each of these T cell types serves different functions in protection against microbes.

MHC genes are codominantly expressed in each individual. In other words, for a given MHC gene, each

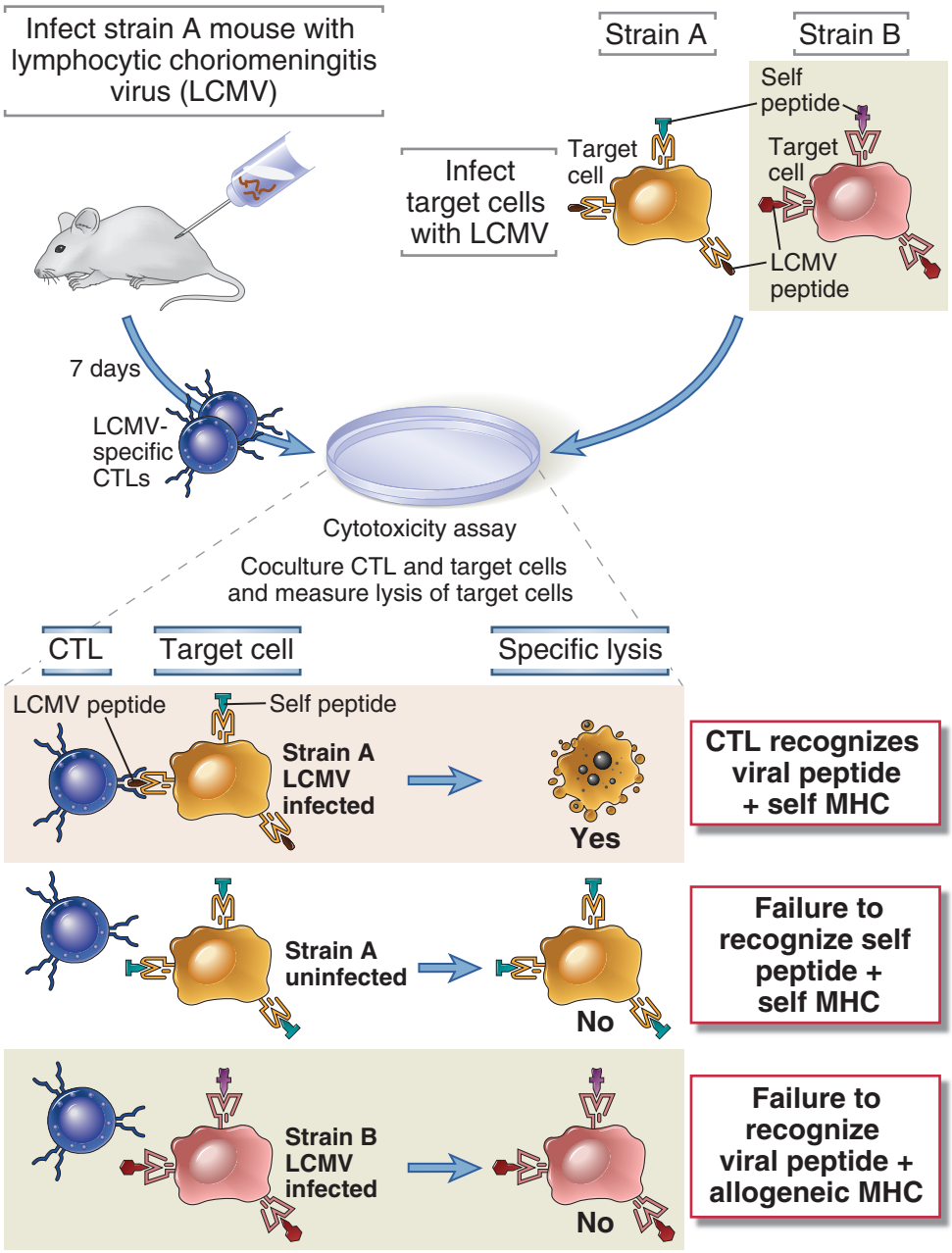


FIGURE 6–6 Experimental demonstration of the phenomenon of MHC restriction of T lymphocytes. Virus-specific cytotoxic T lymphocytes (CTLs) generated from virus-infected strain A mice kill only syngeneic (strain A) target cells infected with that virus. The CTLs do not kill uninfected strain A targets (which express self peptides but not viral peptides) or infected strain B targets (which express different MHC alleles than does strain A). By use of congenic mouse strains that differ only at class I MHC loci, it has been proved that recognition of antigen by $CD8^+$ CTLs is self class I MHC restricted.

individual expresses the alleles that are inherited from each of the two parents. For the individual, this maximizes the number of MHC molecules available to bind peptides for presentation to T cells.

Class I and class II MHC genes are the most polymorphic genes present in the genome. The studies of the mouse MHC were accomplished with a limited number of strains. Although it was appreciated that mouse MHC genes were polymorphic, only about 20 alleles of each MHC gene were identified in the available inbred strains of mice. The human serologic studies were conducted on outbred human populations. A remarkable feature to emerge from the studies of the human MHC genes is the unprecedented and unanticipated extent of their polymorphism. The total number of HLA alleles in the population is estimated to be about 3500, with more than 250 alleles for the HLA-B locus alone. Molecular sequencing has shown that a single serologically defined HLA allele may actually consist of multiple variants that differ slightly. Therefore, the polymorphism is even greater than that predicted from serologic studies. As we shall discuss later in the chapter, the polymorphic residues of

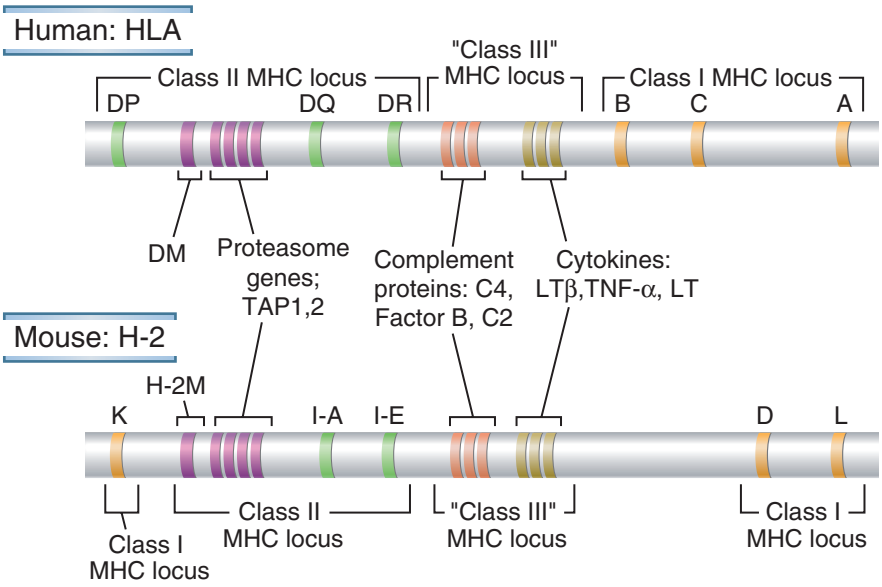


FIGURE 6–7 Schematic maps of human and mouse MHC loci. The basic organization of the genes in the MHC locus is similar in humans and mice. Sizes of genes and intervening DNA segments are not shown to scale. Class II loci are shown as single blocks, but each locus consists of several genes. Class III MHC locus refers to genes that encode molecules other than peptide-display molecules; this term is not used commonly.

MHC molecules determine the specificity of peptide binding and T cell antigen recognition, which has led to the question of why MHC genes are polymorphic. The presence of multiple MHC alleles in the population will ensure that at least some individuals in a population will be able to recognize protein antigens produced by virtually any microbe, and thus reduce the likelihood that a single pathogen can evade host defenses in all the individuals in a given species.

Human and Mouse MHC Loci

In humans, the MHC is located on the short arm of chromosome 6 and occupies a large segment of DNA, extending about 3500 kilobases (kb). (For comparison, a large human gene may extend up to 50 to 100 kb, and the size of the entire genome of the bacterium *Escherichia coli* is approximately 4500 kb.) In classical genetic terms, the MHC locus extends about 4 centimorgans, meaning that crossovers within the MHC occur with a frequency of about 4% at each meiosis. A molecular map of the human MHC is shown in Figure 6-8.

The human class I HLA genes were first defined by serologic approaches (antibody binding). There are three class I MHC genes called *HLA-A, HLA-B,* and *HLA-C,* which encode three class I MHC molecules with the same names. Class II MHC genes were first identified by use of assays in which T cells from one individual would be activated by cells of another individual (called the mixed lymphocyte reaction; see Chapter 16).There are three class II HLA gene loci called *HLA-DP, HLA-DQ,* and *HLA-DR*. Each class II MHC molecule is composed of a heterodimer of α and β polypeptides, and the DP, DQ, and DR loci each contain separate genes designated A or B, encoding α and β chains, respectively. More recently, DNA sequencing methods have been used to more precisely define MHC genes and their differences among individuals. The nomenclature of the HLA locus takes into account the enormous polymorphism (variation among individuals) identified by serologic and molecular methods. Thus, based on modern molecular typing, individual alleles may be called HLA-A*0201, referring to the 01 subtype of HLA-A2, or HLA-DRB1*0401, referring to the 01 subtype of the DR4 allele in the B1 gene, and so on.

The mouse MHC, located on chromosome 17, occupies about 2000 kb of DNA, and the genes are organized in an order slightly different from the human MHC gene. One of the mouse class I genes *(H-2K)* is centromeric to the class II region, but the other class I genes are telomeric to the class II region. There are three mouse class I MHC genes called *H-2K, H-2D,* and *H-2L,* encoding three different class I MHC proteins, *K, D,* and *L*. These genes are homologous to the human HLA-A, B, and C genes. The MHC alleles of particular inbred strains of mice are designated by lowercase letters (e.g., *a, b*), named for the whole set of MHC genes of the mouse strain in which they were first identified. In the parlance of mouse geneticists, the allele of the *H-2K* gene in a strain with the k-type MHC is called K^k (pronounced K of k), whereas the allele of the *H-2K* gene in a strain with d-type MHC is called K^d (K of d). Similar terminology is used for *H-2D* and *H-2L* alleles. Mice have two class II MHC loci called *I-A* and *I-E,* which encode the I-A and I-E molecules, respectively. These are located in the A and E subregions of the *Ir* region of the MHC and were discovered to be the *Ir genes* discussed earlier. The mouse class II genes are homologous to human HLA-DP, DQ, and DR genes. The I-A allele found in the inbred mouse strain with the K^k and D^k alleles is called $I\text{-}A^k$ (pronounced I A of k). Similar terminology is used for the I-E allele. As in humans, there are actually two different genes, designated A and B, in the I-A and I-E loci that encode the α and β chains of each class II MHC molecule.

The set of MHC alleles present on each chromosome is called an MHC **haplotype**. For instance, an HLA haplotype of an individual could be HLA-A2, HLA-B5, HLA-DR3, and so on. All heterozygous individuals, of course, have two HLA haplotypes. Inbred mice, being homozygous, have a single haplotype. Thus, the haplotype of an $H\text{-}2^d$ mouse is $H\text{-}2K^d\ I\text{-}A^d\ I\text{-}E^d\ D^d\ L^d$.

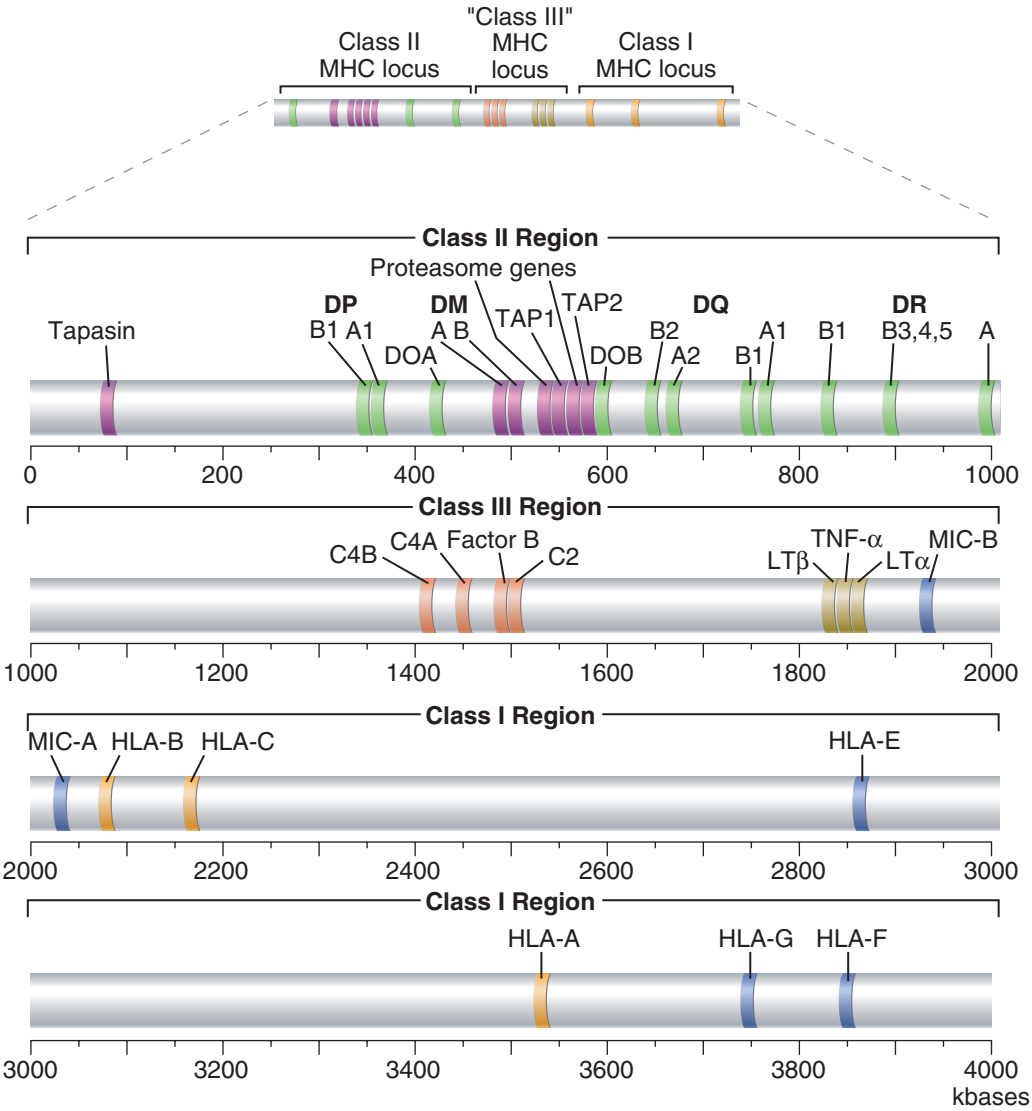


FIGURE 6–8 Map of the human MHC. The genes located within the human MHC locus are illustrated. In addition to the class I and class II MHC genes, HLA-E, HLA-F, and HLA-G and the MIC genes encode class I–like molecules, many of which are recognized by NK cells; C4, C2, and factor B genes encode complement proteins; tapasin, DM, DO, TAP, and proteasome encode proteins involved in antigen processing; LTα, LTβ, and TNF encode cytokines. Many pseudogenes and genes whose roles in immune responses are not established are located in the HLA complex but are not shown to simplify the map.

Expression of MHC Molecules

Because MHC molecules are required to present antigens to T lymphocytes, the expression of these proteins in a cell determines whether foreign (e.g., microbial) antigens in that cell will be recognized by T cells. There are several important features of the expression of MHC molecules that contribute to their role in protecting individuals from diverse microbial infections.

Class I molecules are constitutively expressed on virtually all nucleated cells, whereas class II molecules are expressed only on dendritic cells, B lymphocytes, macrophages, and a few other cell types. This pattern of MHC expression is linked to the functions of class I–restricted and class II–restricted T cells. The effector function of class I–restricted $CD8^+$ CTLs is to kill cells infected with intracellular microbes, such as viruses, as well as tumors that express tumor antigens. The expression of class I MHC molecules on nucleated cells serves provides a display system for viral and tumor antigens. In contrast, class II–restricted $CD4^+$ helper T lymphocytes have a set of functions that require recognizing antigen presented by a more limited number of cell types. In particular, naive $CD4^+$ T cells need to recognize antigens that are captured and presented by dendritic cells in lymphoid organs. Differentiated $CD4^+$ helper T lymphocytes function mainly to activate (or help) macrophages to eliminate extracellular microbes that have been phagocytosed and to activate B lymphocytes to make antibodies that also eliminate extracellular microbes. Class II molecules are expressed mainly on these cell types and provide a system for display of peptides derived from extracellular microbes and proteins.

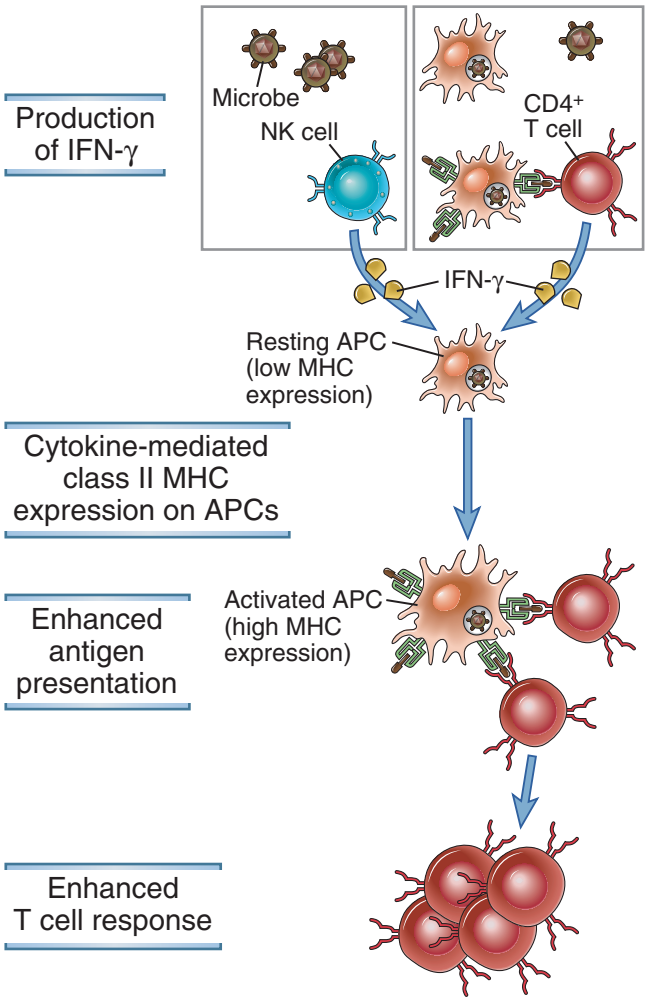


FIGURE 6–9 Enhancement of class II MHC expression by IFN-γ. IFN-γ, produced by NK cells and other cell types during innate immune reactions to microbes or by T cells during adaptive immune reactions, stimulates class II MHC expression on APCs and thus enhances the activation of $CD4^+$ T cells. IFN-γ and type I interferons have a similar effect on the expression of class I MHC molecules and the activation of $CD8^+$ T cells.

The expression of MHC molecules is increased by cytokines produced during both innate and adaptive immune responses (Fig. 6-9). On most cell types, the interferons IFN-α, IFN-β, and IFN-γ increase the level of expression of class I molecules. The interferons are cytokines produced during the early innate immune response to many viruses (see Chapter 4). Thus, innate immune responses to viruses increase the expression of the MHC molecules that display viral antigens to virus-specific T cells. This is one of the mechanisms by which innate immunity stimulates adaptive immune responses.

The expression of class II molecules is also regulated by cytokines and other signals in different cells. IFN-γ is the principal cytokine involved in stimulating expression of class II molecules in APCs such as dendritic cells and macrophages (see Fig. 6-9). IFN-γ may be produced by NK cells during innate immune reactions and by antigen-activated T cells during adaptive immune reactions. The ability of IFN-γ to increase class II MHC expression earlier than APCs is an amplification mechanism in adaptive immunity. As mentioned earlier, the expression of class II molecules also increases in response to signals from Toll-like receptors responding to microbial components, thus promoting the display of microbial antigens. B lymphocytes constitutively express class II molecules and can increase expression in response to antigen recognition and cytokines produced by helper T cells, thus enhancing antigen presentation to helper cells (see Chapter 11). IFN-γ can also increase the expression of MHC molecules on vascular endothelial cells and other nonimmune cell types; the role of these cells in antigen presentation to T lymphocytes is unclear. Some cells, such as neurons, never appear to express class II molecules. Activated human but not mouse T cells express class II molecules after activation; however, no cytokine has been identified in this response, and its functional significance is unknown.

The rate of transcription is the major determinant of the level of MHC molecule synthesis and expression on the cell surface. Cytokines enhance MHC expression by stimulating the transcription of class I and class II genes in a wide variety of cell types. These effects are mediated by the binding of cytokine-activated transcription factors to DNA sequences in the promoter regions of MHC genes. Several transcription factors may be assembled and bind a protein called the class II transcription activator (CIITA), and the entire complex binds to the class II promoter and promotes efficient transcription. By keeping the complex of transcription factors together, CIITA functions as a master regulator of class II gene expression. CIITA is synthesized in response to IFN-γ, explaining how this cytokine increases expression of class II MHC molecules. Mutations in several of these transcription factors have been identified as the cause of human immunodeficiency diseases associated with defective expression of MHC molecules. The best studied of these disorders is the **bare lymphocyte syndrome** (see Chapter 20). Knockout mice lacking CIITA also show reduced or absent class II expression on dendritic cells and B lymphocytes and an inability of IFN-γ to induce class II on all cell types.

The expression of many of the proteins involved in antigen processing and presentation is coordinately regulated. For instance, IFN-γ increases the transcription not only of class I and class II genes but also of several genes whose products are required for class I MHC assembly and peptide display, such as genes encoding the TAP transporter and some of the subunits of proteasomes, discussed later in the chapter.

MHC Molecules

Biochemical studies of MHC molecules culminated in the solution of the crystal structures for the extracellular portions of human class I and class II molecules. Subsequently, many MHC molecules with bound peptides have been crystallized and analyzed in detail. This knowledge has been enormously informative and, because of it, we now understand how MHC molecules display peptides. In this section, we first summarize the functionally important biochemical features that are common to class

TABLE 6–4 Features of Class I and Class II MHC Molecules

Feature	Class I MHC	Class II MHC
Polypeptide chains	α (44-47 kD) β_2- Microglobulin (12 kD)	α and β
Locations of polymorphic residues	α1 and α2 domains	α1 and β1 domains
Binding site for T cell coreceptor	CD8 binds to α3 region	CD4 binds to β2 region
Size of peptide-binding cleft	Accommodates peptides of 8-11 residues	Accommodates peptides of 10-30 residues or more
Nomenclature		
Human	HLA-A, HLA-B, HLA-C	HLA-DR, HLA-DQ, HLA-DP
Mouse	H-2K, H-2D, H-2L	I-A, I-E

I and class II MHC molecules. We then describe the structures of class I and class II proteins, pointing out their important similarities and differences (Table 6-4).

General Properties of MHC Molecules

All MHC molecules share certain structural characteristics that are critical for their role in peptide display and antigen recognition by T lymphocytes.

- ***Each MHC molecule consists of an extracellular peptide-binding cleft, or groove, followed by immunoglobulin (Ig)–like domains and transmembrane and cytoplasmic domains.*** Class I molecules are composed of one polypeptide chain encoded in the MHC and a second, non–MHC-encoded chain, whereas class II molecules are made up of two MHC-encoded polypeptide chains. Despite this difference, the overall three-dimensional structures of class I and class II molecules are similar.
- ***The polymorphic amino acid residues of MHC molecules are located in and adjacent to the peptide-binding cleft.*** This cleft is formed by the folding of the amino termini of the MHC-encoded proteins and is composed of paired α helices resting on a floor made up of an eight-stranded β-pleated sheet. The polymorphic residues, which are the amino acids that vary among different MHC alleles, are located in and around this cleft. This portion of the MHC molecule binds peptides for display to T cells, and the antigen receptors of T cells interact with the displayed peptide and with the α helices of the MHC molecules (see Fig. 6-1). Because of amino acid variability in this region, different MHC molecules bind and display different peptides and are recognized specifically by the antigen receptors of different T cells.
- ***The nonpolymorphic Ig-like domains of MHC molecules contain binding sites for the T cell molecules CD4 and CD8.*** CD4 and CD8 are expressed on distinct subpopulations of mature T lymphocytes and participate, together with antigen receptors, in the recognition of antigen; that is, CD4 and CD8 are T cell "coreceptors" (see Chapter 7). CD4 binds selectively to class II MHC molecules, and CD8 binds to class I molecules. This is why ***$CD4^+$ helper T cells recognize class II MHC molecules displaying peptides, whereas $CD8^+$ T cells recognize class I MHC molecules with bound peptides.*** Stated differently, $CD4^+$ T cells are class II MHC restricted, and $CD8^+$ T cells are class I MHC restricted.

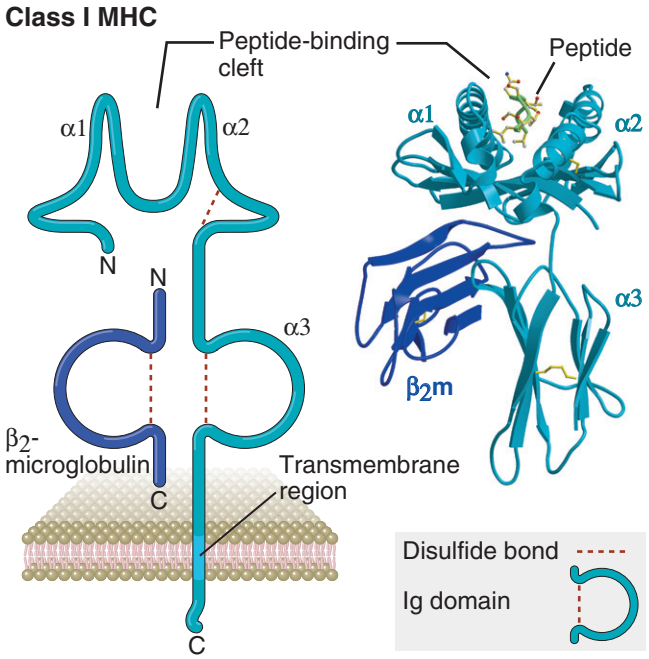


FIGURE 6–10 Structure of a class I MHC molecule. The schematic diagram *(left)* illustrates the different regions of the MHC molecule (not drawn to scale). Class I molecules are composed of a polymorphic α chain noncovalently attached to the nonpolymorphic β_2-microglobulin (β_2m). The α chain is glycosylated; carbohydrate residues are not shown. The ribbon diagram *(right)* shows the structure of the extracellular portion of the HLA-B27 molecule with a bound peptide, resolved by x-ray crystallography. *(Courtesy of Dr. P. Bjorkman, California Institute of Technology, Pasadena.)*

Class I MHC Molecules

Class I molecules consist of two noncovalently linked polypeptide chains, an MHC-encoded 44- to 47-kD α chain (or heavy chain) and a non–MHC-encoded 12-kD subunit called β_2-microglobulin (Fig. 6-10). Each α chain is oriented so that about three quarters of the complete polypeptide extends into the extracellular milieu, a short hydrophobic segment spans the cell membrane, and the carboxyl-terminal residues are located in the cytoplasm. The amino-terminal (N-terminal) α1 and α2 segments of

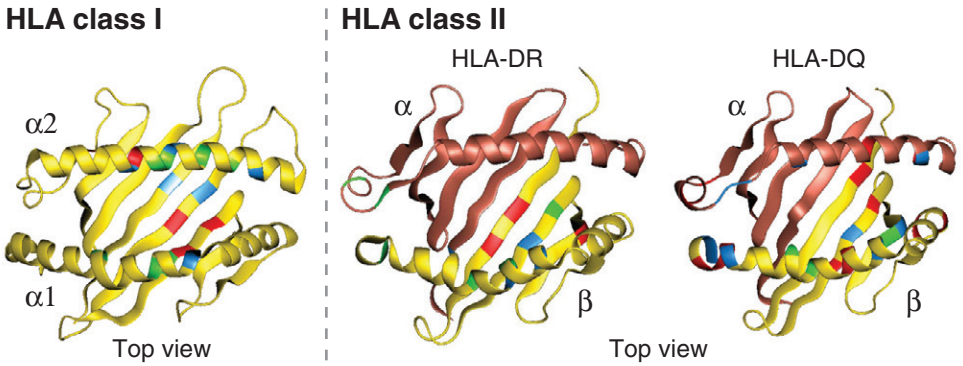


FIGURE 6–11 Polymorphic residues of MHC molecules. The polymorphic residues of class I and class II MHC molecules are located in the peptide-binding clefts and the α helices around the clefts. The regions of greatest variability among different HLA alleles are indicated in red, of intermediate variability in green, and of the lowest variability in blue. *(Reproduced with permission from Margulies DH, K Natarajan, J Rossjohn, and J McCluskey. Major histocompatibility complex [MHC] molecules: structure, function, and genetics. In WE Paul [ed]: Fundamental Immunology, 6th ed. Lippincott Williams & Wilkins, Philadelphia, 2008.)*

the α chain, each approximately 90 residues long, interact to form a platform of an eight-stranded, antiparallel β-pleated sheet supporting two parallel strands of α helix. This forms the peptide-binding cleft of class I molecules. Its size is large enough (~25 Å × 10 Å × 11 Å) to bind peptides of 8 to 11 amino acids in a flexible, extended conformation. The ends of the class I peptide-binding cleft are closed so that larger peptides cannot be accommodated. Therefore, native globular proteins have to be "processed" to generate fragments that are small enough to bind to MHC molecules and to be recognized by T cells (described later). The polymorphic residues of class I molecules are confined to the α1 and α2 domains, where they contribute to variations among different class I alleles in peptide binding and T cell recognition (Fig. 6-11). The α3 segment of the α chain folds into an Ig domain whose amino acid sequence is conserved among all class I molecules. This segment contains the binding site for CD8. At the carboxyl-terminal end of the α3 segment is a stretch of approximately 25 hydrophobic amino acids that traverses the lipid bilayer of the plasma membrane. Immediately following this are approximately 30 residues located in the cytoplasm, which include a cluster of basic amino acids that interact with phospholipid head groups of the inner leaflet of the lipid bilayer and anchor the MHC molecule in the plasma membrane.

β_2-Microglobulin, the light chain of class I molecules, is encoded by a gene outside the MHC and is named for its electrophoretic mobility (β_2), size (micro), and solubility (globulin). β_2-Microglobulin interacts noncovalently with the α3 domain of the α chain. Like the α3 segment, β_2-microglobulin is structurally homologous to an Ig domain and is invariant among all class I molecules.

The fully assembled class I molecule is a heterotrimer consisting of an α chain, β_2-microglobulin, and a bound antigenic peptide, and stable expression of class I molecules on cell surfaces requires the presence of all three components of the heterotrimer. The reason for this is that the interaction of the α chain with β_2-microglobulin is stabilized by binding of peptide antigens to the cleft formed by the α1 and α2 segments, and conversely, the binding of peptide is strengthened by the interaction of β_2-microglobulin with the α chain. Because antigenic peptides are needed to stabilize the MHC molecules, only potentially useful peptide-loaded MHC molecules are expressed on cell surfaces.

Most individuals are heterozygous for MHC genes and therefore express six different class I molecules on every cell, containing α chains encoded by the two inherited alleles of *HLA-A, HLA-B,* and *HLA-C* genes.

Class II MHC Molecules

Class II MHC molecules are composed of two noncovalently associated polypeptide chains, a 32- to 34-kD α chain and a 29- to 32-kD β chain (Fig. 6-12). Unlike class I molecules, the genes encoding both chains of class II molecules are polymorphic and present in the MHC locus.

The amino-terminal α1 and β1 segments of the class II chains interact to form the peptide-binding cleft, which is structurally similar to the cleft of class I molecules. Four strands of the floor of the cleft and one of the α-helical walls are formed by the α1 segment, and the other four strands of the floor and the second wall are formed by the β1 segment. The polymorphic residues are located in the α1 and β1 segments, in and around the peptide-binding cleft, as in class I molecules (see Fig. 6-11). In human class II molecules, most of the polymorphism is in the β chain. In class II molecules, the ends of the peptide-binding cleft are open, so that peptides of 30 residues or more can fit.

The α2 and β2 segments of class II molecules, like class I α3 and β_2-microglobulin, are folded into Ig domains and are nonpolymorphic, that is, they do not vary among alleles of a particular class II gene. The β2 segment of class II molecules contains the binding site for CD4, similar to the binding site for CD8 in the α3 segment of the class I heavy chain. In general, α chains of one class II MHC locus (e.g., DR) most often pair with β chains of the same locus and less commonly with β chains of other loci (e.g., DQ, DP). The carboxyl-terminal ends of the α2 and β2 segments continue into short connecting regions followed by approximately 25–amino acid stretches of

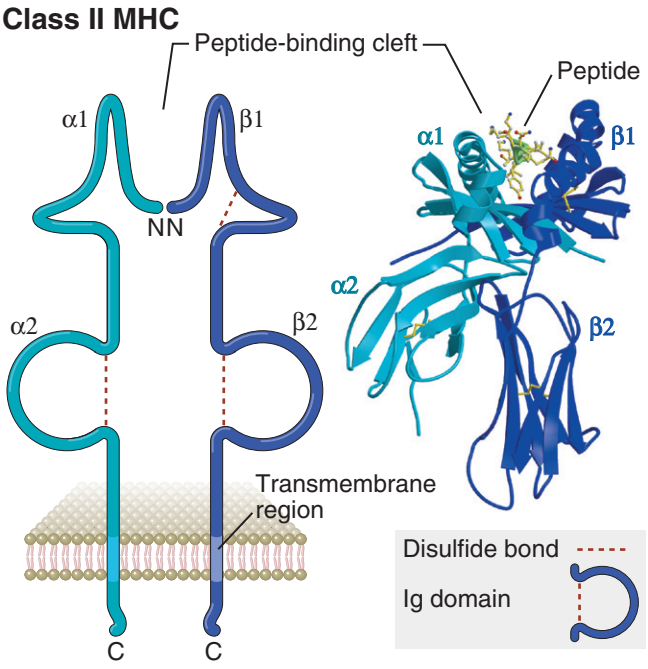


FIGURE 6–12 Structure of a class II MHC molecule. The schematic diagram *(left)* illustrates the different regions of the MHC molecule (not drawn to scale). Class II molecules are composed of a polymorphic α chain noncovalently attached to a polymorphic β chain. Both chains are glycosylated; carbohydrate residues are not shown. The ribbon diagram *(right)* shows the structure of the extracellular portion of the HLA-DR1 molecule with a bound peptide, resolved by x-ray crystallography. *(Courtesy of Dr. P. Bjorkman, California Institute of Technology, Pasadena.)*

hydrophobic transmembrane residues. In both chains, the transmembrane regions end with clusters of basic amino acid residues, followed by short, hydrophilic cytoplasmic tails.

The fully assembled class II molecule is a heterotrimer consisting of an α chain, a β chain, and a bound antigenic peptide, and stable expression of class II molecules on cell surfaces requires the presence of all three components of the heterotrimer. As in class I molecules, this ensures that the MHC molecules that end up on the cell surface are the molecules that are serving their normal function of peptide display.

Humans inherit, from each parent, one DPA1 and one DPB1 gene encoding, respectively, the α and β chains of an HLA-DP molecule; one DQA1 and one DQB1 gene; and one DRA1 gene, a DRB1 gene, and a separate duplicated DRB gene that may encode the alleles DRB3, 4, or 5. Thus, each heterozygous individual inherits six or eight class II MHC alleles, three or four from each parent (one set each of DP and DQ, and one or two of DR). Typically, there is not much recombination between genes of different loci (i.e., DRα with DQβ, and so on), and each haplotype tends to be inherited as a single unit. However, because some haplotypes contain extra DRB loci that produce β chains that assemble with DRα, and some DQα molecules encoded on one chromosome can associate with DQβ molecules produced from the other chromosome, the total number of expressed class II molecules may be considerably more than six.

Binding of Peptides to MHC Molecules

Following the realization that the immunogenicity of proteins depends on the ability of their peptides to be displayed by MHC molecules, considerable effort has been devoted to elucidating the molecular basis of peptide-MHC interactions and the characteristics of peptides that allow them to bind to MHC molecules. These studies have relied on functional assays of helper T cells and CTLs responding to APCs that were incubated with different peptides and direct binding studies of purified MHC molecules with radioactively or fluorescently labeled peptides in solution by methods such as equilibrium dialysis and gel filtration. X-ray crystallographic analysis of peptide-MHC complexes has provided definitive information about how peptides sit in the clefts of MHC molecules and about the residues of each that participate in this binding. In the section that follows, we summarize the key features of the interactions between peptides and class I or class II MHC molecules.

Characteristics of Peptide-MHC Interactions

MHC molecules show a broad specificity for peptide binding, in contrast to the fine specificity of antigen recognition of the antigen receptors of lymphocytes. There are several important features of the interactions of MHC molecules and antigenic peptides.

- ***Each class I or class II MHC molecule has a single peptide-binding cleft that binds one peptide at a time, but each MHC molecule can bind many different peptides.*** One of the earliest lines of evidence supporting this conclusion was the experimental result that different peptides that bind to the same MHC molecule can competitively inhibit one another's presentation, implying that there is only a single peptide-binding cleft in every MHC molecule. The solution of the crystal structures of class I and class II MHC molecules confirmed the presence of a single peptide-binding cleft in these molecules (see Figs. 6-10 and 6-12). It is not surprising that a single MHC molecule can bind multiple peptides because each individual contains only a few different MHC molecules (6 class I and more than 10 to 20 class II molecules in a heterozygous individual), and these must be able to present peptides from the enormous number of protein antigens that one is likely to encounter.
- ***The peptides that bind to MHC molecules share structural features that promote this interaction.*** One of these features is the size of the peptide—class I molecules can accommodate peptides that are 8 to 11 residues long, and class II molecules bind peptides that may be 10 to 30 residues long or longer, the optimal length being 12 to 16 residues. In addition, peptides that bind to a particular allelic form of an MHC molecule contain amino acid residues that allow complementary interactions between the peptide and that allelic MHC molecule. The residues of a peptide that bind to MHC molecules are distinct from those that are recognized by T cells.
- ***MHC molecules acquire their peptide cargo during their biosynthesis and assembly inside cells.*** Therefore,

MHC molecules display peptides derived from microbes that are inside host cells, and this is why MHC-restricted T cells recognize cell-associated microbes and are the mediators of immunity to intracellular microbes. Importantly, class I MHC molecules acquire peptides from cytosolic proteins and class II molecules from proteins in intracellular vesicles. The mechanisms and significance of these processes are discussed later in the chapter.

- ***The association of antigenic peptides and MHC molecules is a saturable interaction with a very slow off-rate.*** In a cell, several chaperones and enzymes facilitate the binding of peptides to MHC molecules (described later). Once formed, most peptide-MHC complexes are stable, and kinetic dissociation constants are indicative of long half-lives that range from hours to many days. This extraordinarily slow off-rate of peptide dissociation from MHC molecules ensures that after an MHC molecule has acquired a peptide, it will display the peptide long enough to maximize the chance that a particular T cell will find the peptide it can recognize and initiate a response.
- ***Very small numbers of peptide-MHC complexes are capable of activating specific T lymphocytes.*** Because APCs continuously present peptides derived from all the proteins they encounter, only a very small fraction of cell surface peptide-MHC complexes will contain the same peptide. It has been estimated that as few as 100 complexes of a particular peptide with a class II MHC molecule on the surface of an APC can initiate a specific T cell response. This represents less than 0.1% of the total number of class II molecules likely to be present on the surface of the APC.
- ***The MHC molecules of an individual do not discriminate between foreign peptides (e.g., those derived from microbial proteins) and peptides derived from the proteins of that individual (self antigens).*** Thus, MHC molecules display both self peptides and foreign peptides, and T cells survey these displayed peptides for the presence of foreign antigens. In fact, if the peptides being displayed normally by APCs are purified, most of them turn out to be derived from self proteins. The inability of MHC molecules to discriminate between self antigens and foreign antigens raises two questions. First, how can a T cell recognize and be activated by any foreign antigen if normally all APCs are displaying mainly self peptide–MHC complexes? The answer, as mentioned before, is that T cells are remarkably sensitive and need to specifically recognize very few peptide-MHC complexes to be activated. Thus, a newly introduced antigen may be processed into peptides that load enough MHC molecules of APCs to activate T cells specific for that antigen, even though most of the MHC molecules are occupied with self peptides. Second, if individuals process their own proteins and present them in association with their own MHC molecules, why do we normally not develop immune responses against self proteins? The answer to this question is that self peptide–MHC complexes are formed but do not induce autoimmunity because T cells specific for such complexes are killed or inactivated. Therefore, T cells cannot normally respond to self antigens (see Chapter 14).

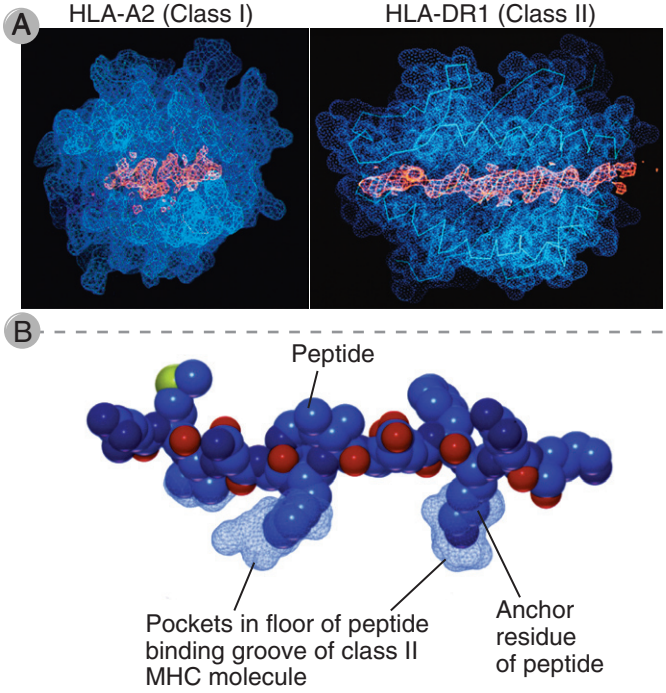


FIGURE 6–13 Peptide binding to MHC molecules. A, These top views of the crystal structures of MHC molecules show how peptides lie in the peptide-binding clefts. The class I molecule shown is HLA-A2, and the class II molecule is HLA-DR1. The cleft of the class I molecule is closed, whereas that of the class II molecule is open. As a result, class II molecules accommodate longer peptides than do class I molecules. *(Reprinted with permission of Macmillan Publishers Ltd. from Bjorkman PJ, MA Saper, B Samraoui, WS Bennett, JL Strominger, and DC Wiley. Structure of the human class I histocompatibility antigen HLA-A2. Nature 329:506-512, 1987; and Brown J, TS Jardetzky, JC Gorga, LJ Stern, RG Urban, JL Strominger, and DC Wiley. Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. Nature 364:33-39, 1993.)* **B,** The side view of a cutout of a peptide bound to a class II MHC molecule shows how anchor residues of the peptide hold it in the pockets in the cleft of the MHC molecule. *(From Scott CA, PA Peterson, L Teyton, and IA Wilson. Crystal structures of two I-A^d–peptide complexes reveal that high affinity can be achieved without large anchor residues. Immunity 8:319-329, 1998. Copyright 1998, with permission from Elsevier Science.)*

Structural Basis of Peptide Binding to MHC Molecules

The binding of peptides to MHC molecules is a noncovalent interaction mediated by residues both in the peptides and in the clefts of the MHC molecules. As we shall see later, protein antigens are proteolytically cleaved in APCs to generate the peptides that will be bound and displayed by MHC molecules. These peptides bind to the clefts of MHC molecules in an extended conformation. Once bound, the peptides and their associated water molecules fill the clefts, making extensive contacts with the amino acid residues that form the β strands of the floor and the α helices of the walls of the cleft (Fig. 6-13). In the case of class I MHC molecules, association of a peptide with the MHC groove depends on the binding of the positively charged N terminus and the negatively charged C terminus of the peptide to the MHC molecule by

electrostatic interactions. In most MHC molecules, the β strands in the floor of the cleft contain "pockets." Many class I molecules have a hydrophobic pocket that recognizes one of the following hydrophobic amino acids—valine, isoleucine, leucine, or methionine—at the C-terminal end of the peptide. Some class I molecules have a predilection for a basic residue (lysine or arginine) at the C terminus. In addition, other amino acid residues of a peptide may contain side chains that fit into specific pockets and bind to complementary amino acids in the MHC molecule through electrostatic interactions (charge-based salt bridges), hydrogen bonding, or van der Waals interactions. Such residues of the peptide are called anchor residues because they contribute most of the favorable interactions of the binding (i.e., they anchor the peptide in the cleft of the MHC molecule). Each MHC-binding peptide usually contains only one or two anchor residues, and this presumably allows greater variability in the other residues of the peptide, which are the residues that are recognized by specific T cells. In the case of some peptides binding to MHC molecules, especially class II molecules, specific interactions of peptides with the α-helical sides of the MHC cleft also contribute to peptide binding by forming hydrogen bonds or charge interactions. Class II MHC molecules accommodate larger peptides than class I MHC molecules. These longer peptides extend at either end beyond the floor of the groove.

Because many of the residues in and around the peptide-binding cleft of MHC molecules are polymorphic (i.e., they differ among various MHC alleles), different alleles favor the binding of different peptides. This is the structural basis for the function of MHC genes as "immune response genes"; only animals that express MHC alleles that can bind a particular peptide and display it to T cells can respond to that peptide.

The antigen receptors of T cells recognize both the antigenic peptide and the MHC molecules, with the peptide being responsible for the fine specificity of antigen recognition and the MHC residues accounting for the MHC restriction of the T cells. A portion of the bound peptide is exposed from the open top of the cleft of the MHC molecule, and the amino acid side chains of this portion of the peptide are recognized by the antigen receptors of specific T cells. The same T cell receptor also interacts with polymorphic residues of the α helices of the MHC molecule itself (see Fig. 6-1). Predictably, variations in either the peptide antigen or the peptide-binding cleft of the MHC molecule will alter presentation of that peptide or its recognition by T cells. In fact, one can enhance the immunogenicity of a peptide by incorporating into it a residue that strengthens its binding to commonly inherited MHC molecules in a population.

Because MHC molecules can bind only peptides but most antigens are large proteins, there must be ways by which these proteins are converted into peptides. The conversion is called **antigen processing** and is the focus of the remainder of the chapter.

PROCESSING OF PROTEIN ANTIGENS

The pathways of antigen processing convert protein antigens present in the cytosol or internalized from the extracellular environment into peptides and load these peptides onto MHC molecules for display to T lymphocytes (Fig. 6-14). The mechanisms of antigen processing are designed to generate peptides that have the structural

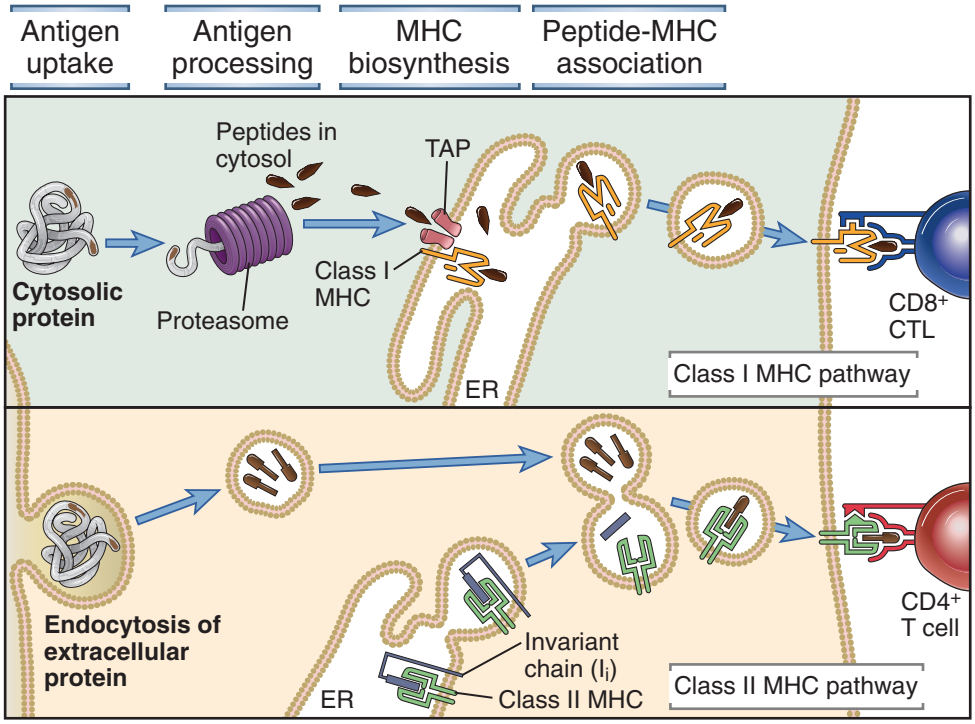


FIGURE 6–14 Pathways of antigen processing and presentation. In the class I MHC pathway *(top panel)*, protein antigens in the cytosol are processed by proteasomes, and peptides are transported into the endoplasmic reticulum (ER), where they bind to class I MHC molecules. In the class II MHC pathway *(bottom panel)*, extracellular protein antigens are endocytosed into vesicles, where the antigens are processed and the peptides bind to class II MHC molecules. Details of these processing pathways are in Figures 6-16 and 6-17.

TABLE 6–5 Comparative Features of Class I and Class II MHC Pathways of Antigen Processing and Presentation

Feature	Class I MHC Pathway	Class II MHC Pathway
Composition of stable peptide-MHC complex	Polymorphic α chain, β_2-microglobulin, peptide 	Polymorphic α and β chains, peptide 
Types of APCs	All nucleated cells	Dendritic cells, mononuclear phagocytes, B lymphocytes; endothelial cells, thymic epithelium
Responsive T cells	$CD8^+$ T cells	$CD4^+$ T cells
Source of protein antigens	Cytosolic proteins (mostly synthesized in the cell; may enter cytosol from phagosomes)	Endosomal and lysosomal proteins (mostly internalized from extracellular environment)
Enzymes responsible for peptide loading of MHC	Cytosolic proteasome	Endosomal and lysosomal proteases (e.g., cathepsins)
Site of peptide loading of MHC	Endoplasmic reticulum	Specialized vesicular compartment
Molecules involved in transport of peptides and loading of MHC molecules	Chaperones, TAP in ER	Chaperones in ER; invariant chain in ER, Golgi and MIIC/CIIV; DM

APC, antigen-presenting cell; CIIV, class II vesicle; ER, endoplasmic reticulum; MHC, major histocompatibility complex; MIIC, MHC class II compartment; TAP, transporter associated with antigen processing.

characteristics required for associating with MHC molecules and to place these peptides in the same cellular location as the appropriate MHC molecules with available peptide-binding clefts. Peptide binding to MHC molecules occurs before cell surface expression and is an integral component of the biosynthesis and assembly of MHC molecules. In fact, as mentioned earlier, peptide association is required for the stable assembly and surface expression of class I and class II MHC molecules.

Protein antigens that are present in the cytosol (usually synthesized in the cell) generate class I–associated peptides that are recognized by $CD8^+$ T cells, whereas antigens internalized from the extracellular environment into the vesicles of APCs generate peptides that are displayed by class II MHC molecules and recognized by $CD4^+$ T cells. The different fates of cytosolic and vesicular antigens are due to the segregated pathways of biosynthesis and assembly of class I and class II MHC molecules (see Fig. 6-14 and Table 6-5). This fundamental difference between cytosolic and vesicular antigens has been demonstrated experimentally by analyzing the presentation of the same antigen introduced into APCs in different ways (Fig. 6-15). If a protein antigen is produced in the cytoplasm of APCs as the product of a transfected gene (modified so its protein product cannot enter the secretory pathway) or introduced directly into the cytoplasm of the APCs by osmotic shock, it is presented in the form of class I–associated peptides that are recognized by $CD8^+$ T cells. In contrast, if the same protein is added in soluble form to APCs and endocytosed into the vesicles of the APCs, it is subsequently presented as class II–associated peptides and is recognized by antigen-specific $CD4^+$ T cells.

We first describe these two pathways of antigen processing and then their functional significance.

The Class I MHC Pathway for Processing and Presentation of Cytosolic Proteins

Class I MHC–associated peptides are produced by the proteolytic degradation of cytosolic proteins, the transport of the generated peptides into the endoplasmic reticulum (ER), and their binding to newly synthesized class I molecules. This sequence of events is illustrated in Figure 6-16, and the individual steps are described next.

Sources of Cytosolic Protein Antigens

Most cytosolic protein antigens are synthesized within cells, and some are phagocytosed and transported into the cytosol. Foreign antigens in the cytosol may be the products of viruses or other intracellular microbes that infect such cells. In tumor cells, various mutated or overexpressed genes may produce protein antigens that are recognized by class I–restricted CTLs (see Chapter 17). Peptides that are presented in association with class I molecules may also be derived from microbes and other particulate antigens that are internalized into phagosomes but escape into the cytosol. Some microbes are able to damage phagosome membranes and create pores through which the microbes and their antigens enter the cytosol. For instance, pathogenic strains of *Listeria monocytogenes* produce a protein, called listeriolysin, that enables bacteria to escape from vesicles into the cytosol. (This escape is a mechanism that the bacteria may have evolved to resist killing by the microbicidal mechanisms of phagocytes, most of which are concentrated in phagolysosomes.) Once the antigens of the phagocytosed microbes are in the cytosol, they are processed like other cytosolic antigens. In dendritic cells, some antigens that are ingested into vesicles enter the cytosolic class I

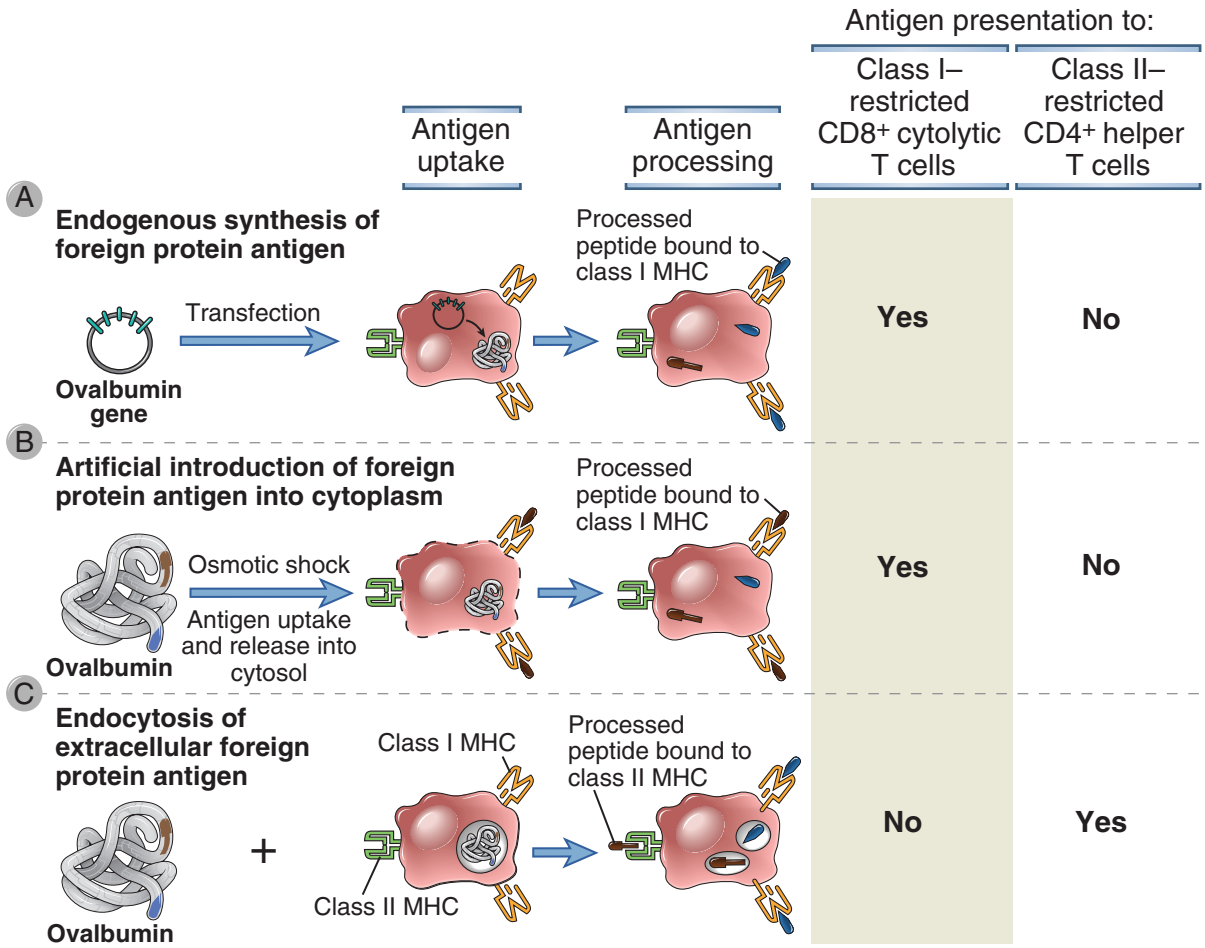


FIGURE 6–15 Experimental demonstration of presentation of cytosolic and extracellular antigens. When a model protein antigen, ovalbumin, is synthesized intracellularly as a result of transfection of its gene modified to lack the N-terminal signal sequences **(A)** or when it is introduced into the cytoplasm through membranes made leaky by osmotic shock **(B)**, ovalbumin-derived peptides are presented in association with class I MHC molecules. When ovalbumin is added as an extracellular antigen to an APC that expresses both class I and class II MHC molecules, ovalbumin-derived peptides are presented only in association with class II molecules **(C)**. The measured response of class I–restricted CTLs is killing of the APCs, and the measured response of class II–restricted helper T cells is cytokine secretion.

pathway, in the process called cross-presentation that is described later. Other important sources of peptides in the cytosol are misfolded proteins in the ER that are translocated into the cytosol and degraded like other cytosolic proteins; this process is called ER-associated degradation.

Proteolytic Digestion of Cytosolic Proteins

The major mechanism for the generation of peptides from cytosolic protein antigens is proteolysis by the proteasome. Proteasomes are large multiprotein enzyme complexes with a broad range of proteolytic activity that are found in the cytoplasm and nuclei of most cells. The proteasome appears as a cylinder composed of a stacked array of two inner β rings and two outer α rings, each ring being composed of seven subunits, with a cap-like structure at either end of the cylinder. The proteins in the outer α rings are structural and lack proteolytic activity; in the inner β rings, three of the seven subunits (β1, β2, and β5) are the catalytic sites for proteolysis.

The proteasome performs a basic housekeeping function in cells by degrading many damaged or improperly folded proteins. Protein synthesis normally occurs at a rapid rate, about six to eight amino acid residues being incorporated into elongating chains every second. The process is error prone, and it is estimated that approximately 20% of newly synthesized proteins are misfolded. These defective ribosomal products as well as older effete proteins are targeted for proteasomal degradation by covalent linkage of several copies of a small polypeptide called ubiquitin. Ubiquitinated proteins, with chains of four or more ubiquitins, are recognized by the proteasomal cap and are then unfolded, the ubiquitin is removed, and the proteins are "threaded" through proteasomes, where they are degraded into peptides. The proteasome has broad substrate specificity and can generate a wide variety of peptides from cytosolic proteins (but usually does not degrade proteins completely into single amino acids). Interestingly, in cells treated with the cytokine IFN-γ, there is increased transcription and synthesis of three novel catalytic subunits of the proteasome known as β1i, β2i, and β5i, which replace the three catalytic subunits of the β ring of the proteasome. This results in a change in the substrate specificity of the proteasome

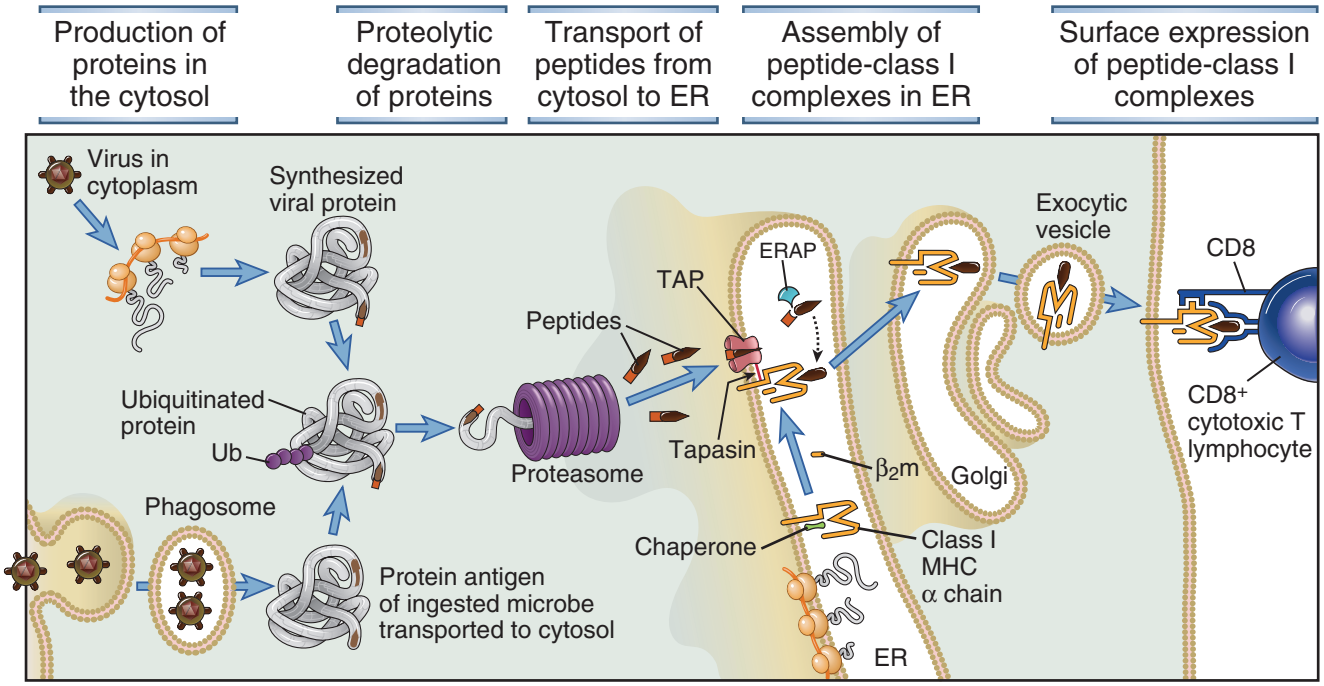


FIGURE 6–16 The class I MHC pathway of antigen presentation. The stages in the processing of cytosolic proteins are described in the text. ERAP, endoplasmic reticulum associated peptidase; ER, endoplasmic reticulum; β_2m, β_2-microglobulin; TAP, transporter associated with antigen processing; Ub, ubiquitin.

so that the peptides produced usually contain carboxyl-terminal hydrophobic amino acids such as leucine, valine, isoleucine, and methionine or basic residues such as lysine or arginine. These kinds of C termini are typical of peptides that are transported into the class I pathway and bind to class I molecules. This is one mechanism by which IFN-γ enhances antigen presentation, another mechanism being increased expression of MHC molecules (see Fig. 6-9). Thus, proteasomes are excellent examples of organelles whose basic cellular function has been adapted for a specialized role in antigen presentation.

Some protein antigens apparently do not require ubiquitination or proteasomes to be presented by the class I MHC pathway and are presumably degraded by cytosolic proteases. In addition, the signal sequences of membrane and secreted proteins are usually cleaved by signal peptidase and degraded proteolytically soon after synthesis and translocation into the ER. This ER processing generates class I–binding peptides without a need for proteolysis in the cytosol.

Transport of Peptides from the Cytosol to the Endoplasmic Reticulum

Peptides generated in the cytosol are translocated by a specialized transporter into the ER, where newly synthesized class I MHC molecules are available to bind the peptides. Because antigenic peptides for the class I pathway are generated in the cytosol but class I MHC molecules are synthesized in the ER, a mechanism is needed to deliver cytosolic peptides into the ER. This transport is mediated by a dimeric protein called transporter associated with antigen processing (TAP), which is homologous to the ABC transporter family of proteins that mediate ATP-dependent transport of low-molecular-weight compounds across cellular membranes. The TAP protein is located in the ER membrane, where it mediates the active, ATP-dependent transport of peptides from the cytosol into the ER lumen. Although the TAP heterodimer has a broad range of specificities, it optimally transports peptides ranging from 8 to 16 amino acids in length and containing carboxyl termini that are basic (in humans) or hydrophobic (in humans and mice). As mentioned before, these are the characteristics of the peptides that are generated in the proteasome and are able to bind to class I MHC molecules.

On the luminal side of the ER membrane, the TAP protein associates with a protein called tapasin, which also has an affinity for newly synthesized empty class I MHC molecules. Tapasin thus brings the TAP transporter into a complex with the class I MHC molecules that are awaiting the arrival of peptides.

Assembly of Peptide–Class I MHC Complexes in the Endoplasmic Reticulum

Peptides translocated into the ER bind to class I MHC molecules that are associated with the TAP dimer through tapasin. The synthesis and assembly of class I molecules involve a multistep process in which peptide binding plays a key role. Class I α chains and β_2-microglobulin are synthesized in the ER. Appropriate folding of the nascent α chains is assisted by chaperone proteins, such as the membrane chaperone calnexin and the luminal chaperone calreticulin. Within the ER, the newly formed empty class I dimers remain linked to the TAP complex. Empty class I MHC molecules, tapasin, and TAP are part of a larger peptide-loading complex in the ER that also

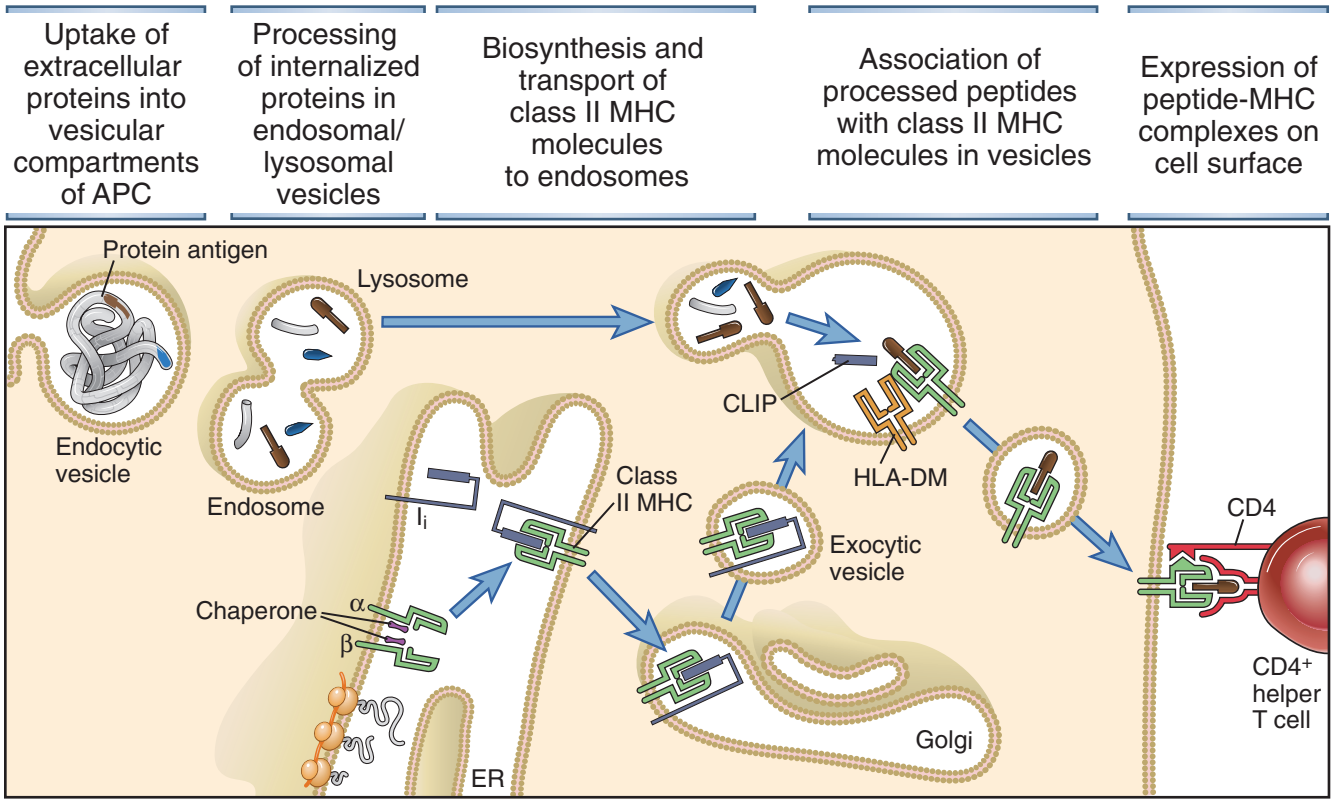


FIGURE 6–17 The class II MHC pathway of antigen presentation. The stages in the processing of extracellular antigens are described in the text. CLIP, class II–associated invariant chain peptide; ER, endoplasmic reticulum; I_i, invariant chain.

includes calnexin, calreticulin, and the oxidoreductase Erp57, all of which contribute to class I assembly and loading. Peptides that enter the ER through TAP and peptides produced in the ER, such as signal peptides, are often trimmed to the appropriate size for MHC binding by the ER-resident aminopeptidase ERAP. The peptide is then able to bind to the cleft of the adjacent class I molecule. Once class I MHC molecules are loaded with peptide, they no longer have an affinity for tapasin, so the peptide–class I complex is released from tapasin, and it is able to exit the ER and be transported to the cell surface. In the absence of bound peptide, many of the newly formed α chain–β_2-microglobulin dimers are unstable and cannot be transported efficiently from the ER to the Golgi. These misfolded empty class I MHC complexes are transported into the cytosol and are degraded in proteasomes.

Peptides transported into the ER preferentially bind to class I but not class II MHC molecules, for two reasons. First, newly synthesized class I molecules are attached to the luminal aspect of the TAP complex, and they capture peptides rapidly as the peptides are transported into the ER by the TAP. Second, as discussed later, in the ER, the peptide-binding clefts of newly synthesized class II molecules are blocked by the associated I_i.

Surface Expression of Peptide–Class I MHC Complexes

Class I MHC molecules with bound peptides are structurally stable and are expressed on the cell surface. Stable peptide–class I MHC complexes that were produced in the ER move through the Golgi complex and are transported to the cell surface by exocytic vesicles. Once expressed on the cell surface, the peptide–class I complexes may be recognized by peptide antigen–specific $CD8^+$ T cells, with the CD8 coreceptor playing an essential role by binding to nonpolymorphic regions of the class I molecule. In later chapters, we will return to a discussion of the role of class I–restricted CTLs in protective immunity. Several viruses have evolved mechanisms that interfere with class I assembly and peptide loading, emphasizing the importance of this pathway for antiviral immunity (see Chapter 15).

The Class II MHC Pathway for Processing and Presentation of Vesicular Proteins

The generation of class II MHC–associated peptides from endocytosed antigens involves the proteolytic degradation of internalized proteins in endocytic vesicles and the binding of peptides to class II MHC molecules in these vesicles. This sequence of events is illustrated in Figure 6-17, and the individual steps are described next.

Generation of Vesicular Proteins

Most class II–associated peptides are derived from protein antigens that are captured from the extracellular environment and internalized into endosomes by specialized APCs. The initial steps in the presentation of an extracellular protein antigen are the binding of the native antigen

to an APC and the internalization of the antigen. Different APCs can bind protein antigens in several ways and with varying efficiencies and specificities. Dendritic cells and macrophages express a variety of surface receptors that recognize structures shared by many microbes (see Chapter 4). These APCs use the receptors to bind and internalize microbes efficiently. Macrophages also express receptors for the Fc portions of antibodies and receptors for the complement protein C3b, which bind antigens with attached antibodies or complement proteins and enhance their internalization. Another example of specific receptors on APCs is the surface immunoglobulin on B cells, which, because of its high affinity for antigens, can effectively mediate the internalization of proteins present at very low concentrations in the extracellular fluid (see Chapter 11).

After their internalization, protein antigens become localized in intracellular membrane-bound vesicles called endosomes. The endosomal pathway of intracellular protein traffic communicates with lysosomes, which are more dense membrane-bound enzyme-containing vesicles. A subset of class II MHC–rich late endosomes plays a special role in antigen processing and presentation by the class II pathway; this is described later. Particulate microbes are internalized into vesicles called phagosomes, which may fuse with lysosomes, producing vesicles called phagolysosomes or secondary lysosomes. Some microbes, such as mycobacteria and *Leishmania,* may survive and even replicate within phagosomes or endosomes, providing a persistent source of antigens in vesicular compartments.

Proteins other than those ingested from the extracellular milieu can also enter the class II MHC pathway. Some protein molecules destined for secretion may end up in the same vesicles as class II MHC molecules and may be processed instead of being secreted. Less often, cytoplasmic and membrane proteins may be processed and displayed by class II molecules. In some cases, this may result from the enzymatic digestion of cytoplasmic contents, referred to as **autophagy**. In this pathway, cytoplasmic proteins are trapped within membrane-bound vesicles called autophagosomes; these vesicles fuse with lysosomes, and the cytoplasmic proteins are proteolytically degraded. The peptides generated by this route may be delivered to the same class II–bearing vesicular compartment as are peptides derived from ingested antigens. Autophagy is primarily a mechanism for degrading cellular proteins and recycling their products as sources of nutrients during times of stress. It also participates in the destruction of intracellular microbes, which are enclosed in vesicles and delivered to lysosomes. It is therefore predictable that peptides generated by autophagy will be displayed for T cell recognition. Some peptides that associate with class II molecules are derived from membrane proteins, which may be recycled into the same endocytic pathway as are extracellular proteins. Thus, even viruses, which replicate in the cytoplasm of infected cells, may produce proteins that are degraded into peptides that enter the class II MHC pathway of antigen presentation. This may be a mechanism for the activation of viral antigen–specific $CD4^+$ helper T cells.

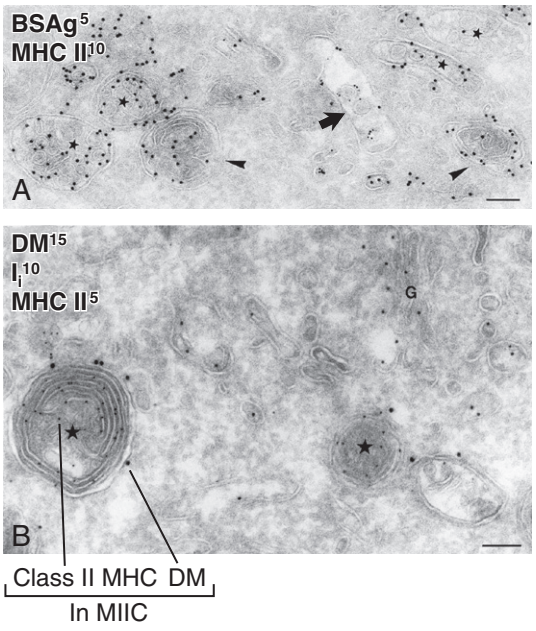


FIGURE 6–18 Morphology of class II MHC–rich endosomal vesicles. A, Immunoelectron micrograph of a B lymphocyte that has internalized bovine serum albumin into early endosomes (labeled with 5-nm gold particles, *arrow*) and contains class II MHC molecules (labeled with 10-nm gold particles, *arrowheads*) in MIICs. The internalized albumin will reach the MIICs ultimately. *(From Kleijmeer MJ, S Morkowski, JM Griffith, AY Rudensky, and HJ Geuze. Major histocompatibility complex class II compartments in human and mouse B lymphoblasts represent conventional endocytic compartments. Reproduced from The Journal of Cell Biology 139:639-649, 1997, by copyright permission of The Rockefeller University Press.)* **B,** Immunoelectron micrograph of a B cell showing location of class II MHC molecules and DM in MIICs *(stars)* and invariant chain concentrated in the Golgi (G) complex. In this example, there is virtually no invariant chain detected in the MIIC, presumably because it has been cleaved to generate CLIP. *(Courtesy of Drs. H. J. Geuze and M. Kleijmeer, Department of Cell Biology, Utrecht University, The Netherlands.)*

Proteolytic Digestion of Proteins in Vesicles

Internalized proteins are degraded enzymatically in late endosomes and lysosomes to generate peptides that are able to bind to the peptide-binding clefts of class II MHC molecules. The degradation of protein antigens in vesicles is an active process mediated by proteases that have acidic pH optima. The most abundant proteases of late endosomes are cathepsins, which are thiol and aspartyl proteases with broad substrate specificities. Several cathepsins contribute to the generation of peptides for the class II pathway. Partially degraded or cleaved proteins bind to the open-ended clefts of class II MHC molecules and are then trimmed enzymatically to their final size. Immunoelectron microscopy and subcellular fractionation studies have defined a class II–rich subset of late endosomes that plays an important role in antigen presentation (Fig. 6-18). In macrophages and human B cells, it is called the MHC class II compartment, or MIIC. (In some mouse B cells, a similar organelle containing class II molecules has been identified and named the class II vesicle.) The MIIC has a characteristic multilamellar appearance by electron microscopy. Importantly, it contains all the components required for peptide–class II

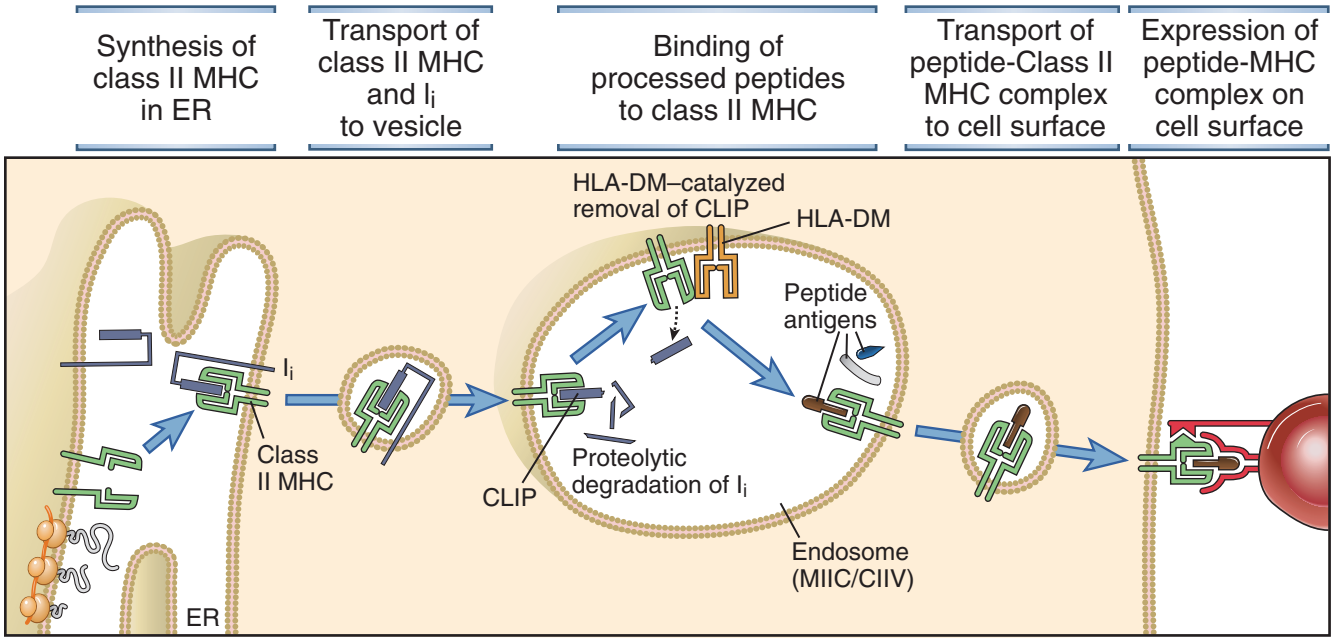


FIGURE 6–19 The functions of class II MHC–associated invariant chain and HLA-DM. Class II molecules with bound invariant chain, or CLIP, are transported into vesicles, where the I_i is degraded and the remaining CLIP is removed by the action of DM. Antigenic peptides generated in the vesicles are then able to bind to the class II molecules. Another class II–like protein, called HLA-DO, may regulate the DM-catalyzed removal of CLIP. CIIV, class II vesicle.

association, including the enzymes that degrade protein antigens, the class II molecules, and two molecules involved in peptide loading of class II molecules, the invariant chain and HLA-DM, whose functions are described later.

Biosynthesis and Transport of Class II MHC Molecules to Endosomes

Class II MHC molecules are synthesized in the ER and transported to endosomes with an associated protein, the invariant chain (I_i), which occupies the peptide-binding clefts of the newly synthesized class II molecules (Fig. 6-19). The α and β chains of class II MHC molecules are coordinately synthesized and associate with each other in the ER. Nascent class II dimers are structurally unstable, and their folding and assembly are aided by ER-resident chaperones, such as calnexin. A protein called the invariant chain (I_i) promotes folding and assembly of class II molecules and directs newly formed class II molecules to the late endosomes and lysosomes where internalized proteins have been proteolytically degraded into peptides. The I_i is a trimer composed of three 30-kD subunits, three of which bind one newly synthesized class II αβ heterodimer in a way that blocks the peptide-binding cleft and prevents it from accepting peptides. As a result, class II MHC molecules cannot bind and present peptides they encounter in the ER, leaving such peptides to associate with class I molecules (described before). The class II MHC molecules are transported in exocytic vesicles toward the cell surface. During this passage, the vesicles taking class II molecules out of the ER meet and fuse with the endocytic vesicles containing internalized and processed antigens. Thus, class II molecules encounter antigenic peptides that have been generated by proteolysis of endocytosed proteins, and the peptide-MHC association occurs in the vesicles.

Association of Processed Peptides with Class II MHC Molecules in Vesicles

Within the endosomal vesicles, the I_i dissociates from class II MHC molecules by the combined action of proteolytic enzymes and the HLA-DM molecule, and antigenic peptides are then able to bind to the available peptide-binding clefts of the class II molecules (see Fig. 6-19). Because the I_i blocks access to the peptide-binding cleft of a class II MHC molecule, it must be removed before complexes of peptide and class II molecules can form. The same proteolytic enzymes, such as cathepsins, that generate peptides from internalized proteins also act on the I_i, degrading it and leaving only a 24–amino acid remnant called class II–associated invariant chain peptide (CLIP), which sits in the peptide-binding cleft in the same way that other peptides bind to class II MHC molecules. Next, CLIP has to be removed so that the cleft becomes accessible to antigenic peptides produced from extracellular proteins. This removal is accomplished by the action of a molecule called HLA-DM (or H-2M in the mouse), which is encoded within the MHC, has a structure similar to that of class II MHC molecules, and colocalizes with class II molecules in the MIIC endosomal compartment. HLA-DM molecules differ from class II MHC molecules in several respects; they are not polymorphic, and they are not expressed on the cell surface. HLA-DM acts as a peptide exchanger, facilitating the removal of CLIP and the addition of other peptides to class II MHC molecules.

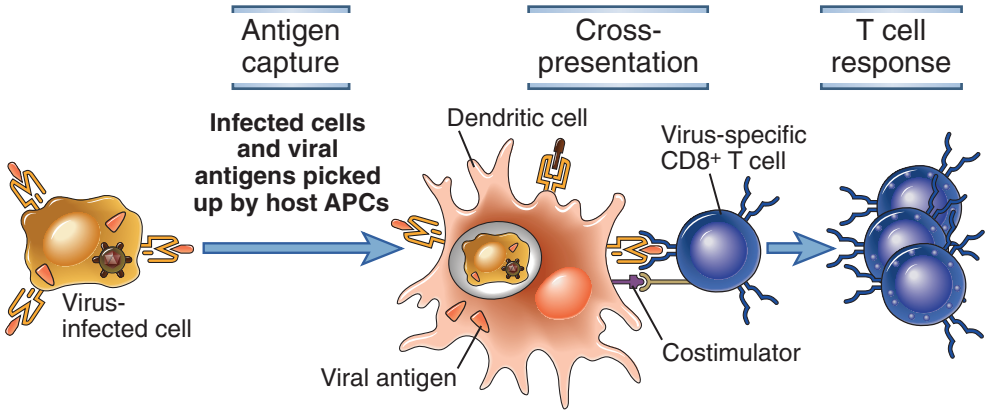


FIGURE 6–20 Cross-presentation of antigens to $CD8^+$ T cells. Cells infected with intracellular microbes, such as viruses, are ingested by dendritic cells, and the antigens of the infectious microbes are processed and presented in association with class I MHC molecules to $CD8^+$ T cells. Thus, dendritic cells are able to present endocytosed vesicular antigens by the class I pathway. Note that the same cross-presenting APCs may display class II MHC–associated antigens from the microbe for recognition by $CD4^+$ helper T cells.

Once CLIP is removed, peptides generated by proteolysis of internalized protein antigens are able to bind to class II MHC molecules. The HLA-DM molecule may accelerate the rate of peptide binding to class II molecules. Because the ends of the class II MHC peptide-binding cleft are open, large peptides may bind and are then "trimmed" by proteolytic enzymes to the appropriate size for T cell recognition. As a result, the peptides that are actually presented attached to cell surface class II MHC molecules are usually 10 to 30 amino acids long and typically have been generated by this trimming step.

Expression of Peptide–Class II MHC Complexes on the Cell Surface

Class II MHC molecules are stabilized by the bound peptides, and the stable peptide–class II complexes are delivered to the surface of the APC, where they are displayed for recognition by $CD4^+$ T cells. The transport of class II MHC–peptide complexes to the cell surface is believed to occur by fusion of vesiculotubular extensions from the lysosome with the plasma membrane, resulting in delivery of the loaded class II MHC complexes to the cell surface. Once expressed on the APC surface, the peptide–class II complexes are recognized by peptide antigen–specific $CD4^+$ T cells, with the CD4 coreceptor playing an essential role by binding to nonpolymorphic regions of the class II molecule. Interestingly, whereas peptide-loaded class II molecules traffic from the late endosomes and lysosomes to the cell surface, other molecules involved in antigen presentation, such as DM, stay in the vesicles and are not expressed in the plasma membrane. The mechanism of this selective traffic is unknown.

Cross-Presentation

Some dendritic cells have the ability to capture and to ingest virus-infected cells or tumor cells and present the viral or tumor antigens to naive $CD8^+$ T lymphocytes (Fig. 6-20). In this pathway, the ingested antigens are transported from vesicles to the cytosol, from where peptides enter the class I pathway. As we discussed before, most ingested proteins do not enter the cytosolic class I pathway of antigen presentation. This permissiveness for protein traffic from endosomal vesicles to the cytosol is unique to dendritic cells. (At the same time, the dendritic cells can present class II MHC–associated peptides generated in the vesicles to $CD4^+$ helper T cells, which are often required to induce full responses of $CD8^+$ cells [see Chapter 9].) This process is called **cross-presentation**, or **cross-priming**, to indicate that one cell type (the dendritic cell) can present antigens from another cell (the virus-infected or tumor cell) and prime, or activate, T cells specific for these antigens. The process of cross-presentation seems to violate the rule that vesicular antigens are presented bound to class II MHC molecules and cytosolic antigens with class I. However, it is a normal function of dendritic cells, because of which dendritic cells can activate naive $CD8^+$ T cells even if the antigens are produced in cells incapable of presenting the antigen, as long as the antigen-containing cells can be internalized by dendritic cells into vesicles.

Cross-presentation involves the fusion of phagosomes containing the ingested antigens with the ER. Ingested proteins are then translocated from the ER to the cytosol by poorly defined pathways that are reminiscent of ER-associated degradation. The proteins that were initially internalized in the phagosome are therefore delivered to the compartment (the cytosol) where proteolysis for the class I pathway normally occurs. These phagocytosed proteins thus undergo proteasomal degradation, and peptides derived from them are transported by TAP back into the ER, where they are assembled with newly synthesized class I MHC molecules as described for the conventional class I pathway.

Physiologic Significance of MHC-Associated Antigen Presentation

So far, we have discussed the specificity of $CD4^+$ and $CD8^+$ T lymphocytes for MHC-associated foreign protein antigens and the mechanisms by which complexes of peptides and MHC molecules are produced. In this

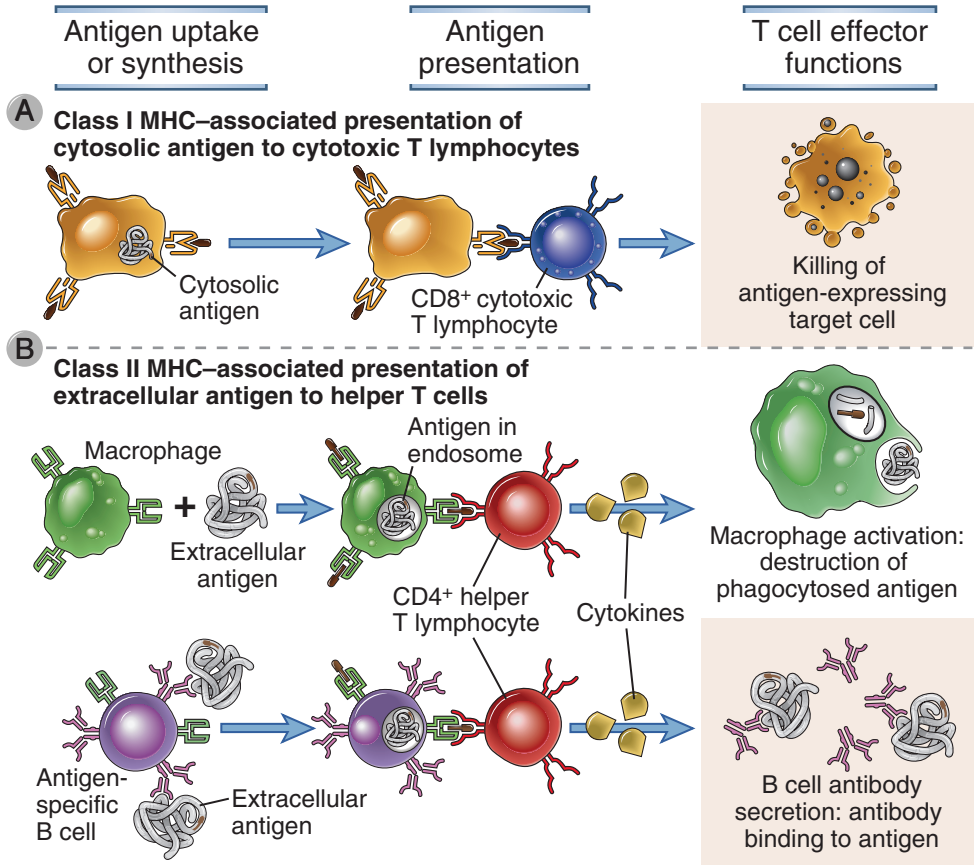


FIGURE 6–21 Presentation of extracellular and cytosolic antigens to different subsets of T cells. A, Cytosolic antigens are presented by nucleated cells to $CD8^+$ CTLs, which kill (lyse) the antigen-expressing cells. **B,** Extracellular antigens are presented by macrophages or B lymphocytes to $CD4^+$ helper T lymphocytes, which activate the macrophages or B cells and eliminate the extracellular antigens.

section, we consider how the central role of the MHC in antigen presentation influences the nature of T cell responses to different antigens and the types of antigens that T cells recognize.

Nature of T Cell Responses

The presentation of cytosolic versus vesicular proteins by the class I or class II MHC pathways, respectively, determines which subsets of T cells will respond to antigens found in these two pools of proteins and is intimately linked to the functions of these T cells (Fig. 6-21). Endogenously synthesized antigens, such as viral and tumor proteins, are located in the cytoplasm and are recognized by class I–restricted $CD8^+$ CTLs, which kill the cells producing the intracellular antigens. Conversely, extracellular antigens usually end up in endosomal vesicles and activate class II–restricted $CD4^+$ T cells because vesicular proteins are processed into class II–binding peptides. $CD4^+$ T cells function as helpers to stimulate B cells to produce antibodies and macrophages to enhance their phagocytic activity, both mechanisms that serve to eliminate extracellular antigens. Thus, antigens from microbes that reside in different cellular locations selectively stimulate the T cell responses that are most effective at eliminating that type of microbe. This is especially important because the antigen receptors of CTLs and helper T cells cannot distinguish between extracellular and intracellular microbes. By segregating peptides derived from these types of microbes, the MHC molecules guide $CD4^+$ and $CD8^+$ subsets of T cells to respond to the microbes that each subset can best combat.

Immunogenicity of Protein Antigens

MHC molecules determine the immunogenicity of protein antigens in two related ways.

- ***The epitopes of complex proteins that elicit the strongest T cell responses are the peptides that are generated by proteolysis in APCs and bind most avidly to MHC molecules.*** If an individual is immunized with a protein antigen, in many instances the majority of the responding T cells are specific for only one or a few linear amino acid sequences of the antigen. These are called the **immunodominant** epitopes or determinants. The proteases involved in antigen processing produce a variety of peptides from natural proteins, and only some of these peptides possess the characteristics that enable them to bind to the MHC molecules present in each individual (Fig. 6-22). It is important to define the structural basis of immunodominance because this may permit the efficient manipulation of the immune system with synthetic peptides. An application of such

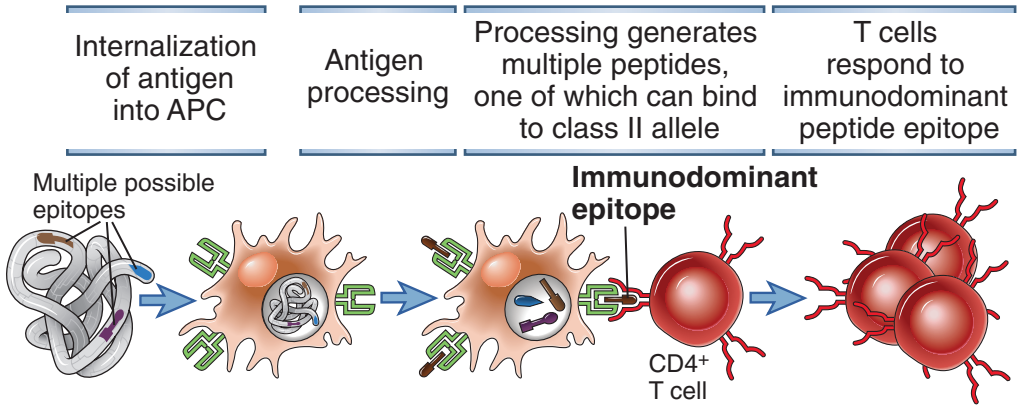


FIGURE 6–22 Immunodominance of peptides. Protein antigens are processed to generate multiple peptides; immunodominant peptides are the ones that bind best to the available class I and class II MHC molecules. The illustration shows an extracellular antigen generating a class II–binding peptide, but this also applies to peptides of cytosolic antigens that are presented by class I MHC molecules.

knowledge is the design of vaccines. For example, a viral protein could be analyzed for the presence of amino acid sequences that would form typical immunodominant epitopes capable of binding to MHC molecules with high affinity. Synthetic peptides containing these epitopes may be effective vaccines for eliciting T cell responses against the viral peptide expressed on an infected cell. Conversely, some individuals do not respond to vaccines (such as hepatitis B virus surface antigen vaccine), presumably because their HLA molecules cannot bind and display the major peptides of the antigen.

- ***The expression of particular class II MHC alleles in an individual determines the ability of that individual to respond to particular antigens.*** As discussed earlier, the immune response (Ir) genes that control antibody responses are the class II MHC genes. They influence immune responsiveness because various allelic class II MHC molecules differ in their ability to bind different antigenic peptides and therefore to stimulate specific helper T cells.

PRESENTATION OF NONPROTEIN ANTIGENS TO SUBSETS OF T CELLS

Several small populations of T cells are able to recognize nonprotein antigens without the involvement of class I or class II MHC molecules. Thus, these populations are exceptions to the rule that T cells can see only MHC-associated peptides. The best defined of these populations are NKT cells and γδ T cells.

NKT cells express markers that are characteristic of both natural killer (NK) cells and T lymphocytes and express αβ T cell receptors with very limited diversity (see Chapter 10). NKT cells recognize lipids and glycolipids displayed by the class I–like "non-classical" MHC molecule called CD1. There are several CD1 proteins expressed in humans and mice. Although their intracellular traffic pathways differ in subtle ways, all the CD1 molecules bind and display lipids by a unique pathway. Newly synthesized CD1 molecules pick up cellular lipids and carry these to the cell surface. From here, the CD1-lipid complexes are endocytosed into endosomes or lysosomes, where lipids that have been ingested from the external environment are captured and the new CD1-lipid complexes are returned to the cell surface. Thus, CD1 molecules acquire endocytosed lipid antigens during recycling and present these antigens without apparent processing. The NKT cells that recognize the lipid antigens may play a role in defense against microbes, especially mycobacteria (which are rich in lipid components).

γδ T cells are a small population of T cells that express antigen receptor proteins that are similar but not identical to those of $CD4^+$ and $CD8^+$ T cells (see Chapter 10). γδ T cells recognize many different types of antigens, including some proteins and lipids, as well as small phosphorylated molecules and alkyl amines. These antigens are not displayed by MHC molecules, and γδ cells are not MHC restricted. It is not known if a particular cell type or antigen display system is required for presenting antigens to these cells.

SUMMARY

- T cells recognize antigens only in the form of peptides displayed by the products of self MHC genes on the surface of APCs. $CD4^+$ helper T lymphocytes recognize antigens in association with class II MHC gene products (class II MHC–restricted recognition), and $CD8^+$ CTLs recognize antigens in association with class I gene products (class I MHC–restricted recognition).
- Specialized APCs, such as dendritic cells, macrophages, and B lymphocytes, capture extracellular protein antigens, internalize and process them, and display class II–associated peptides to $CD4^+$ T cells. Dendritic cells are the most efficient APCs for initiation of primary responses by activating naive T cells, and macrophages and B lymphocytes present antigens to differentiated helper T cells in

the effector phase of cell-mediated immunity and in humoral immune responses, respectively. All nucleated cells can present class I–associated peptides, derived from cytosolic proteins such as viral and tumor antigens, to $CD8^+$ T cells.

- The MHC is a large genetic region coding for class I and class II MHC molecules as well as for other proteins. MHC genes are highly polymorphic. Class I MHC molecules are composed of an α (or heavy) chain in a noncovalent complex with a nonpolymorphic polypeptide called β_2-microglobulin. The class II molecules contain two MHC-encoded polymorphic chains, an α chain and a β chain. Both classes of MHC molecules consist of an extracellular peptide-binding cleft, a nonpolymorphic Ig-like region, a transmembrane region, and a cytoplasmic region. The peptide-binding cleft of MHC molecules has α-helical sides and an eight-stranded antiparallel β-pleated sheet floor. The peptide-binding cleft of class I molecules is formed by the α1 and α2 segments of the α chain, and that of class II molecules by the α1 and β1 segments of the two chains. The Ig-like domains of class I and class II molecules contain the binding sites for the T cell coreceptors CD8 and CD4, respectively. The polymorphic residues of MHC molecules are localized to the peptide-binding domain.
- The function of MHC-encoded class I and class II molecules is to bind peptide antigens and display them for recognition by antigen-specific T lymphocytes. Peptide antigens associated with class I molecules are recognized by $CD8^+$ T cells, whereas class II–associated peptide antigens are recognized by $CD4^+$ T cells. MHC molecules bind only one peptide at a time, and all the peptides that bind to a particular MHC molecule share common structural motifs. Every MHC molecule has a broad specificity for peptides and can bind multiple peptides that have common structural features, such as anchor residues.
- The peptide-binding cleft of class I molecules can accommodate peptides that are 6 to 16 amino acid residues in length, whereas the cleft of class II molecules allows larger peptides (up to 30 amino acid residues in length or more) to bind. Some polymorphic MHC residues determine the binding specificities for peptides by forming structures, called pockets, that interact with complementary residues of the bound peptide, called anchor residues. Other polymorphic MHC residues and some residues of the peptide are not involved in binding to MHC molecules but instead form the structure recognized by T cells.
- Class I molecules are expressed on all nucleated cells, whereas class II molecules are expressed mainly on specialized APCs, such as dendritic cells, macrophages, and B lymphocytes, and a few other cell types, including endothelial cells and thymic epithelial cells. The expression of MHC gene products is enhanced by inflammatory and immune stimuli, particularly cytokines like IFN-γ, which stimulate the transcription of MHC genes.
- Antigen processing is the conversion of native proteins into MHC-associated peptides. This process consists of the introduction of exogenous protein antigens into vesicles of APCs or the synthesis of antigens in the cytosol, the proteolytic degradation of these proteins into peptides, the binding of peptides to MHC molecules, and the display of the peptide-MHC complexes on the APC surface for recognition by T cells. Thus, both extracellular and intracellular proteins are sampled by these antigen-processing pathways, and peptides derived from both normal self proteins and foreign proteins are displayed by MHC molecules for surveillance by T lymphocytes.
- For class I–associated antigen presentation, cytosolic proteins are proteolytically degraded in the proteasome, generating peptides with features that enable them to bind to class I molecules. These peptides are delivered from the cytoplasm to the ER by an ATP-dependent transporter called TAP. Newly synthesized class I MHC–β_2-microglobulin dimers in the ER are associated with the TAP complex and receive peptides transported into the ER. Stable complexes of class I MHC molecules with bound peptides move out of the ER, through the Golgi complex, to the cell surface.
- For class II–associated antigen presentation, extracellular proteins are internalized into endosomes, where these proteins are proteolytically cleaved by enzymes that function at acidic pH. Newly synthesized class II MHC molecules associated with the I_i are transported from the ER to the endosomal vesicles. Here the I_i is proteolytically cleaved, and a small peptide remnant of the I_i, called CLIP, is removed from the peptide-binding cleft of the MHC molecule by the DM molecules. The peptides that were generated from extracellular proteins then bind to the available cleft of the class II MHC molecule, and the trimeric complex (class II MHC α and β chains and peptide) moves to and is displayed on the surface of the cell.
- These pathways of MHC-restricted antigen presentation ensure that most of the body's cells are screened for the possible presence of foreign antigens. The pathways also ensure that proteins from extracellular microbes preferentially generate peptides bound to class II MHC molecules for recognition by $CD4^+$ helper T cells, which activate effector mechanisms that eliminate extracellular antigens. Conversely, proteins synthesized by intracellular (cytosolic) microbes generate peptides bound to class I MHC molecules for recognition by $CD8^+$ CTLs, which function to eliminate cells harboring intracellular infections. The immunogenicity of foreign protein antigens depends on the ability of antigen-processing pathways to generate peptides from these proteins that bind to self MHC molecules.

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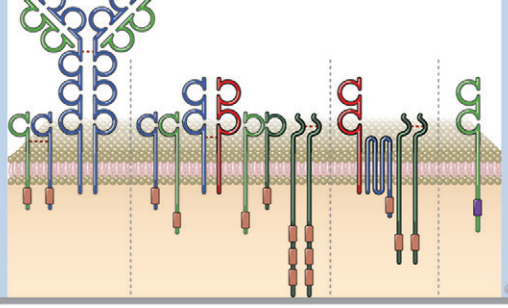
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CHAPTER

7

Immune Receptors and Signal Transduction

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The idea that cells may have specific surface receptors that can be triggered by external ligands came from one of the founders of modern immunology. Paul Ehrlich, in his "side chain theory," published in 1897, conceived of antibodies on the surface of immune cells that recognize antigens and instruct the immune cell to secrete more of the same antibody. Cell surface receptors for hormones were discovered many decades later in the second half of the 20th century but well before the identification of antigen receptors on lymphocytes in the early 1980s.

Cell surface receptors serve two major functions—the induction of intracellular signaling and the adhesion of one cell to another or to the extracellular matrix. **Signal transduction** broadly refers to the intracellular biochemical responses of cells after the binding of ligands to specific receptors. Most but not all signaling receptors are located on the plasma membrane. Signaling initiated by these receptors typically involves an **initial cytosolic phase** when the receptor or proteins that interact with the receptor may be post-translationally modified. This often leads to the activation or nuclear translocation of transcription factors that are silent in resting cells, followed by a **nuclear phase** when transcription factors orchestrate changes in gene expression (Fig. 7-1). Some signal transduction pathways stimulate cell motility or activate granule exocytosis from the cytoplasm independent of a nuclear phase. Signal transduction can result in a number of different consequences for a cell, including acquisition of new functions, induction of differentiation, commitment to a specific lineage, protection from cell death, initiation of proliferative and growth responses, and induction of cell cycle arrest or of death by apoptosis. Antigen receptors on B and T lymphocytes are among the most sophisticated signaling machines known, and they will form a large part of the focus of this chapter.

We will initially provide a broad overview of signal transduction, followed by a discussion of signaling mediated by clonally distributed antigen receptors in lymphocytes and by structurally related immune receptors found mainly in cells of the innate immune system. When discussing antigen receptors in T and B cells, we will examine the role of coreceptors in lymphocyte activation, consider signaling through costimulatory receptors in each lymphocyte lineage, and discuss the role of inhibitory receptors in T, B, and NK cells. We will also consider different

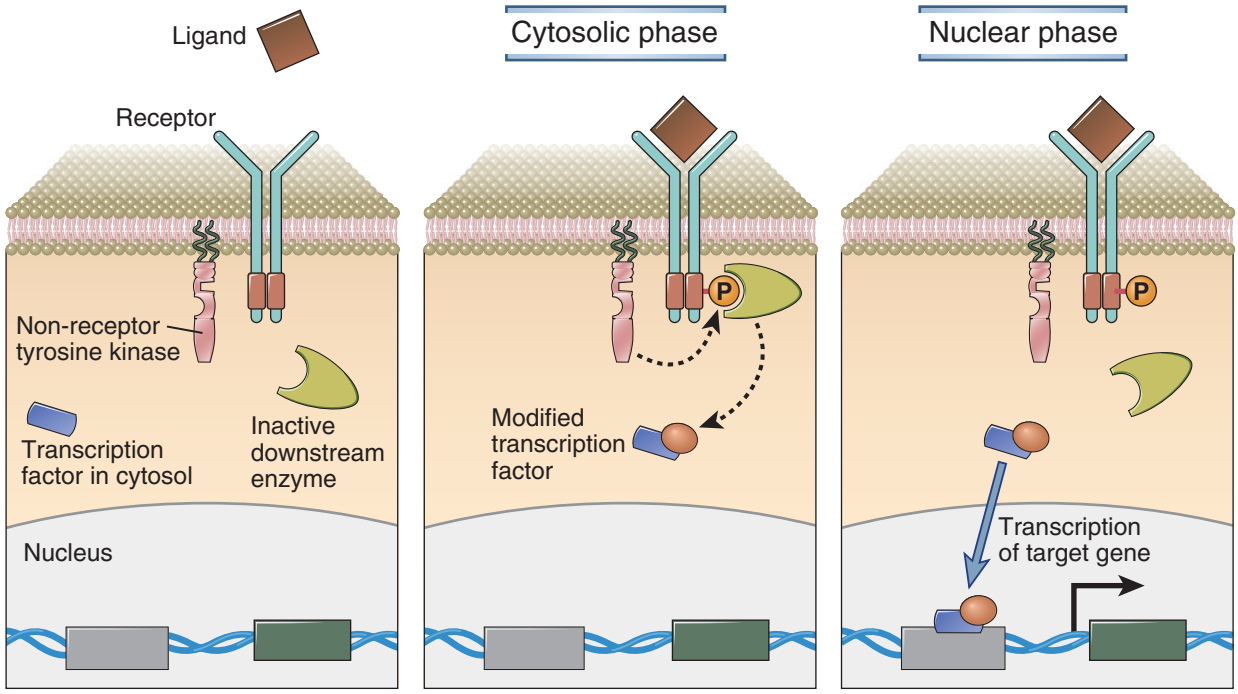


FIGURE 7–1 Signaling from the cell surface involves cytosolic and nuclear phases. A generic receptor that activates a non-receptor tyrosine kinase after it binds ligand is shown. In the cytosolic signaling phase, the non-receptor kinase phosphorylates a key tyrosine residue on the cytoplasmic tail of the receptor, as a result of which the phosphotyrosine-containing receptor tail is able to recruit a downstream enzyme that is activated once it is recruited. In the cytosolic phase, this activated downstream enzyme post-translationally modifies a specific transcription factor that is located in the cytoplasm. In the nuclear phase, this modified transcription factor enters the nucleus and induces the expression of target genes that all have a binding site in the promoter or in some other regulatory region that can bind to this modified transcription factor and facilitate transcription.

categories of cytokine receptors and signal transduction mechanisms initiated by these receptors and finally examine the major pathway that leads to the activation of NF-κB, a transcription factor of relevance to both innate and adaptive immunity.

AN OVERVIEW OF SIGNAL TRANSDUCTION

Receptors that initiate signaling responses are generally integral membrane proteins present on the plasma membrane, where their extracellular domains recognize soluble secreted ligands or structures that are attached to the plasma membrane of a neighboring cell or cells. One distinct category of receptors, nuclear receptors, are actually transcription factors that are functionally activated by lipid-soluble ligands that can easily cross the plasma membrane.

The initiation of signaling from a cell surface receptor may require ligand-induced clustering of the receptor, known as receptor cross-linking, or may involve a conformational alteration of the receptor that is induced by its association with ligand. Both mechanisms of signal initiation typically result in the creation of a novel geometric shape in the cytosolic portion of the receptor that promotes interactions with other signaling molecules. This change in receptor geometry may sometimes result from enzymatic addition of a bulky phosphate residue on a key tyrosine, serine, or threonine side chain on the cytosolic portion of a receptor component or on a distinct adaptor protein. The enzymes that add phosphate groups onto amino acid side chains are called **protein kinases**. Many of the initiating events in lymphocyte signaling depend on protein kinases that phosphorylate key tyrosine residues, and these enzymes are therefore called **protein tyrosine kinases**. Other protein kinases that are involved in distinct signaling pathways are **serine/threonine kinases**, enzymes that phosphorylate protein substrates on serine or threonine residues. Some enzymes activated downstream of signaling receptors phosphorylate lipid substrates; they are therefore known as **lipid kinases**. For every type of phosphorylation event, there is a specific **phosphatase**, an enzyme that can remove a phosphate residue and thus modulate signaling. These phosphatases play important, usually inhibitory roles in signal transduction. Phosphorylation of proteins is not the only post-translational modification that drives signal transduction. Many other modifications are known to facilitate signaling events. Some transcription factors as well as histones can be regulated by **acetylation** and **methylation**, for instance. A type of modification that we will describe later in this chapter is protein **ubiquitination**, the addition of ubiquitin molecules that either target proteins for degradation or drive signal transduction in many cells, including lymphocytes. Many important signaling molecules are modified by the addition of lipids that may help localize the protein in the plasma membrane, or sometimes to a specialized region of the plasma membrane that is rich in signaling molecules.

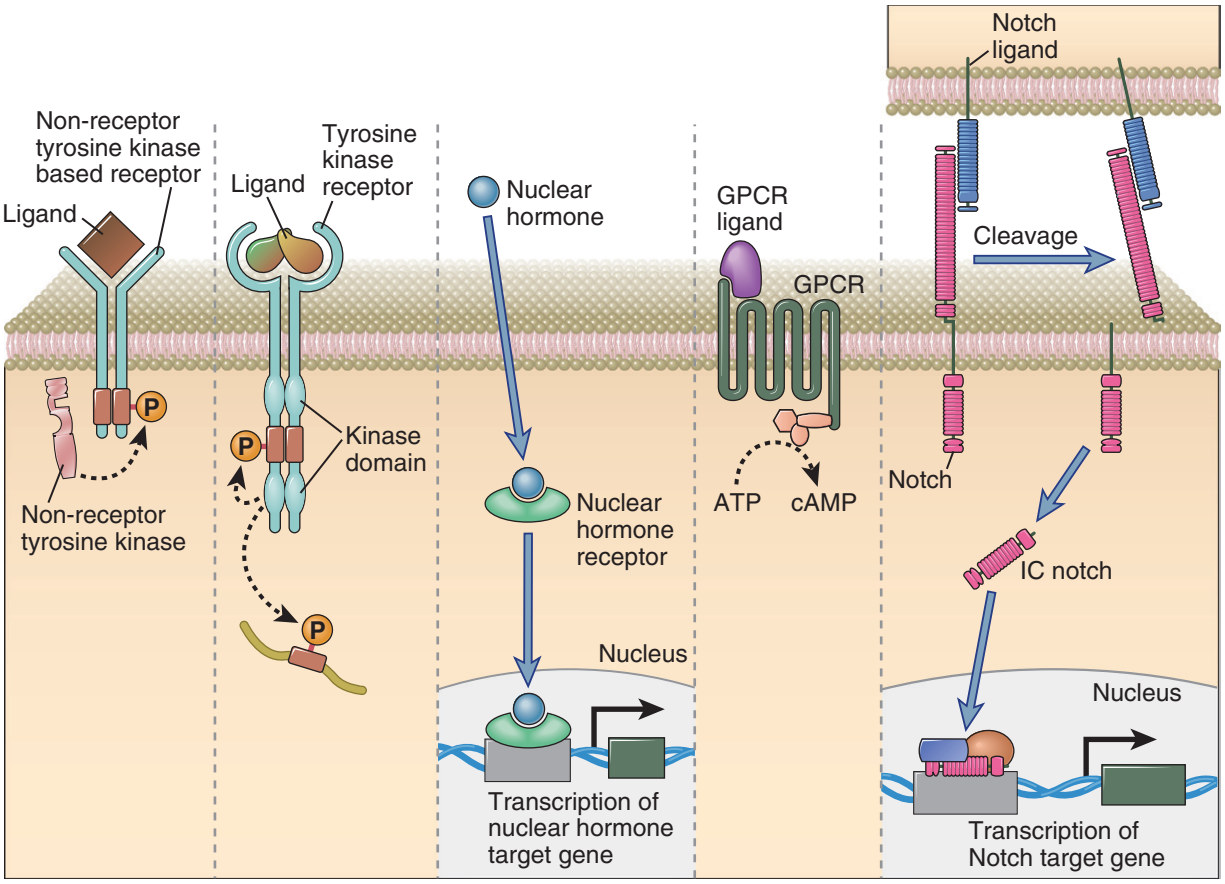


FIGURE 7–2 Major categories of signaling receptors in the immune system. Depicted here are a receptor that uses a non-receptor tyrosine kinase, a receptor tyrosine kinase, a nuclear receptor that binds its ligand and can then influence transcription, a seven-transmembrane receptor linked to heterotrimeric G proteins, and Notch, which recognizes a ligand on a distinct cell and is cleaved, yielding an intracellular fragment (IC Notch) that can enter the nucleus and influence transcription of specific target genes.

Cellular receptors are grouped into several categories based on the signaling mechanisms they use and the intracellular biochemical pathways they activate (Fig. 7-2):

- Receptors that use **non-receptor tyrosine kinases**. In this category of membrane receptors the ligand-binding chains have no intrinsic catalytic activity, but a separate intracellular tyrosine kinase, known as a non-receptor tyrosine kinase, participates in receptor activation by phosphorylating specific motifs on the receptor or on other proteins associated with the receptor (see Fig. 7-1). A family of receptors called immune receptors, some of which recognize antigens while others recognize the Fc portions of antibodies, all use non-receptor tyrosine kinases to initiate signaling. Apart from the immune receptor family, some cytokine receptors, discussed later in this chapter, also use non-receptor tyrosine kinases. Integrins, key adhesion receptors in the immune system, also signal by activating non-receptor tyrosine kinases.
- **Receptor tyrosine kinases (RTKs)** are integral membrane proteins that activate an intrinsic tyrosine kinase domain (or domains) located in their cytoplasmic tails when they are cross-linked by multivalent extracellular ligands (see Fig. 7-2). An example of an RTK relevant to blood cell formation is the c-Kit protein. This RTK has extracellular Ig domains that bind to a ligand known as stem cell factor. Interaction with stem cell factor leads to dimerization of c-Kit and activation of the cytosolic kinase domains of the dimerized receptor. Signaling through c-Kit contributes to the initiation of hematopoiesis and lymphopoiesis. Other examples of RTKs include the insulin receptor, the epidermal growth factor receptor, and the platelet-derived growth factor receptor.
- **Nuclear receptors.** The binding of a lipid-soluble ligand to its nuclear receptor (see Fig. 7-2) results in the ability of the latter either to induce transcription or to repress gene expression. **Nuclear hormone receptors**, such as the vitamin D receptor and the glucocorticoid receptor, can influence events that range from the development of the immune system to the modulation of cytokine gene expression.
- **Seven-transmembrane** receptors are polypeptides that traverse the plasma membrane seven times, because of which they are sometimes called serpentine receptors (see Fig. 7-2). Because these receptors function by activating associated GTP-binding proteins (G proteins), they are also commonly called **G protein–coupled receptors (GPCRs)**. A

conformational change induced by the binding of ligand to this type of receptor permits the activation of an associated heterotrimeric G protein, which initiates downstream signaling events. Examples of this category of receptors that are relevant to immunity and inflammation include receptors for leukotrienes, prostaglandins, histamine, complement fragments C3a and C5a, bacterial f-met-leu-phe peptide, and all chemokines (see Chapter 3). Different types of G proteins linked to distinct GPCRs may activate or inhibit different downstream effectors. The two major enzymes that GPCRs activate are adenylate cyclase, which converts ATP to the effector molecule cAMP, capable of activating numerous cellular responses, and phospholipase C, which also triggers multiple signals as discussed later.

- **Other classes of receptors.** Other categories of receptors have long been known to be important in embryonic development and in certain mature tissues, and their functions in the immune system have more recently begun to emerge. Receptor proteins of the **Notch** family (see Fig. 7-2) are involved in development in a wide range of species. The association of specific ligands with receptors of this family leads to proteolytic cleavage of the receptor and the nuclear translocation of the cleaved cytoplasmic domain (intracellular Notch), which functions as a component of a transcription complex. Notch proteins contribute to cell fate determination during lymphocyte development (see Chapter 8) and may also influence the activation of mature lymphocytes. A group of ligands called **Wnt** proteins can influence lymphopoiesis. Signaling through transmembrane receptors for these proteins can regulate the levels of **β-catenin**, which facilitates the transcriptional activity of proteins that contribute to B and T cell development, as discussed in Chapter 8. Numerous other signaling receptors and pathways first discovered in non–immune cell populations are now beginning to be analyzed in the context of lymphocyte biology. We will not attempt to comprehensively consider all these pathways in this chapter.

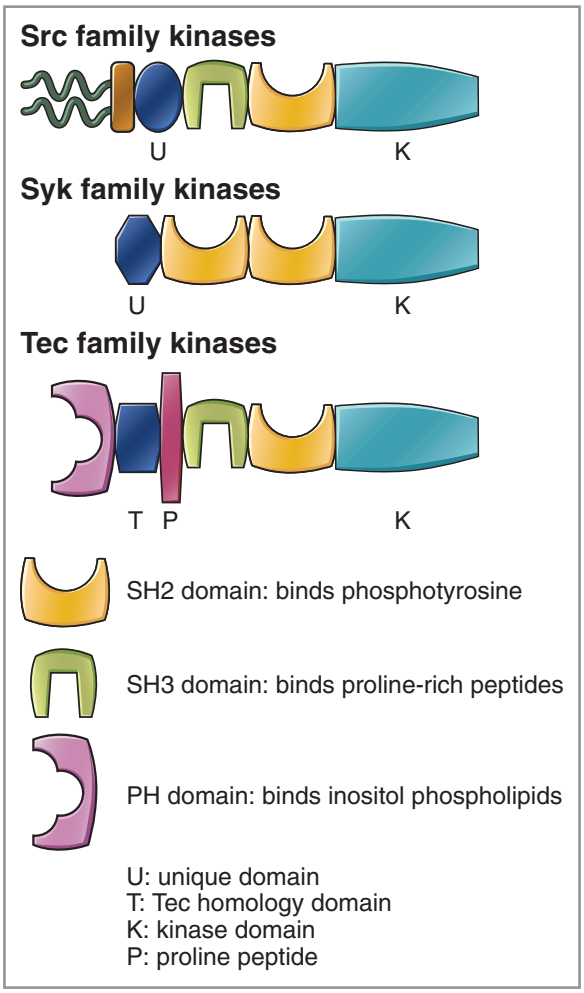


FIGURE 7–3 The modular structure of tyrosine kinases that influence lymphocyte activation. Modules include SH2 domains that bind specific phosphotyrosine-containing polypeptides, SH3 domains that recognize proline-rich stretches in polypeptides, PH domains that recognize PIP3 or other phosphatidylinositol-derived lipids, and Tec homology domains found in tyrosine kinases of the Tec family. Tyrosine kinase families depicted are the Src family kinases, which include c-Src, Lyn, Fyn, and Lck; the Syk family kinases, which include Syk and ZAP-70; and the Tec family kinases, which include Tec, Btk, and Itk.

Modular Signaling Proteins and Adaptors

Signaling molecules are often composed of distinct modules, each with a specific binding or catalytic function. The discovery of tyrosine phosphorylation represented a major breakthrough in the study of cellular signaling pathways. It was subsequently discovered that the sequence surrounding specific phosphorylated tyrosine residues contributes to the interaction of tyrosine-phosphorylated proteins with other signaling molecules. An appreciation that signaling molecules contain modules or domains that each have defined functions was obtained from the study of non-receptor tyrosine kinases. The cellular homologue of the transforming protein of the Rous sarcoma virus, called c-Src, is the prototype for an immunologically important family of non-receptor tyrosine kinases known as **Src family kinases**. c-Src contains unique domains, including **Src homology 2** (SH2) and **Src homology 3** (SH3) domains described later. It also contains a catalytic tyrosine kinase domain and an N-terminal lipid addition domain that facilitates the covalent addition of a myristic acid molecule to the protein. The myristate helps target Src family kinases to the plasma membrane. The modular structures of three families of tyrosine kinases that are important in the immune system are depicted in Figure 7-3.

SH2 domains are composed of about 100 amino acids folded into a particular conformation, and they recognize specific phosphotyrosine-containing peptides. In antigen receptor signaling, Src family kinases phosphorylate tyrosine residues present in particular motifs in the cytoplasmic tails of proteins that are part of the receptor complex (described later). These phosphotyrosine motifs in the antigen receptor complex are then recognized by SH2 domains present in tyrosine kinases of the Syk family, such as Syk and ZAP-70 (see Fig. 7-3). The recruitment

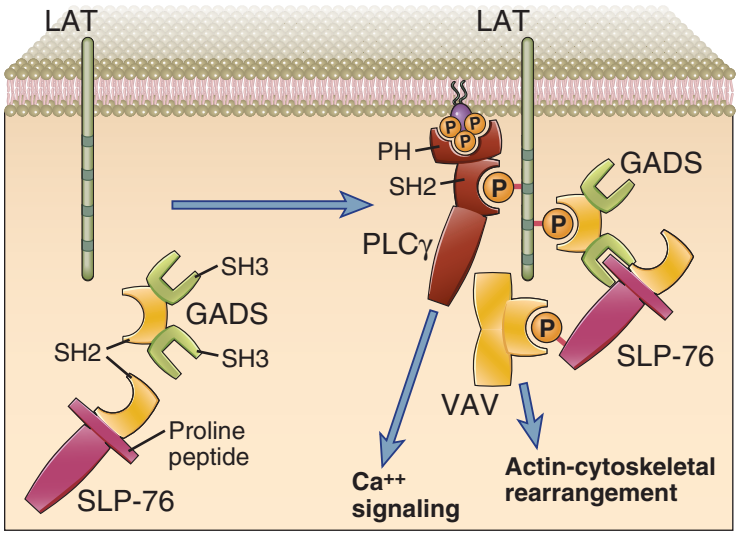


FIGURE 7–4 Selected adaptors that participate in lymphocyte activation. On the left, LAT, an integral membrane protein that functions as an adaptor, and two cytosolic adaptors, GADS and SLP-76, are shown in a nonactivated T cell. On the right, after T cell activation, LAT is tyrosine phosphorylated and is shown to have recruited PLCγ and the GADS adaptor, both of which contain SH2 domains. A proline-rich amino acid stretch in SLP-76 associates with an SH3 domain of GADS, and tyrosine-phosphorylated SLP-76 recruits Vav.

of a Syk family kinase to an antigen receptor by means of a specific SH2 domain–phosphotyrosine interaction is a key step in antigen receptor activation.

SH3 domains are also about 100 amino acids in length, and they help mediate protein-protein interactions by binding to proline-rich stretches in certain proteins. Another type of modular domain, called a **pleckstrin homology** (PH) domain, can recognize specific phospholipids. The PH domains in a number of signaling molecules including the TEC family tyrosine kinase Btk, recognize phosphatidylinositol trisphosphate (PIP3), a lipid moiety on the inner leaflet of the plasma membrane.

Adaptor proteins function as molecular hubs that physically link different enzymes and promote the assembly of complexes of signaling molecules. Adaptors may be integral membrane proteins like LAT (linker for the activation of T cells) (Fig. 7-4), or they may be cytosolic proteins such as BLNK (B cell linker), SLP-76 (SH2 domain–containing linker protein of 76 kD), and GADS (Grb-2–related adaptor protein downstream of Shc). A typical adaptor may contain a few specific domains that mediate protein-protein interactions, such as SH2 and SH3 domains, among others (there are many more types of modular domains not mentioned here). Adaptors may also contain some proline-rich stretches (that can bind other proteins that contain SH3 domains), and they also often contain critical tyrosine residues that may be phosphorylated by tyrosine kinases. The amino acid residues that are close to a tyrosine moiety that is phosphorylated determine which specific SH2 domains may bind that site. For instance, an adaptor with a YxxM motif (where Y represents tyrosine, M represents methionine, and x refers to any amino acid) will bind an SH2 domain in the lipid kinase phosphatidylinositol 3-kinase (PI3-kinase). The same adaptor protein may recruit a tyrosine kinase with a specific SH3 domain to a proline-rich stretch, and tyrosine phosphorylation of the adaptor may thus result in a tyrosine kinase and PI3-kinase being perched next to each other, resulting in the phosphorylation and activation of PI3-kinase. Signal transduction can therefore be visualized as a kind of social networking phenomenon. An initial signal (tyrosine phosphorylation, for instance) results in proteins being brought close to one another at designated hubs (adaptors), resulting in the activation of specific enzymes that eventually influence the nuclear localization or activity of specific downstream transcription factors or induce other cellular events, such as actin polymerization.

THE IMMUNE RECEPTOR FAMILY

Immune receptors are a unique family of receptor complexes typically made up of integral membrane proteins of the immunoglobulin (Ig) superfamily that are involved in ligand recognition, associated with other transmembrane signaling proteins that have unique tyrosine-containing motifs in their cytoplasmic tails. Whereas the signaling components are generally separate proteins from those involved in ligand recognition, in a few members of the family, the receptor consists of a single chain in which the extracellular domain is involved in ligand recognition and the cytoplasmic tail contains tyrosine residues that contribute to signaling. The signaling proteins of the immune receptor family are often positioned close to non-receptor tyrosine kinases of the Src family. The latter also possess N-terminal lipid anchors that tether them to the inner leaflet of the plasma membrane. The cytoplasmic tyrosine-containing motifs on the signaling proteins of the immune receptor family are generally one of two different types. **ITAMs** (immunoreceptor tyrosine-based activating motifs) are found on receptors involved in cell activation and have the sequence $YxxL/I(x)_{6\text{-}8}YxxL/I$, where Y represents a tyrosine residue, L represents leucine, I represents isoleucine, and x refers to any amino acid. ITAM motifs can be phosphorylated on both tyrosine residues that are present in this motif by Src family kinases when immune receptors are activated. Tyrosine-phosphorylated ITAMs recruit a distinct tyrosine kinase of the Syk/ZAP-70 family, which contains tandem SH2 domains that each bind to one of the two phosphorylated YxxL/I motifs of the ITAM. Binding of Syk (or ZAP-70) to

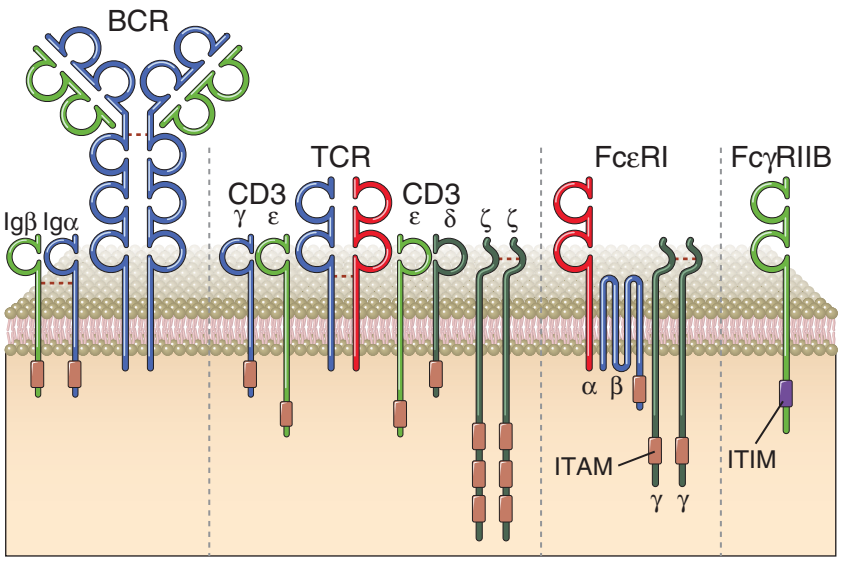


FIGURE 7–5 Selected members of the immune receptor family. Four selected members of the immune receptor family are depicted. Typically, immune receptors that activate immune cells have separate chains for recognition and associated chains that contain cytosolic ITAMs. Examples shown here include the B cell receptor (BCR), the T cell receptor (TCR), and the high-affinity receptor for IgE (FcεRI). Inhibitory receptors in the immune system typically have ITIM motifs on the cytosolic portion of the same chain that uses its extracellular domain for ligand recognition. The inhibitory receptor shown, FcγRIIB, is found on B cells and myeloid cells.

a phospho-ITAM results in a conformational change in this kinase and its activation. The activated Syk or ZAP-70 kinase then drives immune cell activation. Some immune receptors inhibit cellular responses, and signaling chains in these receptors may contain a slightly different tyrosine-containing motif that is called an **ITIM** (immunoreceptor tyrosine-based inhibitory motif), which has the consensus sequence V/L/IxYxxL, where V refers to valine. Phosphorylated ITIMs recruit tyrosine or inositol lipid phosphatases, enzymes that remove phosphate residues from phosphotyrosine moieties or from certain lipid phosphates and thus counteract ITAM-based immune receptor activation.

Members of the immune receptor family include antigen receptors on both B cells and T cells, the IgE receptor on mast cells, and activating and inhibitory Fc receptors on innate immune cells and B lymphocytes (Fig. 7-5). ITAMs are found in the cytoplasmic tails of several immune receptor complexes that are involved in signal transduction, including the ζ chain and CD3 proteins of the T cell receptor (TCR) complex, Igα and Igβ proteins associated with membrane Ig molecules (the antigen receptors) of B cells, and components of several Fc receptors and of the NKG2D activating receptor on natural killer (NK) cells (see Chapter 4). ITIM-containing inhibitory receptors include CD22, FcγRIIB, and several inhibitory NK cell receptors.

General Features of Antigen Receptor Signaling

Signaling downstream of the T and B cell antigen receptors is characterized by a similar sequence of events, consisting of the following.

- Receptor ligation typically involves the clustering of receptors by multivalent ligands, resulting in activation of an associated Src family kinase. Receptor ligation may also result in the unfolding of the cytoplasmic tail of a polypeptide chain that is part of the receptor. The unfolding event (or conformational change) may allow previously hidden tyrosine residues of a cytosolic ITAM motif to become available for phosphorylation by a Src family kinase.
- The activated Src family kinase phosphorylates available tyrosines in the ITAMs of signaling proteins that are part of the receptor complex.
- The two phosphorylated tyrosines in a single ITAM are recognized by a Syk family tyrosine kinase that has tandem SH2 domains that each recognize an ITAM phosphotyrosine.
- Recruitment of the Syk family kinase to the phosphorylated ITAM results in the activation of this tyrosine kinase and the subsequent tyrosine phosphorylation of adaptor proteins and enzymes that activate distinct signaling pathways downstream of the immune receptor.

This sequence of events is described in more detail in the context of T cell and B cell receptor signaling later in the chapter.

Alterations in the strength of TCR and B cell receptor (BCR) signaling influence the fates of lymphocytes during their development and activation. In other words, the presence of different numbers of activated signaling molecules induced by antigen-ligated receptors is interpreted differently by lymphocytes. For instance, during maturation of T cells in the thymus, weak antigen receptor signals are required for positive selection, the process that preserves useful cells by matching coreceptors to the appropriate MHC molecules, and gradations of signal strength may determine positive selection of developing T cells into the CD4 or CD8 lineage (see Chapter 8). In contrast, strong antigen receptor signals during maturation may contribute to lymphocyte death by apoptosis. The strength of TCR and BCR signaling may also differentially influence the type of immune response that is generated by a given antigen.

Antigen receptor signaling is fine-tuned and modulated by three mechanisms that are unique to this class of receptors.

- ***Progressive ITAM use.*** One of the ways in which different quantities of signal output might be generated by antigen receptors is the phosphorylation of different numbers of ITAM tyrosines after receptor engagement. The TCR complex has six signaling chains and ten ITAMs, and increasing numbers of ITAMs may be phosphorylated as the affinity of different ligands for the TCR increases. The number of ITAMs phosphorylated may therefore provide a cytosolic interpretation of the affinity of the antigen that binds to the TCR, and antigen affinity can thus influence the nature of the cellular response at different stages of differentiation and activation. The BCR has only two ITAMs, but because this number increases when the receptor is cross-linked by multivalent antigens, the degree of cross-linking by antigens may determine the number of ITAMs that might be used and thus generate different responses to antigens of differing affinity and valency.
- ***Increased cellular activation by coreceptors.*** A **coreceptor** is a transmembrane signaling protein on a lymphocyte that can facilitate antigen receptor activation by simultaneously binding to the same antigen complex that is recognized by the antigen receptor. The coreceptor brings with it signaling enzymes linked to its cytoplasmic tail and can thereby facilitate ITAM phosphorylation and activation of the antigen receptor when antigen draws it into the vicinity of the antigen receptor. Coreceptors on T cells are the CD4 and CD8 proteins that demarcate the two functionally distinct subsets. Complement receptor type 2 (CR2/CD21) is the coreceptor on B cells.
- ***Modulation of signaling by inhibitory receptors.*** Key **inhibitory receptors** in T cells include CTLA-4 and PD-1, whereas important inhibitory signals in B cells are delivered through receptors such as CD22 and FcγRIIB, among others. The roles of these inhibitors are mentioned later in this chapter.

In addition, antigen receptor signals may, in some circumstances, cooperate with signals from receptors, known as **costimulatory receptors**, that add yet another level of control to the process of lymphocyte activation. Costimulatory receptors provide "second signals" for lymphocytes (antigen recognition provides the first signal) and ensure that immune responses are optimally triggered by infectious pathogens and substances that mimic microbes. Unlike coreceptors, costimulatory receptors do not recognize components of the same ligands as do antigen receptors; signal outputs downstream of costimulatory receptors are integrated with the signals derived from the antigen receptor, and these signals cooperate to fully activate lymphocytes. The prototypic costimulatory receptor is CD28 on T cells, which is activated by the costimulatory molecules B7-1 and B7-2 (CD80 and CD86), ligands induced on antigen-presenting cells (APCs) as a result of their exposure to microbes (see Chapter 9).

The T Cell Receptor Complex and T Cell Signaling

The TCR was discovered in the early 1980s, around the same time that the structure of major histocompatibility complex (MHC) molecules associated with peptides, the ligands for T cells, was being defined (see Chapter 6). A number of separate approaches were used to molecularly identify the TCR. One approach depended on the identification of genes that were expressed specifically in T cells and that also could be shown to have undergone a gene rearrangement event specifically in these cells (a characteristic feature of antigen receptor genes, described in Chapter 8). The first gene thus identified was homologous to Ig genes and proved to be a chain of the heterodimeric γδ TCR. In another approach, clonal populations of T cells were created and monoclonal antibodies were generated against different T cell clones. Monoclonal antibodies that each recognized only a specific T cell clone were identified. These clonotype-specific antibodies identified a chain of the TCR. In yet another study, one chain of the TCR was identified serendipitously, when sequencing of a T cell–specific library of cDNAs unexpectedly revealed a novel gene with homology to immunoglobulins. We now know that the TCR is similar to antibodies, but there are important differences between these two types of antigen receptors (Table 7-1).

TABLE 7–1 Properties of Lymphocyte Antigen Receptors: T Cell Receptor and Immunoglobulins

	T Cell Receptor (TCR)	Immunoglobulin (Ig)
Components	α and β chains	Heavy and light chains
Number of Ig domains	One V domain and one C domain in each chain	Heavy chain: one V domain, three or four C domains Light chain: one V domain and one C domain
Number of CDRs	Three in each chain for antigen binding	Three in each chain
Associated signaling molecules	CD3 and ζ	Igα and Igβ
Affinity for antigen (K_d)	10^{-5}-10^{-7} M	10^{-7}-10^{-11} M (secreted Ig)
Changes after cellular activation		
Production of secreted form	No	Yes
Isotope switching	No	Yes
Somatic mutations	No	Yes

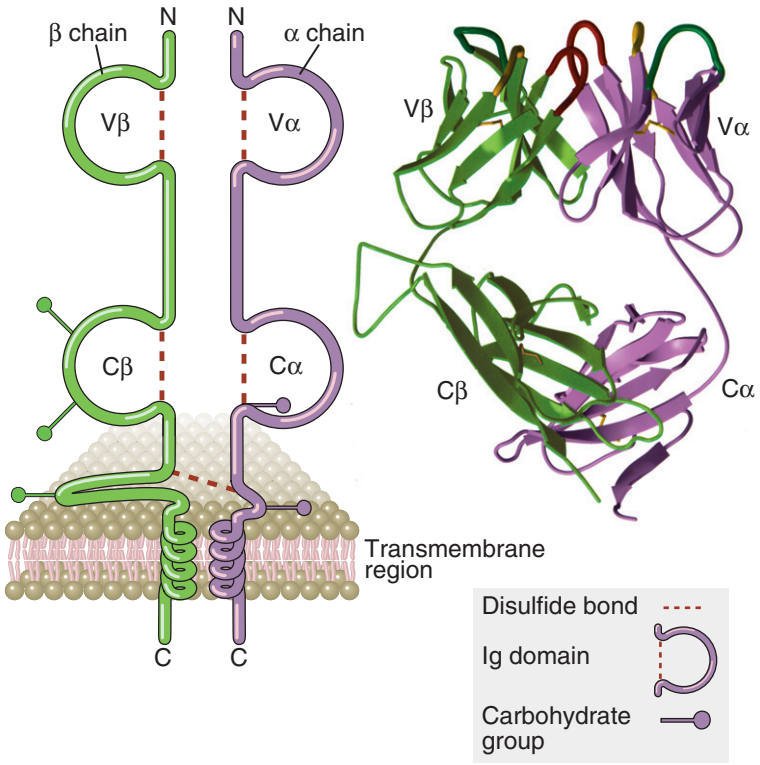


FIGURE 7–6 Structure of the T cell receptor. The schematic diagram of the αβ TCR *(left)* shows the domains of a typical TCR specific for a peptide-MHC complex. The antigen-binding portion of the TCR is formed by the V_β and V_α domains. The ribbon diagram *(right)* shows the structure of the extracellular portion of a TCR as revealed by x-ray crystallography. The hypervariable segment loops that form the peptide-MHC binding site are at the top. *(Modified from Bjorkman PJ. MHC restriction in three dimensions: a view of T cell receptor/ligand interactions. Cell 89:167-170, 1997. Copyright Cell Press.)*

The Structure of the T Cell Receptor for Antigen

The antigen receptor of MHC-restricted CD4+ helper T cells and CD8+ cytotoxic T lymphocytes (CTLs) is a heterodimer consisting of two transmembrane polypeptide chains, designated TCR α and β, covalently linked to each other by a disulfide bridge between extracellular cysteine residues (Fig. 7-6). These T cells are called αβ T cells. A less common type of TCR, found on γδ T cells, is composed of TCR γ and δ chains. Each TCR α and β chain consists of one Ig-like N-terminal variable (V) domain, one Ig-like constant (C) domain, a hydrophobic transmembrane region, and a short cytoplasmic region. Thus, the extracellular portion of the TCR αβ heterodimer is structurally similar to the antigen-binding fragment (Fab) of an Ig molecule, which is made up of the V and C regions of a light chain and the V region and one C region of a heavy chain (see Chapter 5).

The V regions of the TCR α and β chains contain short stretches of amino acids where the variability between different TCRs is concentrated, and these form the hypervariable or complementarity-determining regions (CDRs). Three CDRs in the α chain and three similar regions in the β chain together form the part of the TCR that specifically recognizes peptide-MHC complexes (Fig. 7-7). The β chain V domain contains a fourth hypervariable region that does not appear to participate in antigen recognition but is the binding site for microbial products called superantigens (see Chapter 15). Each TCR chain, like Ig heavy and light chains, is encoded by multiple gene segments that undergo somatic rearrangements during the maturation of the T lymphocytes (see Chapter 8).

The C regions of both α and β chains continue into short hinge regions, which contain cysteine residues that contribute to a disulfide bond linking the two chains. The hinge is followed by hydrophobic transmembrane portions, an unusual feature of which is the presence of positively charged amino acid residues, including a lysine residue (in the α chain) or a lysine and an arginine residue (in the β chain). These residues interact with negatively charged residues present in the transmembrane portions of other polypeptides (those of the CD3 complex and ζ) that are part of the TCR complex. Both TCR α and β chains have carboxyl-terminal cytoplasmic tails that are 5 to 12 amino acids long. Like membrane Ig on B cells (see later), these cytoplasmic regions are too small to transduce signals, and specific molecules physically associated with the TCR serve the signal-transducing functions of this antigen receptor complex.

The CD3 and ζ proteins are noncovalently associated with the TCR αβ heterodimer, and when the TCR recognizes antigen, these associated proteins transduce the signals that lead to T cell activation. The components of the TCR complex are illustrated in Figures 7-8 and 7-9. The CD3 proteins and the ζ chain are identical in all T cells regardless of specificity, which is consistent with their role in signaling and not in antigen recognition. The CD3 proteins are required not only for signaling in T cells but for surface expression of the functionally complete receptor complex on T cells.

The CD3 γ, δ, and ε proteins are homologous to each other. The N-terminal extracellular regions of the γ, δ, and ε chains each contains a single Ig-like domain, and therefore these three proteins are members of the Ig superfamily. The transmembrane segments of all three

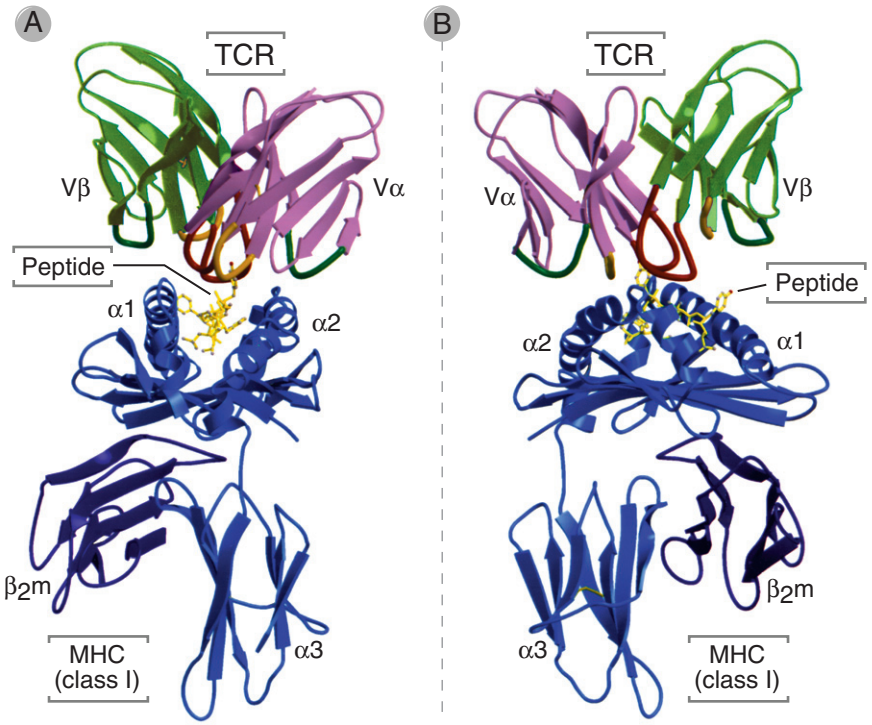


FIGURE 7–7 Binding of a TCR to a peptide-MHC complex. The V domains of a TCR are shown interacting with a human class I MHC molecule, HLA-A2, presenting a viral peptide (in yellow). **A** is a front view and **B** is a side view of the x-ray crystal structure of the trimolecular MHC-peptide-TCR complex. *(From Bjorkman PJ. MHC restriction in three dimensions: a view of T cell receptor/ligand interactions. Cell 89:167-170, 1997. Copyright Cell Press.)*

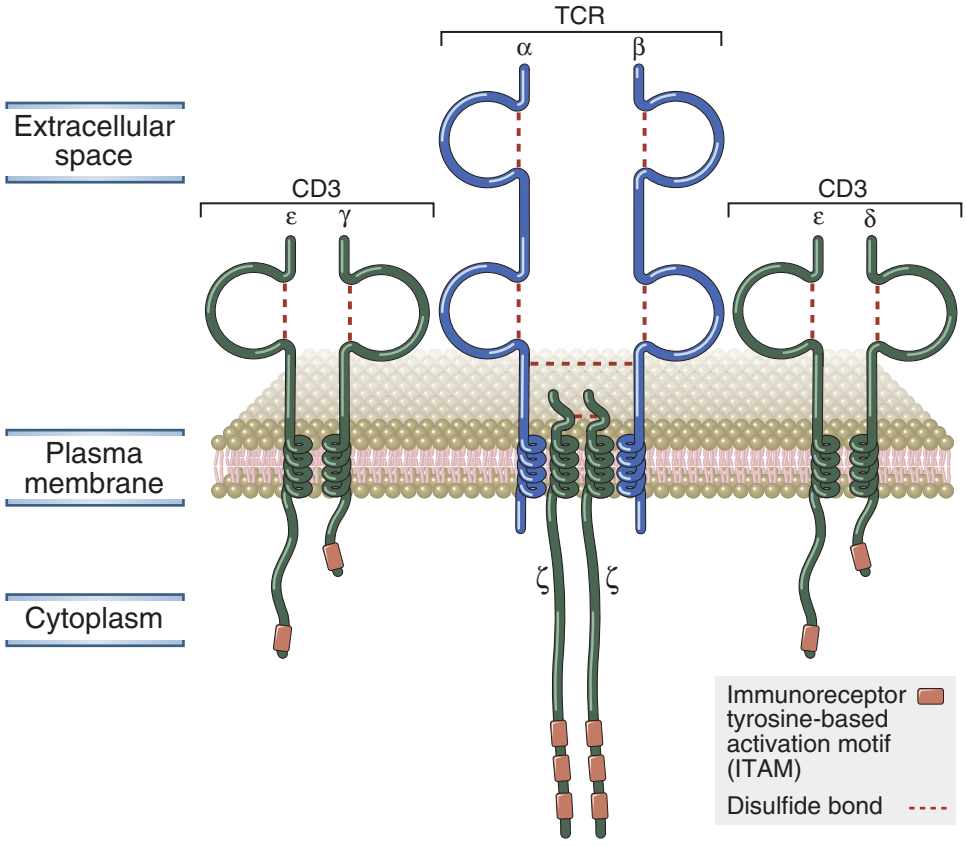
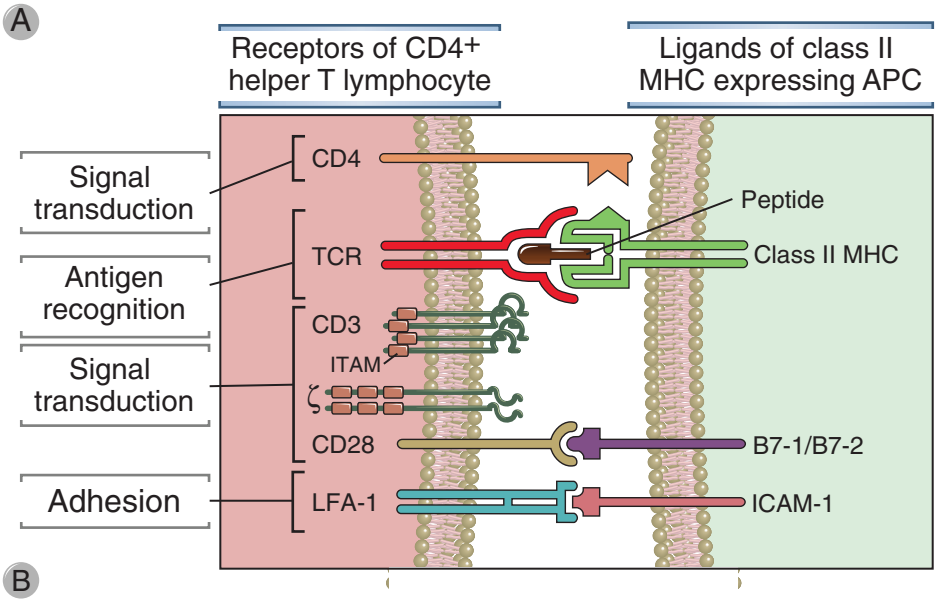


FIGURE 7–8 Components of the TCR complex. The TCR complex of MHC-restricted T cells consists of the αβ TCR noncovalently linked to the CD3 and ζ proteins. The association of these proteins with one another is mediated by charged residues in their transmembrane regions, which are not shown.

A



B

T cell accessory molecule	Function	Ligand	
		Name	Expressed on
CD3	Signal transduction by TCR complex	None	
ζ	Signal transduction by TCR complex	None	
CD4	Signal transduction	Class II MHC	Antigen presenting cells
CD8	Signal transduction	Class I MHC	Antigen presenting cells, CTL target cells
CD28	Signal transduction (costimulation)	B7-1/B7-2	Antigen presenting cells
CTLA-4	Signal transduction (negative regulation)	B7-1/B7-2	Antigen presenting cells
LFA-1	Adhesion	ICAM-1	Antigen presenting cells, endothelium
VLA-4	Adhesion	VCAM-1	Endothelium

FIGURE 7–9 Ligand-receptor pairs involved in T cell activation. A, The major surface molecules of $CD4^+$ T cells involved in the activation of these cells (the receptors) and the molecules on APCs (the ligands) recognized by the receptors are shown. $CD8^+$ T cells use most of the same molecules, except that the TCR recognizes peptide–class I MHC complexes, and the coreceptor is CD8, which recognizes class I MHC. Immunoreceptor tyrosine-based activation motifs (ITAMs) are the regions of signaling proteins that are phosphorylated on tyrosine residues and become docking sites for other signaling molecules. CD3 is composed of three polypeptide chains, named γ, δ, and ε, arranged in two pairs (γε and δε); we show CD3 as three protein chains. **B,** The important properties of the major "accessory" molecules of T cells, so called because they participate in responses to antigens but are not the receptors for antigen, are summarized. CTLA-4 (CD152) is a receptor for B7 molecules that delivers inhibitory signals; its role in shutting off T cell responses is described in Chapter 9. VLA molecules are integrins involved in leukocyte binding to endothelium (see Chapter 3). APC, antigen-presenting cell; ICAM-1, intercellular adhesion molecule 1; LFA-1, leukocyte function-associated antigen 1; MHC, major histocompatibility complex; TCR, T cell receptor; VLA, very late antigen.

CD3 chains contain a negatively charged aspartic acid residue that binds to positively charged residues in the transmembrane domains of the TCR α and β chains. Each TCR complex contains one TCR αβ heterodimer associated with one CD3 γε heterodimer, one CD3 δε heterodimer, and one disulfide-linked ζζ homodimer.

The cytoplasmic domains of the CD3 γ, δ, and ε proteins range from 44 to 81 amino acid residues in length, and each of these domains contains one ITAM. The ζ chain has a short extracellular region of nine amino acids, a transmembrane region containing a negatively charged aspartic acid residue (similar to the CD3 chains), and a long cytoplasmic region (113 amino acids) that contains three ITAMs. It is normally expressed as a homodimer. The ζ chain is also associated with signaling receptors on lymphocytes other than T cells, such as the Fcγ receptor (FcγRIII) of NK cells.

Signal Initiation by the T Cell Receptor

Ligation of the TCR by MHC-peptide ligands results in the clustering of coreceptors with the antigen receptor and phosphorylation of ITAM tyrosine residues. Phosphorylation of ITAM tyrosines initiates signal transduction and the activation of downstream tyrosine kinases, which in turn phosphorylate tyrosine residues on other adaptor proteins. The subsequent steps in signal transduction are generated by the specific recruitment of key enzymes that each initiate distinct downstream signaling pathways.

It is thought that the TCR, like other immune receptors, is activated when multiple receptor molecules are brought together by binding to adjacent antigenic epitopes. However, cross-linking of the TCR poses a challenge because the induction of receptor clustering would require a high density of identical MHC-peptide complexes on APCs, and APCs generally express very few peptide-MHC complexes, perhaps as few as 100 per cell, that may be recognized by a given TCR (see Chapter 6). How, then, is the signal from the TCR initiated? It is known that antigen recognition by the TCR induces ITAM phosphorylation by active Src family kinases, but the actual mechanism of signal initiation remains to be conclusively determined. There is growing evidence that ITAMs in the TCR complex are "folded" and unavailable before the TCR recognizes antigen. Recognition of MHC-peptide complexes may induce a conformational change in the TCR, making the ITAMs associated with the linked CD3 or ζ chains available for tyrosine phosphorylation by Src family kinases. Alternatively, the activity of Src family kinases may be enhanced after receptor ligation (Fig. 7-10). The CD4 and CD8 coreceptors (described next) greatly facilitate the activation process by bringing Lck (which is loosely associated with the tail of the coreceptor proteins) close to the CD3 and ζ ITAMs (see Fig. 7-10). Eventually, a relatively stable interface is formed between the T cell and the APC, and this interface is known as the immunologic synapse (discussed later).

The Role of the CD4 and CD8 Coreceptors in T Cell Activation

CD4 and CD8 are T cell coreceptors that bind to nonpolymorphic regions of MHC molecules and facilitate signaling by the TCR complex during T cell activation (see Fig. 7-9). These proteins are called **coreceptors** because they bind to MHC molecules and thus recognize a part of the same ligand (peptide-MHC complexes) that interacts with the TCR. Mature αβ T cells express either CD4 or CD8, but not both. CD8 and CD4 interact with class I and class II MHC molecules, respectively, and are responsible for the class I or class II MHC restriction of these subsets of T cells (see Fig. 7-9 and Chapter 6).

CD4 and CD8 are transmembrane glycoprotein members of the Ig superfamily (Fig. 7-11). CD4 is expressed as a monomer on the surface of peripheral T cells and thymocytes and is also present on mononuclear phagocytes and some dendritic cells. It is the receptor on T cells for the envelope protein of the human immunodeficiency virus. CD4 has four extracellular Ig-like domains, a hydrophobic transmembrane region, and a highly basic cytoplasmic tail 38 amino acids long. The two N-terminal Ig-like domains of the CD4 protein bind to the nonpolymorphic β2 domain of the class II MHC molecule.

Most CD8 molecules exist as disulfide-linked heterodimers composed of two related chains called CD8α and CD8β (see Fig. 7-11). Both the α chain and the β chain have a single extracellular Ig domain, a hydrophobic transmembrane region, and a highly basic cytoplasmic tail about 25 amino acids long. The Ig domain of CD8 binds to the nonpolymorphic α3 domain of class I MHC molecules. Some T cells express CD8 αα homodimers, but this different form appears to function like the more common CD8 αβ heterodimers. These homodimers are also present on a subset of murine dendritic cells (see Chapter 6).

The cytoplasmic tails of both CD4 and CD8 bind the Src family kinase Lck. The ability of these coreceptors to bind to MHC molecules helps these proteins to be drawn adjacent to the TCR that contacts the same MHC-peptide complex on the APC. As a result, on the cytosolic face of the membrane, Lck is drawn very close to the ITAMs in CD3 and ζ proteins and phosphorylates the ITAMs, thus facilitating the subsequent recruitment and activation of the kinase ZAP-70.

Activation of Tyrosine Kinases and a Lipid Kinase During T Cell Activation

Phosphorylation of residues in proteins and lipids plays a central role in the transduction of signals from the TCR complex and coreceptors. Within seconds of TCR ligation, many of the tyrosine residues within the ITAMs of the CD3 and ζ chains become phosphorylated (see Fig.7-10). In addition to coreceptor-associated Lck, another Src family kinase that is found in physical association with the TCR complex is CD3-associated Fyn, and it may play a role similar to that of Lck. Knockout mice lacking Lck show some defects in T cell development, and double knockout mice lacking both Lck and Fyn show even more severe defects.

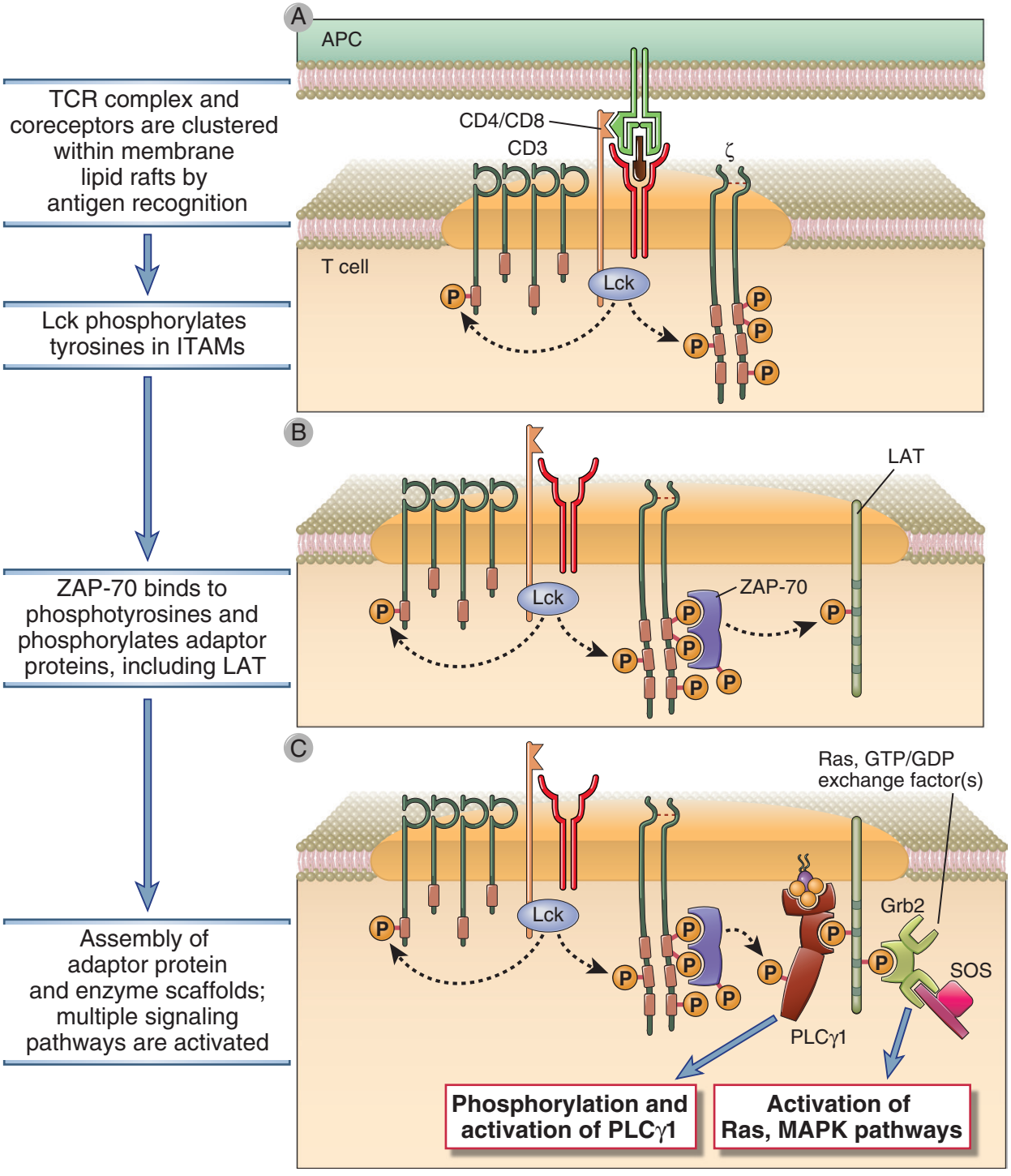


FIGURE 7–10 Early tyrosine phosphorylation events in T cell activation. On antigen recognition, there is clustering of TCR complexes with coreceptors (CD4, in this case). CD4-associated Lck becomes active and phosphorylates tyrosines in the ITAMs of CD3 and ζ chains **(A)**. ZAP-70 binds to the phosphotyrosines of the ζ chains and is itself phosphorylated and activated. (The illustration shows one ZAP-70 molecule binding to two phosphotyrosines of one ITAM in the ζ chain, but it is likely that initiation of a T cell response requires the assembly of multiple ZAP-70 molecules on each ζ chain.) Active ZAP-70 then phosphorylates tyrosines on various adaptor molecules, such as LAT **(B)**. The adaptors become docking sites for cellular enzymes such as PLCγ1 and exchange factors that activate Ras and other small G proteins upstream of MAP kinases **(C)**, and these enzymes activate various cellular responses.

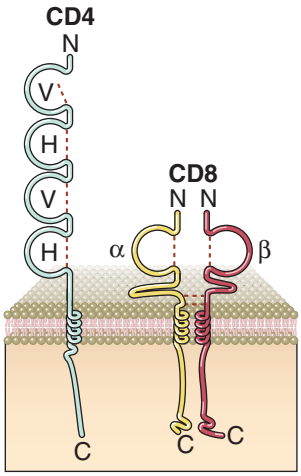


FIGURE 7–11 A schematic view of the structure of the CD4 and CD8 coreceptors. The CD4 protein is an integral membrane monomer consisting of four extracellular Ig domains, a transmembrane domain, and a cytoplasmic tail. The CD8 protein is either a disulfide-linked αβ integral membrane heterodimer or a disulfide linked αα homodimer (not shown). Each chain has a single extracellular Ig domain. The cytoplasmic portions of both CD4 and CD8 can associate with Lck (not shown).

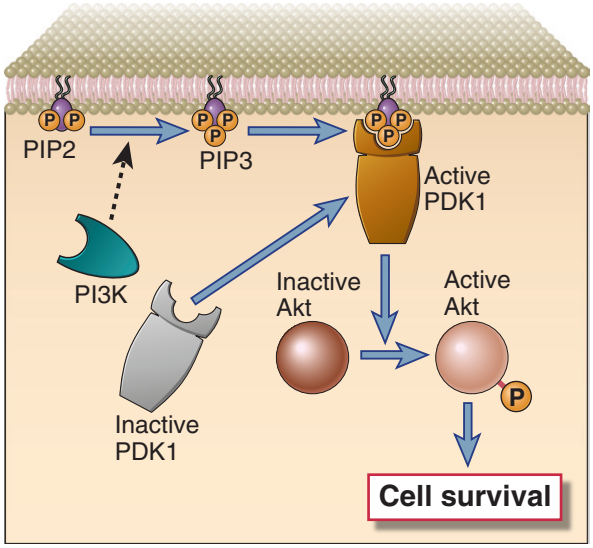


FIGURE 7–12 Role of PI3-kinase in T cell responses. Membrane PIP3, generated by PI3-kinase (PI3K), activates PDK1, which phosphorylates and activates the Akt kinase. This enzyme phosphorylates downstream targets that are involved in cell survival.

The tyrosine-phosphorylated ITAMs in the ζ chain become "docking sites" for the Syk family tyrosine kinase called **ZAP-70** (ζ-associated protein of 70 kD). ZAP-70 contains two SH2 domains that can bind to ITAM phosphotyrosines. Each ITAM has two tyrosine residues, and both of these must be phosphorylated to provide a docking site for one ZAP-70 molecule. The bound ZAP-70 becomes a substrate for the adjacent Lck, which phosphorylates specific tyrosine residues of ZAP-70. As a result, ZAP-70 acquires its own tyrosine kinase activity and is then able to phosphorylate a number of other cytoplasmic signaling molecules. A critical threshold of ZAP-70 activity may be needed before downstream signaling events will proceed, and this threshold is achieved by the recruitment of multiple ZAP-70 molecules to the phosphorylated ITAMs on the ζ chains and on CD3 tails.

Another signaling pathway in T cells involves the activation of PI3-kinase, which phosphorylates a specific membrane-associated inositol lipid (Fig. 7-12). This enzyme is recruited to the TCR complex and associated adaptor proteins and generates phosphatidylinositol trisphosphate (PIP3) from membrane phosphatidylinositol bisphosphate (PIP2) on the inner leaflet of the plasma membrane. Certain signaling proteins in the cytosol have specialized PH domains that have an affinity for PIP3, and as a result, PH domain–containing proteins can bind to the inside of the cell membrane only when PIP3 is generated. Examples of PH domain–containing proteins include kinases such as Itk in T cells and Btk in B cells. Another important PIP3-dependent kinase is PDK1, which is required for the phosphorylation and activation of an important downstream kinase called Akt. Activated Akt phosphorylates crucial targets and contributes to cell survival in a number of ways. Phosphorylation by Akt leads to the inactivation of two proapoptotic members of the Bcl-2 family, BAD and BAX. Akt also inactivates a Forkhead family transcription factor that induces the expression of Fas ligand, and this kinase also targets caspase-9 for degradation.

Recruitment and Modification of Adaptor Proteins

Activated ZAP-70 phosphorylates several adaptor proteins that are able to bind signaling molecules (see Fig. 7-10). A key early event in T cell activation is the ZAP-70–mediated tyrosine phosphorylation of adaptor proteins such as SLP-76 and LAT. Phosphorylated LAT directly binds PLCγ1, a key enzyme in T cell activation (discussed later), and coordinates the recruitment of several other adaptor proteins, including SLP-76, GADS, and Grb-2, to the cluster of TCR and TCR-associated proteins, sometimes referred to as the signalosome. Thus, LAT serves to bring a variety of downstream components of TCR signaling pathways close to their upstream activators. Because the function of many of these adaptors depends on their tyrosine phosphorylation by active ZAP-70, only antigen recognition (the physiologic stimulus for ZAP-70 activation) triggers the signal transduction pathways that lead to functional T cell responses.

Formation of the Immunologic Synapse

When the TCR complex recognizes MHC-associated peptides on an APC, several T cell surface proteins and intracellular signaling molecules are rapidly mobilized to the site of T cell–APC contact (Fig. 7-13). This region of physical contact between the T cell and the APC forms a bull's-eye–like structure that is called an **immunologic synapse** or a supramolecular activation cluster (SMAC). The T cell molecules that are rapidly mobilized to the center of the synapse include the TCR complex (the TCR, CD3, and ζ chains), CD4 or CD8 coreceptors, receptors for

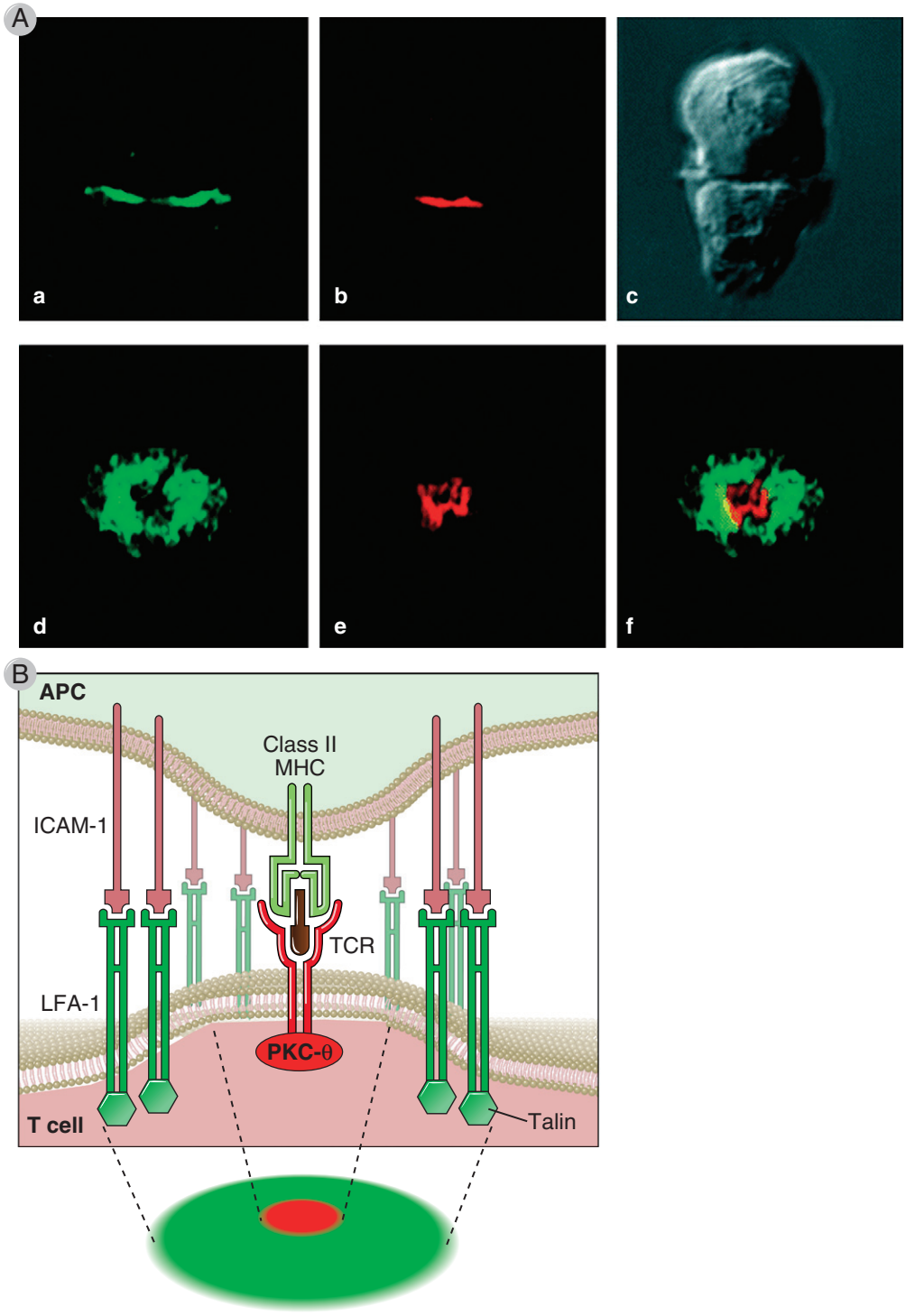


FIGURE 7–13 The immunologic synapse. A, This figure shows two views of the immunologic synapse in a T cell–APC conjugate (shown as a Nomarski image in panel **c**). Talin, a protein that associates with the cytoplasmic tail of the LFA-1 integrin, was revealed by an antibody labeled with a green fluorescent dye, and PKC-θ, which associates with the TCR complex, was visualized by antibodies conjugated to a red fluorescent dye. In panels **a** and **b**, a two-dimensional optical section of the cell contact site along the x-y axis is shown, revealing the central location of PKC-θ and the peripheral location of talin, both in the T cell. In panels **d-f**, a three-dimensional view of the entire region of cell-cell contact along the x-z axis is provided. Note, again, the central location of PKC-θ and the peripheral accumulation of talin. *(Reprinted with permission of Macmillan Publishers Ltd. from Monks CRF, BA Freiburg, H Kupfer, N Sciaky, and A Kupfer. Three dimensional segregation of supramolecular activation clusters in T cells. Nature 395:82-86, copyright 1998.)* **B,** A schematic view of the synapse, showing talin and LFA-1 in the p-SMAC (green) and PKC-θ and the TCR in the c-SMAC (red).

costimulators (such as CD28), enzymes such as PKC-θ, and adaptor proteins that associate with the cytoplasmic tails of the transmembrane receptors. At this portion of the synapse, called the c-SMAC (for central supramolecular activation cluster), the distance between the T cell plasma membrane and that of the APC is about 15 nm. Integrins remain at the periphery of the synapse, where they function to stabilize the binding of the T cell to the APC, forming the peripheral portion of the SMAC called the p-SMAC. In this outer part of the synapse, the two membranes are about 40 nm apart. Many signaling molecules found in synapses are initially localized to regions of the plasma membrane that have a lipid content different from the rest of the cell membrane and are called **lipid rafts** or glycolipid-enriched microdomains. TCR and costimulatory receptor signaling is initiated in these rafts, and signaling initiates cytoskeletal rearrangements that allow rafts to coalesce and form the immunologic synapse.

Immunologic synapses may serve a number of functions during and after T cell activation.

- The synapse forms a stable contact between an antigen-specific T cell and an APC displaying that antigen and becomes the site for assembly of the signaling machinery of the T cell, including the TCR complex, coreceptors, costimulatory receptors, and adaptors. Although TCR signal transduction is clearly initiated before the formation of the synapse and is required for synapse formation, the immunologic synapse itself may provide a unique interface for TCR triggering. T cell activation needs to overcome the problems of a generally low affinity of TCRs for peptide-MHC ligands and the presence of few MHC molecules displaying any one peptide on an APC. The synapse represents a site at which repeated engagement of TCRs may be sustained by this small number of peptide-MHC complexes on the APC, thus facilitating prolonged and effective T cell signaling.
- The synapse may ensure the specific delivery of secretory granule contents and cytokines from a T cell to APCs or targets that are in contact with the T cell. Vectorial delivery of secretory granules containing perforin and granzymes from CTLs to target cells has been shown to occur at the synapse (see Chapter 10). Similarly, CD40L-CD40 interactions are facilitated by the accumulation of these molecules on the T cell and APC interfaces of the immunologic synapse. Some cytokines are also secreted in a directed manner into the synaptic cleft, from where they are preferentially delivered to the cell that is displaying antigen to the T lymphocyte.
- The synapse may also be an important site for the turnover of signaling molecules, primarily by monoubiquitination and delivery to late endosomes and lysosomes. This degradation of signaling proteins may contribute to the termination of T cell activation and is discussed later.

MAP Kinase Signaling Pathways in T Lymphocytes

Small guanine nucleotide–binding proteins (G proteins) activated by antigen recognition stimulate at least three different mitogen-activated protein (MAP) kinases, which in turn activate distinct transcription factors. G proteins are involved in diverse activation responses in different cell types. Two major members of this family activated downstream of the TCR are Ras and Rac. Each activates a different component or set of transcription factors, and together they mediate many cellular responses of T cells.

- ***The Ras pathway is activated in T cells after TCR ligation, leading to the activation of the extracellular receptor-activated kinase (ERK), a prominent member of the MAP kinase family, and eventually to the activation of downstream transcription factors.*** Ras is loosely attached to the plasma membrane through covalently attached lipids. In its inactive form, the guanine nucleotide–binding site of Ras is occupied by guanosine diphosphate (GDP). When the bound GDP is replaced by guanosine triphosphate (GTP), Ras undergoes a conformational change and can then recruit or activate various cellular enzymes, the most important of which is c-Raf. Activation of Ras by GDP/GTP exchange is seen in response to the engagement of many types of receptors in many cell populations, including the TCR complex in T cells. Mutated Ras proteins that are constitutively active (i.e., they constantly assume the GTP-bound conformation) are associated with neoplastic transformation of many cell types. Nonmutated Ras proteins are active GTPases that convert the GTP bound to Ras into GDP, thus returning Ras to its normal, inactive state.

  The mechanism of Ras activation in T cells involves the adaptor proteins LAT and Grb-2 (Fig. 7-14). When LAT is phosphorylated by ZAP-70 at the site of TCR clustering, it serves as the docking site for the SH2 domain of Grb-2. Once attached to LAT, Grb-2 recruits the Ras GTP/GDP exchange factor called SOS (so named because it is the mammalian homologue of a *Drosophila* protein called son of sevenless) to the plasma membrane. SOS catalyzes GTP for GDP exchange on Ras. This generates the GTP-bound form of Ras (written as Ras·GTP), which then activates a "MAP kinase" cascade of three kinases, the first two of which phosphorylate and activate the next kinase in the cascade. The last kinase in the cascade initiated by Ras is a MAP kinase called ERK. Ras·GTP activates a kinase called c-Raf, which then activates a dual-specificity kinase that phosphorylates ERK on closely spaced threonine and tyrosine residues. This dual-specificity kinase is an example of a MAP kinase kinase (a kinase that activates a MAP kinase). The activated ERK MAP kinase translocates to the nucleus and phosphorylates a protein called Elk, and phosphorylated Elk stimulates transcription of c-Fos, a component of the activation protein 1 (AP-1) transcription factor.
- In parallel with the activation of Ras through recruitment of Grb-2 and SOS, the adaptors phosphorylated by TCR-associated kinases also recruit and activate a GTP/GDP exchange protein called Vav that acts on another small guanine nucleotide–binding protein called **Rac** (see Fig. 7-14). The Rac·GTP that is generated initiates a parallel MAP kinase cascade, resulting in the activation of a distinct MAP kinase called c-Jun

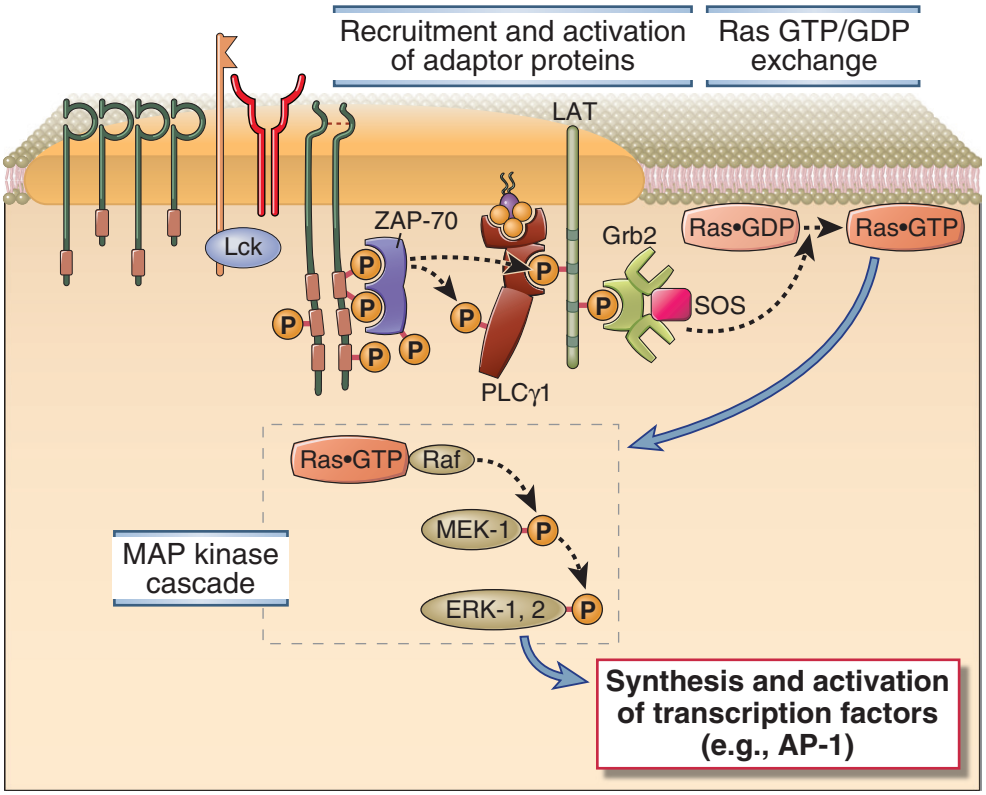


FIGURE 7–14 The Ras-MAP kinase pathway in T cell activation. ZAP-70 that is activated by antigen recognition phosphorylates membrane-associated adaptor proteins (such as LAT), which then bind another adaptor, Grb-2, that provides a docking site for the GTP/GDP exchange factor SOS. SOS converts Ras·GDP to Ras·GTP. Ras·GTP activates a cascade of enzymes, which culminates in the activation of the MAP kinase ERK. A parallel Rac-dependent pathway generates another active MAP kinase, JNK (not shown).

N-terminal kinase (JNK). JNK is sometimes called stress-activated protein (SAP) kinase because in many cells, it is activated by various forms of noxious stimuli such as ultraviolet light, osmotic stress, or proinflammatory cytokines such as tumor necrosis factor (TNF) and IL-1. Activated JNK then phosphorylates c-Jun, the second component of the AP-1 transcription factor. A third member of the MAP kinase family, in addition to ERK and JNK, is p38, and it too is activated by Rac·GTP and in turn activates various transcription factors. Rac·GTP also induces cytoskeletal reorganization and may play a role in the clustering of TCR complexes, coreceptors, and other signaling molecules into the synapse.

The activities of ERK and JNK are eventually shut off by the action of dual-specificity protein tyrosine/threonine phosphatases. These phosphatases are induced or activated by ERK and JNK themselves, providing a negative feedback mechanism to terminate T cell activation.

Calcium- and PKC-Mediated Signaling Pathways in T Lymphocytes

TCR signaling leads to the activation of the γ1 isoform of the enzyme phospholipase C (PLCγ1), and the products of PLCγ1-mediated hydrolysis of membrane lipids activate enzymes that induce specific transcription factors in T cells (Fig. 7-15). PLCγ1 is a cytosolic enzyme specific for inositol phospholipids that is recruited to the plasma membrane by tyrosine-phosphorylated LAT within minutes of ligand binding to the TCR. Here, the enzyme is phosphorylated by ZAP-70 and by other kinases, such as the Tec family kinase called Itk. Phosphorylated PLCγ1 catalyzes the hydrolysis of a plasma membrane phospholipid called PIP2, generating two breakdown products, the soluble sugar triphosphate, inositol 1,4,5-trisphosphate (IP3), and membrane-bound diacylglycerol (DAG). IP3 and DAG then activate two distinct downstream signaling pathways in T cells.

IP3 produces a rapid increase in cytosolic free calcium within minutes after T cell activation. IP3 diffuses through the cytosol to the endoplasmic reticulum, where it binds to its receptor, a ligand-gated calcium channel, and stimulates release of membrane-sequestered calcium stores. The released calcium causes a rapid rise (during a few minutes) in the cytosolic free calcium ion concentration, from a resting level of about 100 nM to a peak of 600 to 1000 nM. The depletion of endoplasmic reticulum calcium is sensed by an endoplasmic reticulum membrane protein called STIM1, which activates a "store-operated" plasma membrane ion channel called a **CRAC** (calcium release–activated calcium) channel. The result is an influx of extracellular calcium that sustains cytosolic levels at about 300 to 400 nM for more than an hour. A key component of the CRAC channel is a protein called **Orai**, which was discovered as a gene that is defective in a rare human immunodeficiency disease. Cytosolic free

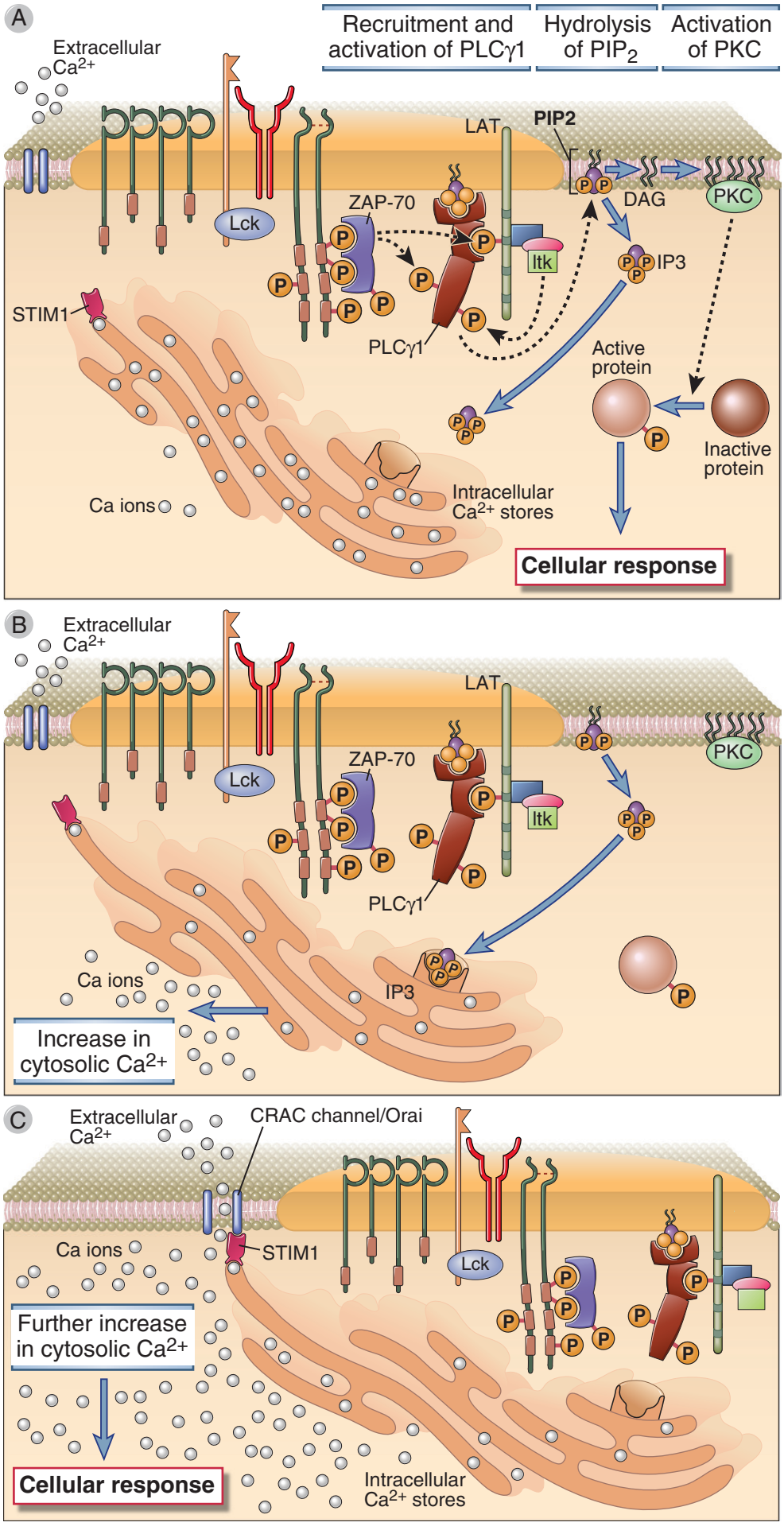


FIGURE 7–15 T cell signaling downstream of PLCγ1. A, The LAT adaptor protein that is phosphorylated on T cell activation binds the cytosolic enzyme PLCγ1, which is phosphorylated by ZAP-70 and other kinases, such as Itk, and activated. Active PLCγ1 hydrolyzes membrane PIP2 to generate IP3, which stimulates an increase in cytosolic calcium, and DAG, which activates the enzyme PKC. **B,** Depletion of endoplasmic reticulum calcium is sensed by STIM1. **C,** STIM1 which induces the opening of the CRAC channel that facilitates entry of extracellular calcium into the cytosol. Orai is a component of the CRAC channel. Increased cytosolic calcium and PKC then activate various transcription factors, leading to cellular responses.

calcium acts as a signaling molecule by binding to a ubiquitous calcium-dependent regulatory protein called calmodulin. Calcium-calmodulin complexes activate several enzymes, including a protein serine/threonine phosphatase called **calcineurin** that is important for transcription factor activation, as discussed later.

Diacylglycerol (DAG), the second breakdown product of PIP2, is a membrane-bound lipid that activates the enzyme protein kinase C (PKC). There are several isoforms of PKC that participate in the generation of active transcription factors, discussed later. The combination of elevated free cytosolic calcium and DAG activates certain isoforms of membrane-associated PKC by inducing a conformational change that makes the catalytic site of the kinase accessible to its substrates. Numerous downstream proteins are phosphorylated by PKC. The PKC-θ isoform localizes to the immunologic synapse and is involved in the activation and nuclear translocation of the nuclear factor κB (NF-κB) transcription factor. Pathways of NF-κB activation are discussed later in this chapter.

So far, we have described several signal transduction pathways initiated by ligand binding to the TCR that result in the activation of different types of enzymes: small G protein–MAP kinase pathways leading to activation of kinases such as ERK and JNK; a PLCγ1-calcium–dependent pathway leading to activation of the phosphatase calcineurin; and a DAG-dependent pathway leading to activation of PKC. Each of these pathways contributes to the expression of genes encoding proteins needed for T cell clonal expansion, differentiation, and effector functions. In the following section, we describe the mechanisms by which these different signaling pathways stimulate the transcription of various genes in T cells.

Activation of Transcription Factors That Regulate T Cell Gene Expression

The enzymes generated by TCR signaling activate transcription factors that bind to regulatory regions of numerous genes in T cells and thereby enhance transcription of these genes (Fig. 7-16). Much of our understanding of the transcriptional regulation of genes in T cells is based on analyses of cytokine gene expression. The transcriptional regulation of most cytokine genes in T cells is controlled by the binding of transcription factors to nucleotide sequences in the promoter and enhancer regions of these genes. For instance, the IL-2 promoter, located 5′ of the coding exons of this gene, contains a segment of approximately 300 base pairs in which are located binding sites for several different transcription factors. All these sites must be occupied by transcription factors for maximal transcription of the IL-2 gene. Different transcription factors are activated by different cytoplasmic signal transduction pathways, and the requirement for multiple transcription factors accounts for the need to activate many signaling pathways after antigen recognition. It is likely that the same principles are true for many genes in T cells, including genes encoding cytokine receptors and effector molecules, although different genes may be responsive to different combinations of transcription factors.

Three transcription factors that are activated in T cells by antigen recognition and appear to be critical for most T cell responses are nuclear factor of activated T cells (NFAT), AP-1, and NF-κB.

- **NFAT** is a transcription factor required for the expression of IL-2, IL-4, TNF, and other cytokine genes. NFAT is present in an inactive, serine-phosphorylated form in the cytoplasm of resting T lymphocytes. It is activated by the calcium-calmodulin–dependent phosphatase calcineurin. Calcineurin dephosphorylates cytoplasmic NFAT, thereby uncovering a nuclear localization signal that permits NFAT to translocate into the nucleus. Once it is in the nucleus, NFAT binds to the regulatory regions of IL-2, IL-4, and other cytokine genes, usually in association with other transcription factors, such as AP-1.

  The mechanism of activation of NFAT was discovered indirectly by studies of the mechanism of action of the immunosuppressive drug cyclosporine (see Chapter 16). This drug and the functionally similar compound, FK506, are natural products of fungi and are widely used therapeutic agents to treat allograft rejection. They function largely by blocking T cell cytokine gene transcription. Cyclosporine binds to a cytosolic protein called cyclophilin, and FK506 binds to a protein called FK506-binding protein (FKBP). Cyclophilin and FKBP are also called immunophilins. Cyclosporine-cyclophilin complexes and FK506-FKBP complexes bind to and inhibit calcineurin and thereby block translocation of NFAT into the nucleus.
- **AP-1** is a transcription factor found in many cell types; it is specifically activated in T lymphocytes by TCR-mediated signals. AP-1 is actually the name for a family of DNA-binding factors composed of dimers of two proteins that bind to one another through a shared structural motif called a leucine zipper. The best characterized AP-1 factor is composed of the proteins Fos and Jun. TCR-induced signals lead to the appearance of active AP-1 in the nucleus of T cells. Activation of AP-1 typically involves synthesis of the Fos protein and phosphorylation of preexisting Jun protein. Transcription and synthesis of Fos can be enhanced by the ERK pathway, as described before, and also by PKC. JNK phosphorylates c-Jun, and AP-1 complexes containing the phosphorylated form of Jun have increased transcription-enhancing activity. AP-1 appears to physically associate with other transcription factors in the nucleus, including NFAT, and works best in combination with NFAT. Thus, AP-1 activation represents a convergence point of several TCR-initiated signaling pathways.
- **NF-κB** is a transcription factor that is activated in response to TCR signals and is essential for cytokine synthesis. NF-κB proteins are homodimers or heterodimers of proteins that are homologous to the product of a cellular proto-oncogene called c-*rel* and are important in the transcription of many genes in diverse cell types, particularly in innate immune cells (see Chapter 4). In resting T cells, NF-κB is present in the cytoplasm in a complex with other proteins called inhibitors of κB (IκBs), which make a nuclear

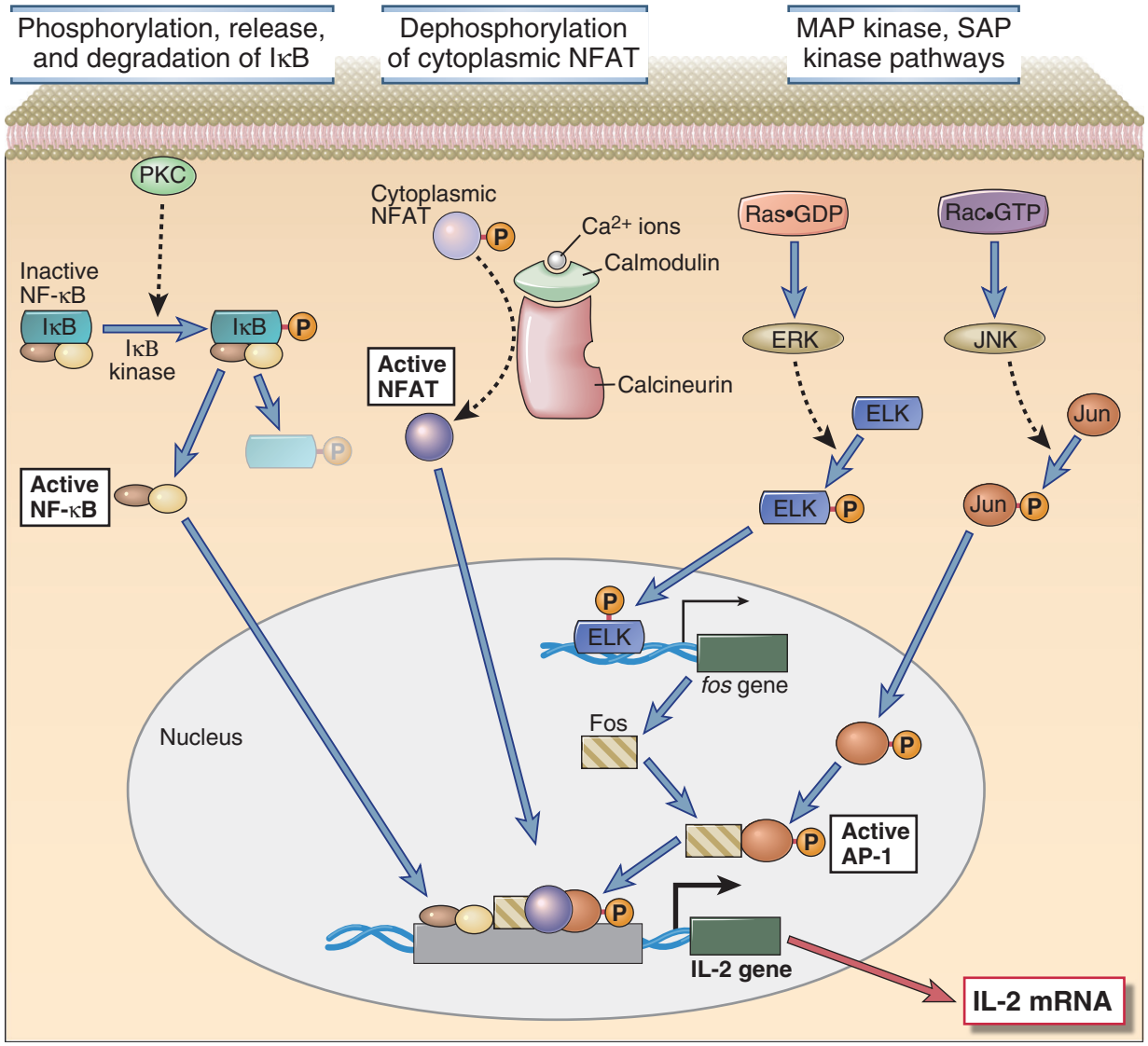


FIGURE 7–16 Activation of transcription factors in T cells. Multiple signaling pathways converge in antigen-stimulated T cells to generate transcription factors that stimulate expression of various genes (in this case, the IL-2 gene). The calcium-calmodulin pathway activates NFAT, and the Ras and Rac pathways generate the two components of AP-1. Less is known about the link between TCR signals and NF-κB activation. (NF-κB is shown as a complex of two subunits, which in T cells are typically the p50 and p65 proteins, named for their molecular sizes in kilodaltons.) PKC is important in T cell activation, and the PKC-θ isoform is particularly important in activating NF-κB. These transcription factors function coordinately to regulate gene expression. Note also that the various signaling pathways are shown as activating unique transcription factors, but there may be considerable overlap, and each pathway may play a role in the activation of multiple transcription factors.

localization signal on NF-κB inaccessible, thus preventing the entry of this factor into the nucleus. TCR signals lead to serine phosphorylation of IκBα and then its ubiquitination and proteasomal degradation. The enzymes responsible for phosphorylation of IκB are called IκB kinases, and these are discussed toward the end of this chapter. Once released from IκB, NF-κB is able to migrate into the nucleus and bind to and regulate the promoters of target genes.

The links between different signaling proteins, activation of transcription factors, and functional responses of T cells are often difficult to establish because there are complex and incompletely understood interactions between signaling pathways. Also, for the sake of simplicity, we often discuss signaling in the context of linear pathways, but it is likely that this does not reflect the more complex and interconnected reality. Finally, we have focused on selected pathways to illustrate how antigen recognition may lead to biochemical alterations, but it is clear that many other signaling molecules are also involved in antigen-induced lymphocyte activation.

Modulation of T Cell Signaling by Protein Tyrosine Phosphatases

Tyrosine phosphatases remove phosphate moieties from tyrosine residues on proteins and generally inhibit TCR signaling. Two tyrosine phosphatases that serve an

important inhibitory role in lymphocytes and other hematopoietic cells are called SHP-1 and SHP-2 (for SH2 domain–containing phosphatases 1 and 2). Inhibitory phosphatases are typically recruited by inhibitory receptors that are induced after a lymphocyte has been activated by tyrosine kinases. These phosphatases inhibit signal transduction by removing phosphates from tyrosine residues in key signaling molecules and thus functionally antagonize tyrosine kinases. Another inhibitory phosphatase that does not act on phosphoproteins but rather is specific for an inositol phospholipid is called SHIP (SH2 domain–containing inositol phosphatase). Like SHP-1 and SHP-2, SHIP binds to phosphorylated ITIM sequences on specific inhibitory receptors. SHIP removes a phosphate group from PIP3, a phospholipid in the inner leaflet of the plasma membrane, and thus antagonizes PI3-kinase signaling in lymphocytes.

Although most phosphatases attenuate lymphocyte signaling, one tyrosine phosphatase, CD45, facilitates lymphocyte activation. The CD45 protein is a receptor tyrosine phosphatase expressed in all hematopoietic cells. It is an integral membrane protein whose cytoplasmic tail contains tandem protein tyrosine phosphatase domains. CD45 dephosphorylates inhibitory tyrosine residues in the Src family kinases Lck and Fyn and thus contributes to the generation of active kinases.

Costimulatory Receptors of T Cells

Costimulatory signals are delivered by receptors that recognize ligands that are induced on APCs by microbes and cooperate with TCR signals to augment signaling and activate T cells. The two-signal hypothesis for T cell activation was introduced in Chapter 1. TCR signaling aided by coreceptors drives the T cell's response to foreign structures. In immunologic jargon, this response by the TCR to MHC and peptide on an APC is referred to as signal 1. T cells are fully activated only when a foreign peptide is recognized in the context of the activation of the innate immune system by a pathogen or some other cause of inflammation. Costimulatory ligands represent the danger signals (or signal 2) induced on antigen-presenting cells by microbes. "Foreignness" must combine with "danger" for optimal T cell activation to occur.

The CD28 Family of Costimulatory Receptors

The best defined costimulators for T lymphocytes are a pair of related proteins, called B7-1 (CD80) and B7-2 (CD86), which are expressed on activated dendritic cells, macrophages, and B lymphocytes. The CD28 molecule on T cells is the principal costimulatory receptor for delivery of second signals for T cell activation. The biologic roles of the B7 and CD28 proteins are considered in more detail in Chapter 9.

Another important activating member of the CD28 family is a receptor called ICOS (inducible costimulator), which plays an important role in T follicular helper cell development and will be discussed in Chapters 9 and 11.

The CD2/SLAM Family of Costimulatory Receptors

Although the best studied and most prominent family of costimulatory receptors on T cells is the CD28 family, other proteins also contribute to optimal T cell activation and differentiation. One important family of proteins that plays a role in the activation of T cells and NK cells is a group of proteins structurally related to a receptor called **CD2** (Fig. 7-17). CD2 is a glycoprotein present on more than 90% of mature T cells, on 50% to 70% of thymocytes, and on NK cells. The molecule contains two extracellular Ig domains, a hydrophobic transmembrane region, and a long (116 amino acid residues) cytoplasmic tail. The principal ligand for CD2 in humans is a molecule called leukocyte function-associated antigen 3 (LFA-3, or CD58), also a member of the CD2 family. LFA-3 is expressed on a wide variety of hematopoietic and nonhematopoietic cells, either as an integral membrane protein or as a phosphatidylinositol-anchored membrane molecule. In mice, the principal ligand for CD2 is CD48, which is also a member of the CD2 family and is distinct from but structurally similar to LFA-3.

CD2 functions both as an intercellular adhesion molecule and as a signal transducer. Some anti-CD2 antibodies increase cytokine secretion by and proliferation of human T cells cultured with anti-TCR/CD3 antibodies, indicating that CD2 signals can enhance TCR-triggered T cell responses. Some anti-CD2 antibodies block conjugate

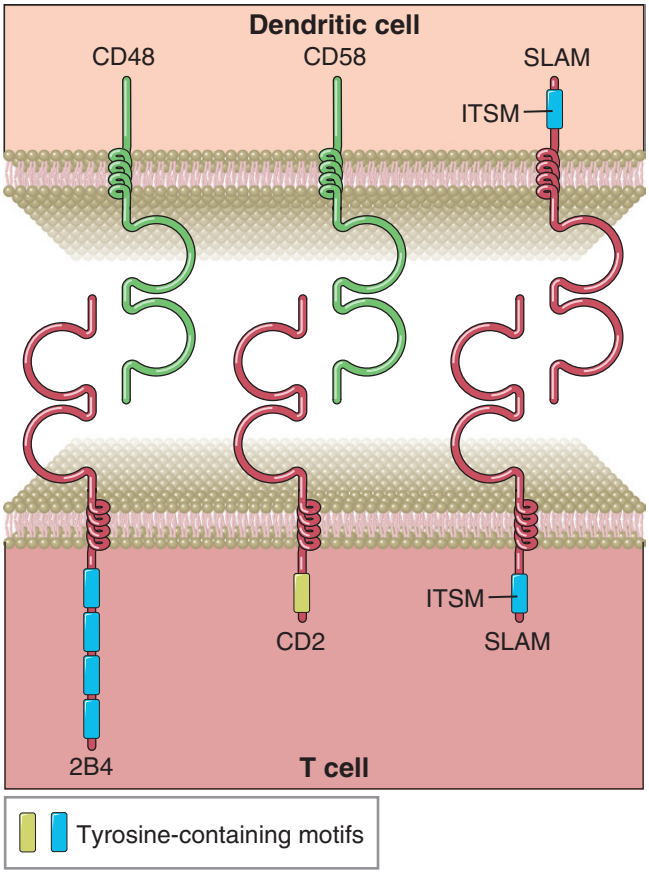


FIGURE 7–17 Selected costimulatory receptors of the CD2 family and their ligands. 2B4, CD2, and SLAM contain two extracellular Ig-like domains, and their cytoplasmic tails also contain tyrosine-containing motifs. The tyrosine-based motif in the tail regions of SLAM and SLAM family members such as 2B4 is called an ITSM and binds to SAP or SAP-like proteins (not shown).

formation between T cells and other LFA-3–expressing cells, indicating that CD2 binding to LFA-3 also promotes cell-cell adhesion. Such antibodies inhibit both CTL activity and antigen-dependent helper T cell responses. Knockout mice lacking both CD28 and CD2 have more profound defects in T cell responses than do mice lacking either molecule alone. This indicates that CD28 and CD2 may compensate for each other, an example of the redundancy of costimulatory receptors of T cells. On the basis of such findings, anti-CD2 antibodies are currently being tested for their efficacy in psoriasis.

A distinct subgroup of the CD2 family of proteins is known as the **SLAM** (signaling lymphocytic activation molecule) family. SLAM, like all members of the CD2 family, is an integral membrane protein that contains two extracellular Ig domains and a relatively long cytoplasmic tail. The cytoplasmic tail of SLAM, but not of CD2, contains a specific tyrosine-based motif, TxYxxV/I (where T is a threonine residue, Y is a tyrosine residue, V is a valine, I is an isoleucine, and x is any amino acid), known as an immunoreceptor tyrosine-based switch motif (ITSM) that is distinct from the ITAM and ITIM motifs found in other activating and inhibitory receptors. It is called a switch motif because in some receptors, this motif can orchestrate a "switch" from the binding of a tyrosine phosphatase, SHP-2, in the absence of an adaptor to the binding of other enzymes in the presence of an adaptor called SAP (SLAM-associated protein), thus potentially mediating a change from an inhibitory to an activating function.

The extracellular Ig domains of SLAM are involved in homophilic interactions. SLAM on a T cell can interact with SLAM on a dendritic cell and, as a result, the cytoplasmic tail of SLAM may deliver signals to T cells. The ITSM motif binds to SAP, and the latter forms a bridge between SLAM and Fyn (a Src family kinase that is also physically linked to CD3 proteins in T cells). SLAM and other members of the SLAM family function as costimulatory receptors in T cells, NK cells, and some B cells. As we shall discuss in Chapter 20, mutations in the *SH2D1A* gene encoding SAP are the cause of a disease called the X-linked lymphoproliferative syndrome (XLP).

An important member of the SLAM family in NK cells, $CD8^+$ T cells, and γδ T cells is called **2B4** (see Fig. 7-17). 2B4 recognizes a known ligand for CD2 called CD48. Like SLAM, the cytoplasmic tail of 2B4 contains ITSM motifs, binds to the SAP adaptor protein, and signals by recruiting Fyn. Defective 2B4 signaling may contribute in a major way to the immune deficit in patients with the X-linked lymphoproliferative syndrome.

THE B LYMPHOCYTE ANTIGEN RECEPTOR COMPLEX

The B lymphocyte antigen receptor is a transmembrane form of an antibody molecule associated with two signaling chains. The structure of antibodies was described in detail in Chapter 5. Here we will focus on some salient features of the membrane forms of Ig and their associated proteins and discuss how they deliver signals to B cells. Because the signaling pathways are similar to those in T cells, we will summarize these without much detail. However, there are both similarities and significant differences between B and T cell antigen receptors (see Table 7-1).

Structure of the B Cell Receptor for Antigen

Membrane IgM and IgD, the antigen receptors of naive B cells, have short cytoplasmic tails consisting of only three amino acids (lysine, valine, and lysine). These tails are too small to transduce signals generated after the recognition of antigen. Ig-mediated signals are transduced by two other molecules, called Igα and Igβ, that are disulfide linked to one another and are expressed in B cells noncovalently associated with membrane Ig (Fig. 7-18). These proteins each contain an ITAM motif in their cytoplasmic tails, are required for the transport of membrane Ig molecules to the cell surface, and together with membrane Ig form the **B cell receptor (BCR) complex**. B cell receptor complexes in class-switched B cells, including memory B cells, contain membrane immunoglobulins that may be of the IgG, IgA, or IgE classes (see Chapter 11).

Signal Initiation by the B Cell Receptor

Signal initiation by antigens occurs by cross-linking of the BCR and is facilitated by the coreceptor for the BCR. It is thought that cross-linking of membrane Ig by multivalent antigens brings Src family kinases together and, by

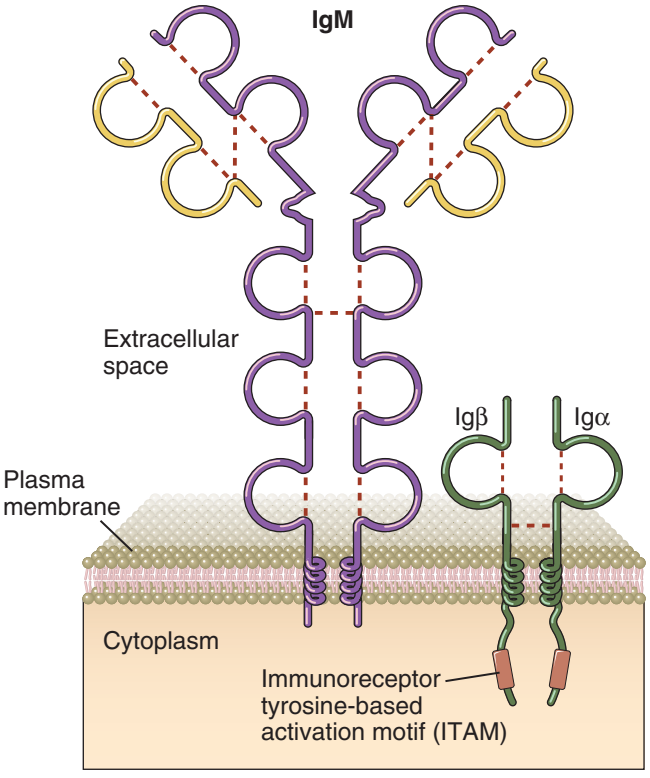


FIGURE 7–18 B cell antigen receptor complex. Membrane IgM (and IgD) on the surface of mature B cells is associated with the invariant Igβ and Igα molecules, which contain ITAMs in their cytoplasmic tails that mediate signaling functions. Note the similarity to the TCR complex.

promoting their physical interaction, fully activates these enzymes, enabling them then to phosphorylate the tyrosine residues on the ITAMs of Igα and Igβ. It is also possible that as in T cells, antigen binding facilitates a conformational change in BCR-associated ITAMs, making them accessible to already active Src family kinases that modify ITAM tyrosines, but there is at present no firm evidence to support such a model. The phosphorylation of ITAM tyrosine residues triggers all subsequent signaling events downstream of the BCR (Fig. 7-19). Cross-linked Ig receptors enter lipid rafts, where many adaptor proteins and signaling molecules are concentrated. Igα and Igβ are loosely connected to Src family tyrosine kinases such as Lyn, Fyn, and Blk, and these enzymes are also linked by lipid anchors to the inside of the plasma membrane. The phosphorylation of the tyrosine residues in the ITAMs of Igα and Igβ provides a docking site for the tandem SH2 domains of the Syk tyrosine kinase. Syk is activated when it associates with phosphorylated tyrosines of ITAMs and may itself be phosphorylated on specific tyrosine residues by BCR-associated Src family kinases, leading to further activation. If the antigen is monovalent and incapable of cross-linking multiple Ig molecules, some signaling may nevertheless occur, but additional activation by helper T cells may be necessary to fully activate B cells, as discussed in Chapter 11.

Role of the CR2/CD21 Complement Receptor as a Coreceptor for B Cells

The activation of B cells is enhanced by signals that are provided by complement proteins and the CD21 coreceptor complex, which link innate immunity to the adaptive humoral immune response (Fig. 7-20). The complement system consists of a collection of plasma proteins that are

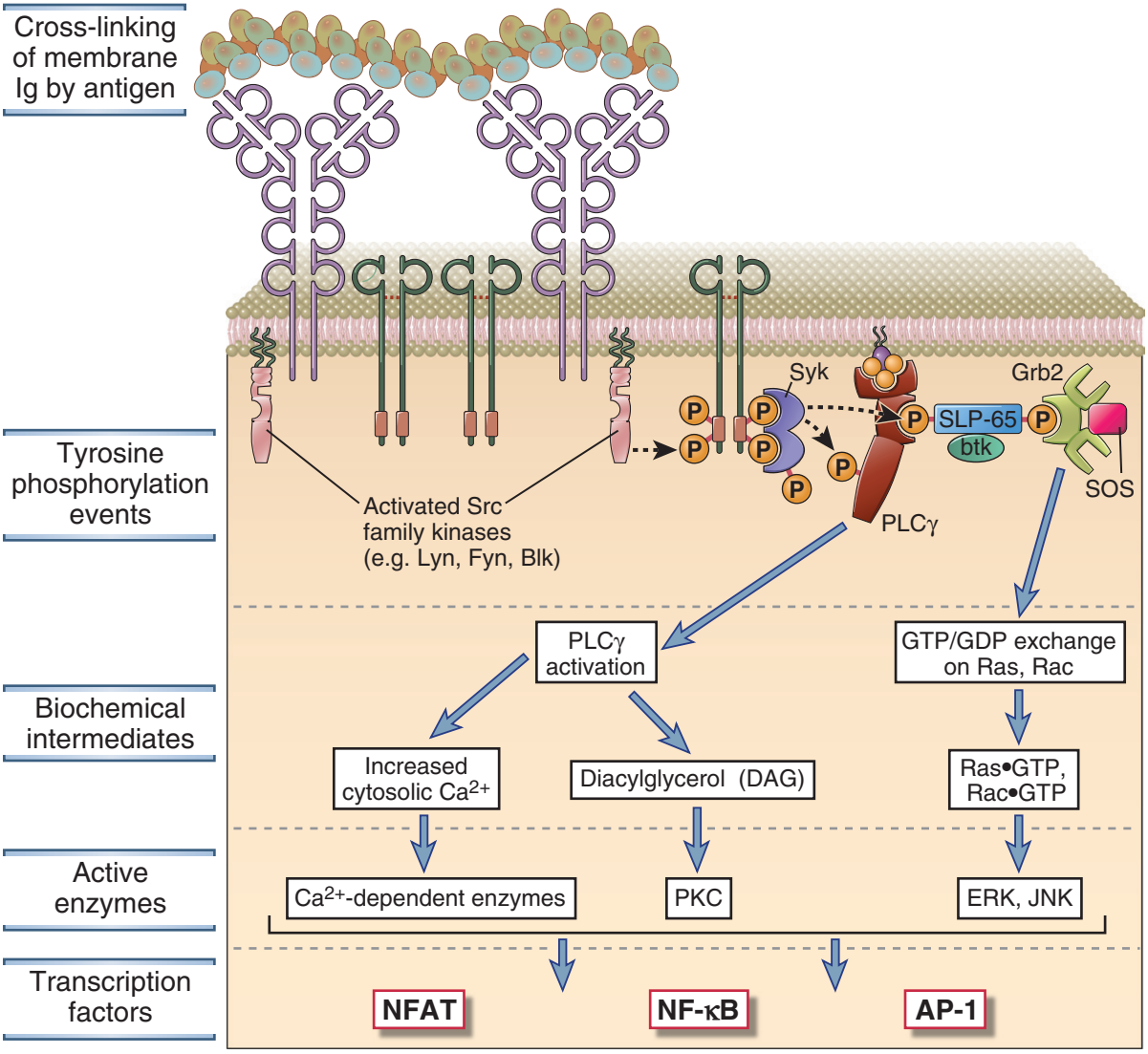


FIGURE 7–19 Signal transduction by the BCR complex. Antigen-induced cross-linking of membrane Ig on B cells leads to clustering and activation of Src family tyrosine kinases and tyrosine phosphorylation of the ITAMs in the cytoplasmic tails of the Igα and Igβ molecules. This leads to docking of Syk and subsequent tyrosine phosphorylation events as depicted. Several signaling cascades follow these events, as shown, leading to the activation of several transcription factors. These signal transduction pathways are similar to those described in T cells.

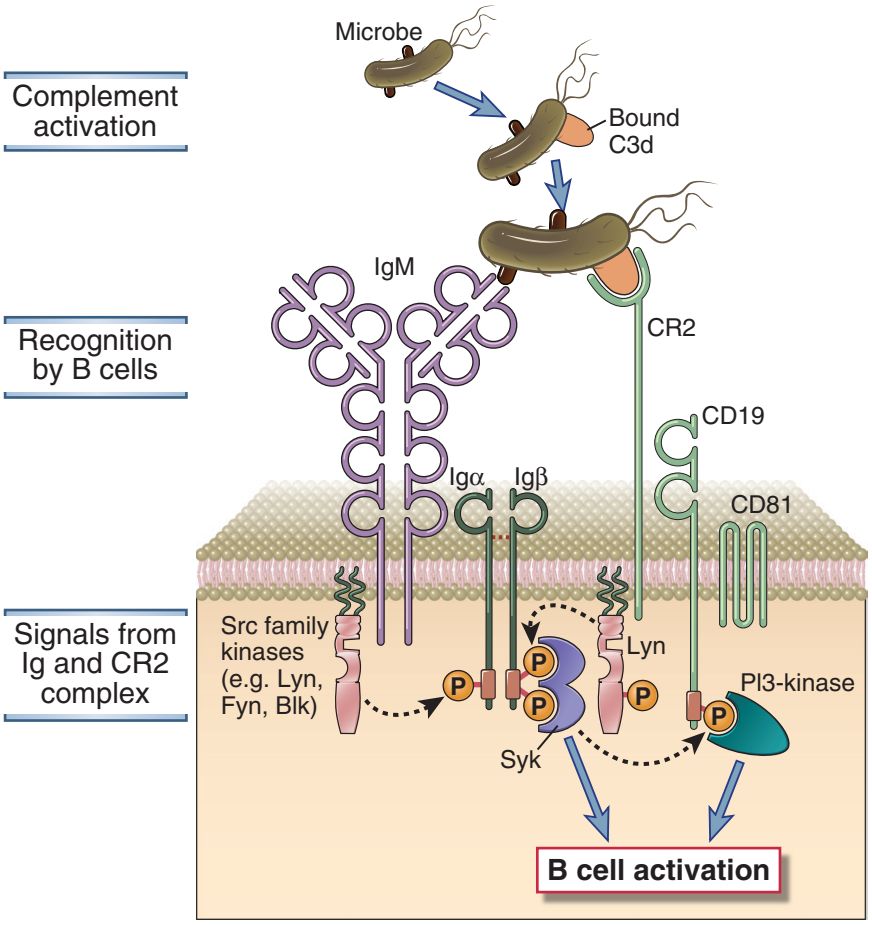


FIGURE 7–20 Role of complement in B cell activation. B cells express a complex of the CR2 complement receptor, CD19, and CD81. Microbial antigens that have bound the complement fragment C3d can simultaneously engage both the CR2 molecule and the membrane Ig on the surface of a B cell. This leads to the initiation of signaling cascades from both the BCR complex and the CR2 complex, because of which the response to C3d-antigen complexes is greatly enhanced compared with the response to antigen alone.

activated either by binding to antigen-complexed antibody molecules (the classical pathway) or by binding directly to some microbial surfaces and polysaccharides in the absence of antibodies (the alternative and lectin pathways) (see Chapters 4 and 12). Thus, polysaccharides and other microbial components may activate the complement system directly, during innate immune responses. Proteins and other antigens that do not activate complement directly may be bound by preexisting antibodies or by antibodies produced early in the response, and these antigen-antibody complexes activate complement by the classical pathway. Recall that complement activation results in the proteolytic cleavage of complement proteins. The key component of the system is a protein called C3, and its cleavage results in the production of a molecule called C3b that binds covalently to the microbe or antigen-antibody complex. C3b is further degraded into a fragment called C3d, which remains bound to the microbial surface or on the antigen-antibody complex. B lymphocytes express a receptor for C3d that is called the type 2 complement receptor (CR2, or CD21). The complex of C3d and antigen or C3d and antigen-antibody complex binds to B cells, with the membrane Ig recognizing antigen and CR2 recognizing the bound C3d (see Fig. 7-20).

CR2 is expressed on mature B cells as a complex with two other membrane proteins, CD19 and CD81 (also called TAPA-1). The CR2-CD19-CD81 complex is often called the B cell coreceptor complex because CR2 binds to antigens through attached C3d at the same time that membrane Ig binds directly to the antigen. Binding of C3d to the B cell complement receptor brings CD19 in proximity to BCR-associated kinases, and the cytoplasmic tail of CD19 rapidly becomes tyrosine phosphorylated. Phosphorylation of the tail of CD19 results in the efficient recruitment of Lyn, a Src family kinase, that can amplify BCR signaling by greatly enhancing the phosphorylation of ITAM tyrosines in Igα and Igβ. Phosphorylated CD19 also activates other signaling pathways, notably one dependent on the enzyme PI3-kinase, which in turn further augment signaling initiated by antigen binding to membrane Ig. PI3-kinase is required for the activation of Btk and PLCγ2 because these enzymes must bind to PIP3 on the inner leaflet of the plasma membrane to be fully activated, in a manner analogous to that shown in Figure 7-12. The net result of coreceptor activation is that the response of the antigen-stimulated B cell is greatly enhanced.

Signaling Pathways Downstream of the B Cell Receptor

After antigen binding to the BCR, Syk and other tyrosine kinases activate numerous downstream signaling

pathways that are regulated by adaptor proteins (see Fig. 7-19). The cross-linking of the BCR or the activation of the BCR by a coreceptor-dependent mechanism results in ITAM phosphorylation and recruitment of Syk to the ITAM, followed by the activation of this dual SH2 domain–containing kinase. Activated Syk phosphorylates critical tyrosine residues on adaptor proteins such as SLP-65 (SH2-binding leukocyte phosphoprotein of 65 kD, also called BLNK, B cell linker protein). This facilitates the recruitment to these adaptor proteins of other SH2 domain– and phosphotyrosine-binding (PTB) domain–containing enzymes, including guanine nucleotide exchange proteins that can separately activate Ras and Rac, PLCγ2, and the Btk tyrosine kinase, among others. Recruitment facilitates the activation of these downstream effectors, each generally contributing to the activation of a distinct signaling pathway.

- ***The Ras–MAP kinase pathway is activated in antigen-stimulated B cells.*** The GTP/GDP exchange factor SOS is recruited to BLNK through the binding of the Grb-2 adaptor protein; Ras is then converted by this exchange factor from an inactive GDP-bound form to an active GTP-bound form. Activated Ras contributes to the activation of the ERK MAP kinase pathway discussed earlier in the context of T cell signaling. In a parallel fashion, the activation of the Rac small GTP protein may contribute to the activation of the JNK MAP kinase pathway.
- ***A specific phosphatidylinositol-specific phospholipase C (PLC) is activated in response to BCR signaling, and this in turn facilitates the activation of downstream signaling pathways.*** In B cells, the dominant isoform of PLC is the γ2 isoform, whereas T cells express the related γ1 isoform of the enzyme. PLCγ2 becomes active when it binds to BLNK and is phosphorylated by Syk and Btk. As described in the context of TCR signaling, active PLC breaks down membrane PIP2 to yield soluble IP3 and leaves DAG in the plasma membrane. IP3 mobilizes calcium from intracellular stores, leading to a rapid elevation of cytoplasmic calcium, which is subsequently augmented by an influx of calcium from the extracellular milieu. In the presence of calcium, DAG activates some isoforms of protein kinase C (mainly PKC-β in B cells), which phosphorylate downstream proteins on serine/threonine residues.
- ***PKC-β activation downstream of the BCR contributes to the activation of NF-κB in antigen-stimulated B cells.*** This process is similar to that in T cells triggered by PKC-θ, the PKC isoform present in T cells, and the pathway of NF-κB activation downstream of PKCs is described later in this chapter.

These signaling cascades ultimately lead to the activation of a number of transcription factors that induce the expression of genes whose products are required for functional responses of B cells. Some of the transcription factors that are activated by antigen receptor–mediated signal transduction in B cells are Fos (downstream of Ras and ERK activation), JunB (downstream of Rac and JNK activation), and NF-κB (downstream of Btk, PLCγ2, and PKC-β activation). These were discussed earlier when we described T cell signaling pathways. These and other transcription factors, many not mentioned here, are involved in stimulating proliferation and differentiation of B cells (see Chapter 11).

As in T cells, our knowledge of antigen-induced signaling pathways in B cells and their links with subsequent functional responses is incomplete. We have described some of these pathways to illustrate the main features, but others may play important roles in B cell activation. The same signaling pathways are used by membrane IgM and IgD on naive B cells and by IgG, IgA, and IgE on B cells that have undergone isotype switching because all these membrane isotypes associate with Igα and Igβ.

THE ATTENUATION OF IMMUNE RECEPTOR SIGNALING

Activation of lymphocytes has to be tightly controlled to limit immune responses against microbes for avoidance of "collateral damage" to host tissues. In addition, the immune system needs mechanisms that will prevent reactions against self antigens. We will describe the biology of these control mechanisms in later chapters, notably Chapter 14. Attenuation of signaling is essential to prevent uncontrolled inflammation and lymphoproliferation. Here we discuss the biochemical mechanisms that serve to limit and terminate lymphocyte activation.

Inhibitory signaling in lymphocytes is mediated primarily by inhibitory receptors and also by enzymes known as E3 ubiquitin ligases that mark certain signaling molecules for degradation. Inhibitory receptors typically recruit and activate phosphatases that counter signaling events induced by antigen receptors (Fig. 7-21). The functional responses of all cells are regulated by a balance between stimulatory and inhibitory signals, and we will first describe, from a broad mechanistic standpoint, how inhibitory receptors may function in NK cells, T cells, and B cells. We will then describe how ubiquitin E3 ligases may attenuate signaling in lymphocytes. The biologic relevance of signal attenuation through inhibitory receptors in NK cells, T cells, and B cells is addressed in Chapters 4, 9, and 11, respectively.

Inhibitory Receptors in NK Cells, B Cells, and T Cells

Most but not all inhibitory receptors in the immune system contain cytosolically oriented ITIM motifs that can recruit SH2 domain–containing phosphatases and thus attenuate signaling in a broadly similar manner (see Fig 7-21). Inhibitory receptors play key roles in NK cells, T cells, and B cells as well as in other cells of innate immunity.

In human NK cells, the key inhibitory receptors can broadly be divided into three groups: KIRs or killer Ig-like receptors (see Chapter 4); ILT (Ig-like transcript) family proteins that are closely related to KIRs; and C-type lectins, the major one being a heterodimer consisting of the NKG2A C-type lectin and CD94. These inhibitory receptors are not restricted to NK cells and may also be

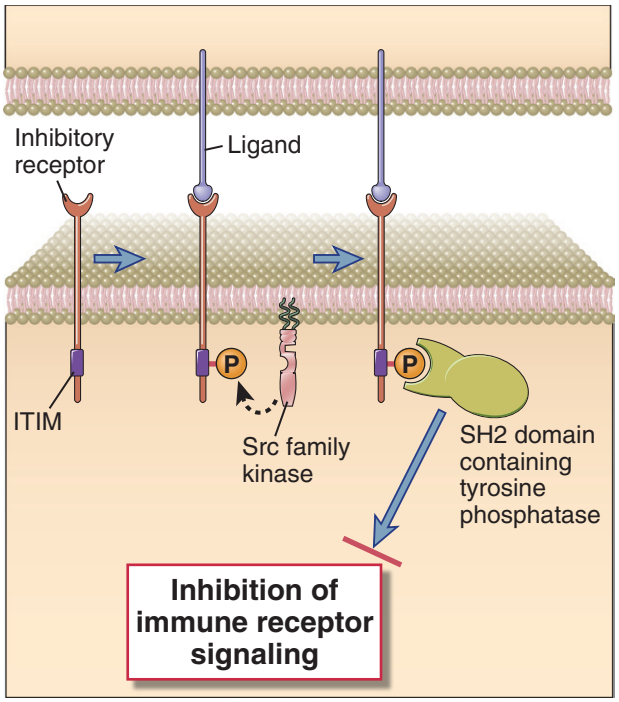


FIGURE 7–21 Inhibitory signaling in lymphocytes. A schematic depiction is provided of an inhibitory receptor with an extracellular ligand-binding domain and a cytosolic ITIM motif. Ligand binding results in phosphorylation of the ITIM tyrosine by a Src family kinase, followed by recruitment of an SH2 domain–containing tyrosine phosphatase that can attenuate immune receptor signaling.

present on some activated T cells. KIRs contain extracellular Ig domains that can recognize class I HLA molecules, and a subset of these receptors contains cytosolic ITIM motifs. ILT-2, part of an evolutionarily older family of inhibitory receptors than KIRs, also has extracellular Ig domains that bind HLA class I and cytosolic ITIM motifs. The CD94/NKG2A dimer binds to an atypical class I MHC molecule called HLA-E, and the NKG2A chain of this dimer contains cytosolic ITIM motifs.

Tyrosine residues on the ITIMs of these and other inhibitory receptors can be phosphorylated by Src family kinases linked to lymphocyte activation and, as described earlier, recruit SH2 domain–containing tyrosine phosphatases such as SHP-1 and SHP-2 and an SH2 domain–containing inositol phosphatase called SHIP. SHP-1 and SHP-2 attenuate tyrosine kinase–initiated signaling from activating receptors in NK cells as well as from the BCR and TCR in B and T cells, respectively. SHIP removes phosphate moieties from PIP3 and thus inhibits PI3-kinase activity in lymphocytes, NK cells, and innate immune cells.

The prototypical inhibitory receptor of the CD28 family, **CTLA-4** (also called CD152), has the ability to inhibit T cell responses induced on activated T cells and has a higher affinity than CD28 for B7 proteins. CTLA-4 is involved in the maintenance of unresponsiveness (tolerance) to self antigens and is discussed in this context in Chapter 14. Another inhibitory receptor of the same family is called **PD-1** (programmed death 1), and this is also discussed in Chapter 14. CTLA-4 contains a tyrosine-containing motif in its tail that may be inhibitory; PD-1 contains cytosolic ITIM and ITSM motifs, and its cytosolic tail is critical for the initiation of inhibitory signals. The key inhibitory receptors in B cells include FcγRIIB and CD22/Siglec-2. FcγRIIB, an important attenuator of signaling in activated B cells as well as in dendritic cells and macrophages, can bind IgG-containing immune complexes through extracellular Ig domains. It primarily recruits SHIP and antagonizes PI3-kinase signaling. This receptor dampens B cell activation in the latter part of a humoral immune response and will be discussed in more detail in Chapter 11.

E3 Ubiquitin Ligases and the Degradation of Signaling Proteins

One of the major ways of degrading cytosolic and nuclear proteins involves the covalent attachment of ubiquitin residues to these proteins. Although ubiquitination of proteins is frequently linked to the degradation of these proteins in proteasomes, proteins can be ubiquitinated in a number of ways, each form of ubiquitination serving a very different function. In the context of signal transduction, different types of ubiquitination mediate signal attenuation on the one hand and signal generation on the other.

Ubiquitination was briefly discussed in Chapter 6 in the context of class I MHC–based antigen processing and presentation. Ubiquitin is a 76–amino acid protein that is activated in an ATP-dependent fashion by an E1 enzyme, then "carried" by an E2 enzyme, and transferred to lysine residues on specific substrates that are recognized by specific E3 ubiquitin ligases. In many cases, after the C terminus of a ubiquitin moiety is covalently linked to a lysine residue on a target protein, the C-terminal ends of subsequent ubiquitin moieties may be covalently attached to lysine residues on the preceding ubiquitin to generate a polyubiquitin chain. The geometric shape of the polyubiquitin chain is very different, depending on which specific lysine residue on the preceding ubiquitin molecule in the chain is the site for covalent binding of the next ubiquitin molecule, and the shape of the ubiquitin chain has important functional consequences. If lysine in position 48 of the first ubiquitin moiety forms an isopeptide bond with the C terminus of the next ubiquitin and so on, a lysine-48 type of ubiquitin chain will be generated that can be recognized by the proteasomal cap, and the protein will be targeted for degradation in the proteasome. Some E3 ligases generate a different type of polyubiquitin chain called a lysine-63 type of chain, which does not target proteins for degradation but instead generates a structure for latching the marked proteins onto other specific proteins; this is important in NF-κB signaling, as discussed later. For some functions, in particular targeting membrane proteins to lysosomes rather than to proteasomes, only a single ubiquitin moiety may need to be attached to a protein target.

Several E3 ligases are found in T cells; some of them are involved in signal activation and others in signal attenuation. The prototype of E3 ligases involved in terminating T cell responses is Cbl-b, but several others

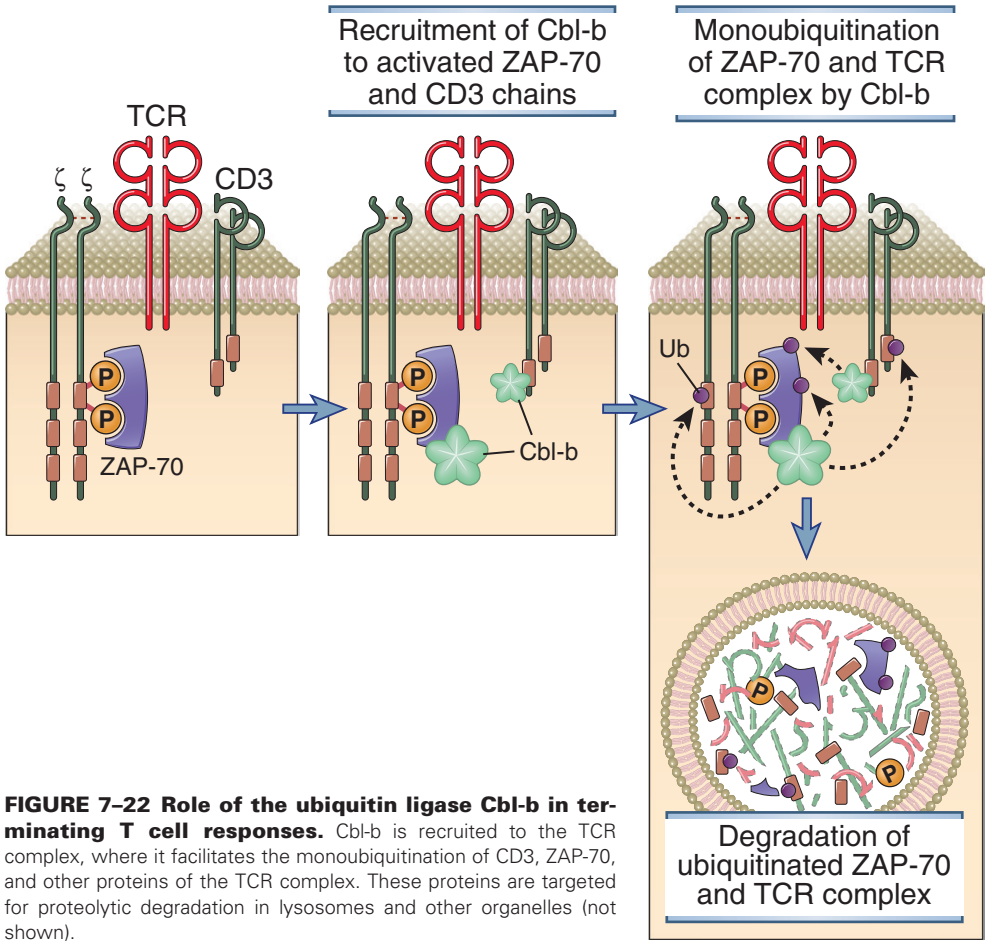


FIGURE 7–22 Role of the ubiquitin ligase Cbl-b in terminating T cell responses. Cbl-b is recruited to the TCR complex, where it facilitates the monoubiquitination of CD3, ZAP-70, and other proteins of the TCR complex. These proteins are targeted for proteolytic degradation in lysosomes and other organelles (not shown).

serve similar functions. Recruitment of Cbl-b to the TCR complex and associated adaptor proteins leads to the monoubiquitination, endocytosis, and lysosomal degradation of the TCR complex, and this may be a mechanism for the attenuation of TCR signaling (Fig. 7-22). CD28 signals block the inhibitory activity of Cbl-b, and this is one mechanism by which costimulation augments TCR signals. In knockout mice lacking Cbl-b, the T cells respond to antigen even without CD28-mediated costimulation and produce abnormally high amounts of IL-2. These mice develop autoimmunity as a result of the enhanced activation of their T cells.

CYTOKINE RECEPTORS AND SIGNALING

Cytokines, the secreted "messenger molecules" of the immune system, were introduced in Chapter 1 and discussed in Chapter 4 in the context of innate immunity; their roles in adaptive T cell–mediated immune responses will be described in Chapters 9 and 10. Here we will describe receptors for cytokines and their mechanisms of signaling.

All cytokine receptors consist of one or more transmembrane proteins whose extracellular portions are responsible for cytokine binding and whose cytoplasmic portions are responsible for initiation of intracellular signaling pathways. For most cytokine receptors, these signaling pathways are typically activated by ligand-induced receptor clustering, bringing together the cytoplasmic portions of two or more receptor molecules, thus inducing the activity of unique non-receptor tyrosine kinases. In the case of the TNF receptor family of cytokine receptors, preformed receptor trimers apparently undergo a conformational change after contacting their cognate trimeric ligands.

Classes of Cytokine Receptors

The most widely used classification of cytokine receptors is based on structural homologies of the extracellular cytokine-binding domains and shared intracellular signaling mechanisms (Fig. 7-23). Signaling through type I and type II cytokine receptors occurs by a similar mechanism, known as JAK-STAT signaling, that is described in more detail later. Cytokine receptors of the TNF receptor family activate a number of pathways, a prominent one being the NF-κB pathway, which will also be considered in detail later. Signaling through the IL-1R and the TLR families uses a common cytoplasmic domain, and a major component downstream is ubiquitin E3 ligase–dependent activation of the NF-κB pathway. Chemokines, which

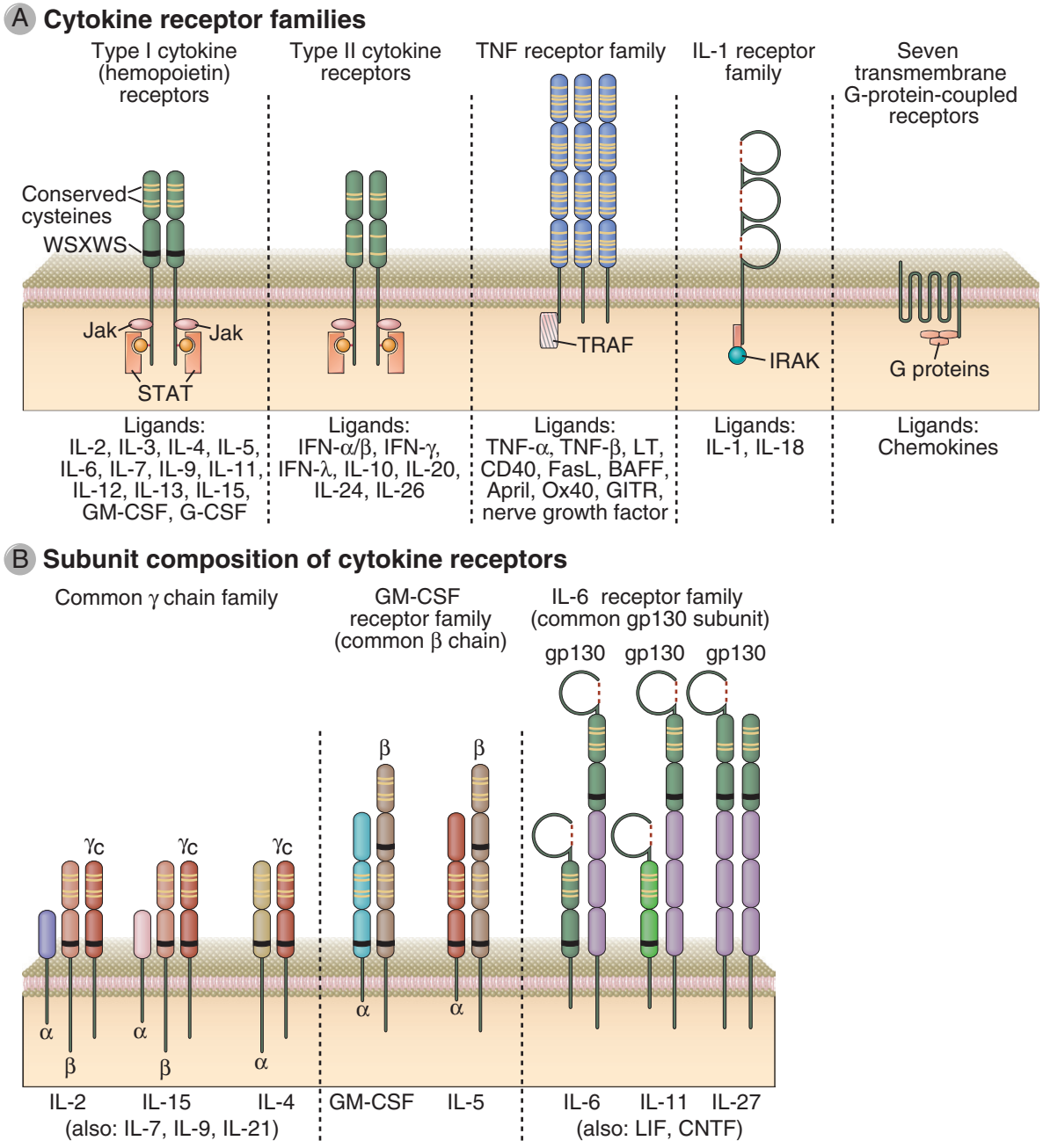


FIGURE 7–23 Structure of cytokine receptors. A, Receptors for different cytokines are classified into families on the basis of conserved extracellular domain structures and signaling mechanisms. The cytokines or other ligands that bind to each receptor family are listed below the schematic drawings. WSXWS, tryptophan-serine-X-tryptophan-serine. **B,** Groups of cytokine receptors share identical or highly homologous subunit chains. Selected examples of cytokine receptors in each group are shown.

are chemotactic cytokines, activate a large subfamily of receptors and have been discussed in Chapter 3. Chemokine receptors are seven-transmembrane GPCRs described in the early part of this chapter and are not elaborated on here.

Type I Cytokine Receptors (Hematopoietin Receptor Family)

Type I cytokine receptors are dimers or trimers that typically consist of unique ligand-binding chains and one or more signal-transducing chains, which are often shared by receptors for different cytokines. These chains contain one or two domains with a conserved pair of cysteine residues and a membrane proximal peptide stretch containing a tryptophan-serine-X-tryptophan-serine (WSXWS) motif, where X is any amino acid (Fig. 7-23A). The conserved sequences of the receptors form structures that bind cytokines that have four α-helical bundles and are referred to as type I cytokines, but the specificity for individual cytokines is determined by amino acid residues that vary from one receptor to another. This

receptor family can be divided into subgroups based on structural homologies or the use of shared signaling polypeptides (Fig. 7-23B). One group contains a signaling component called the common γ chain (CD132); in this group are the receptors for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21. A distinct subgroup of type I receptors includes receptors that share a common β chain (CD131) subunit. This subgroup includes the receptors for IL-3, IL-5, and GM-CSF. Another subgroup of receptors uses the gp130 signaling component, and this includes the receptors for IL-6, IL-11, and IL-27. All the type I cytokine receptors engage JAK-STAT signaling pathways.

Type II Cytokine Receptors (Interferon Receptor Family)

The type II receptors are similar to type I receptors by virtue of possessing two extracellular domains with conserved cysteines, but type II receptors do not contain the WSXWS motif. These receptors consist of one ligand-binding polypeptide chain and one signal-transducing chain. All the type II cytokine receptors, like the type I receptors, engage JAK-STAT signaling pathways. This family includes receptors for type I and type II interferons and for IL-10, IL-20, and IL-26.

TNF Receptor Family

These receptors are part of a large family of preformed trimers (some of which are not considered cytokine receptors) with conserved cysteine-rich extracellular domains and shared intracellular signaling mechanisms that typically stimulate gene expression but in some cases induce apoptosis. Some important receptors of this family, most of which will be discussed in other chapters in their biologic contexts, include the TNF receptors TNFRI and TNFRII, the CD40 protein, Fas, the lymphotoxin receptor, and the BAFF receptor family. The ligands for these receptors also form trimers. Some of these ligands are membrane bound, whereas others are soluble.

Binding of the ligands to the preformed trimeric receptors typically induces a conformational change and recruits adaptor proteins to the receptor complex. These adaptors in turn recruit enzymes that include both E3 ubiquitin ligases, which mediate nondegradatory polyubiquitination, and protein kinases, which initiate downstream signaling. In the case of the TNF receptor illustrated in Figure 7-24, the receptor recruits the adaptor protein TRADD (TNF receptor–associated death domain), and TRADD in turn can recruit proteins called TRAFs (TNF receptor associated factors), which possess a unique type of E3 ligase activity that will be discussed in the section on NF-κB signaling. The type I TNF receptor (there are two different receptors for TNF) and Fas (CD95) can also recruit adaptors that induce the activation of caspase-8, and these receptors, in certain cells, can thereby induce apoptosis.

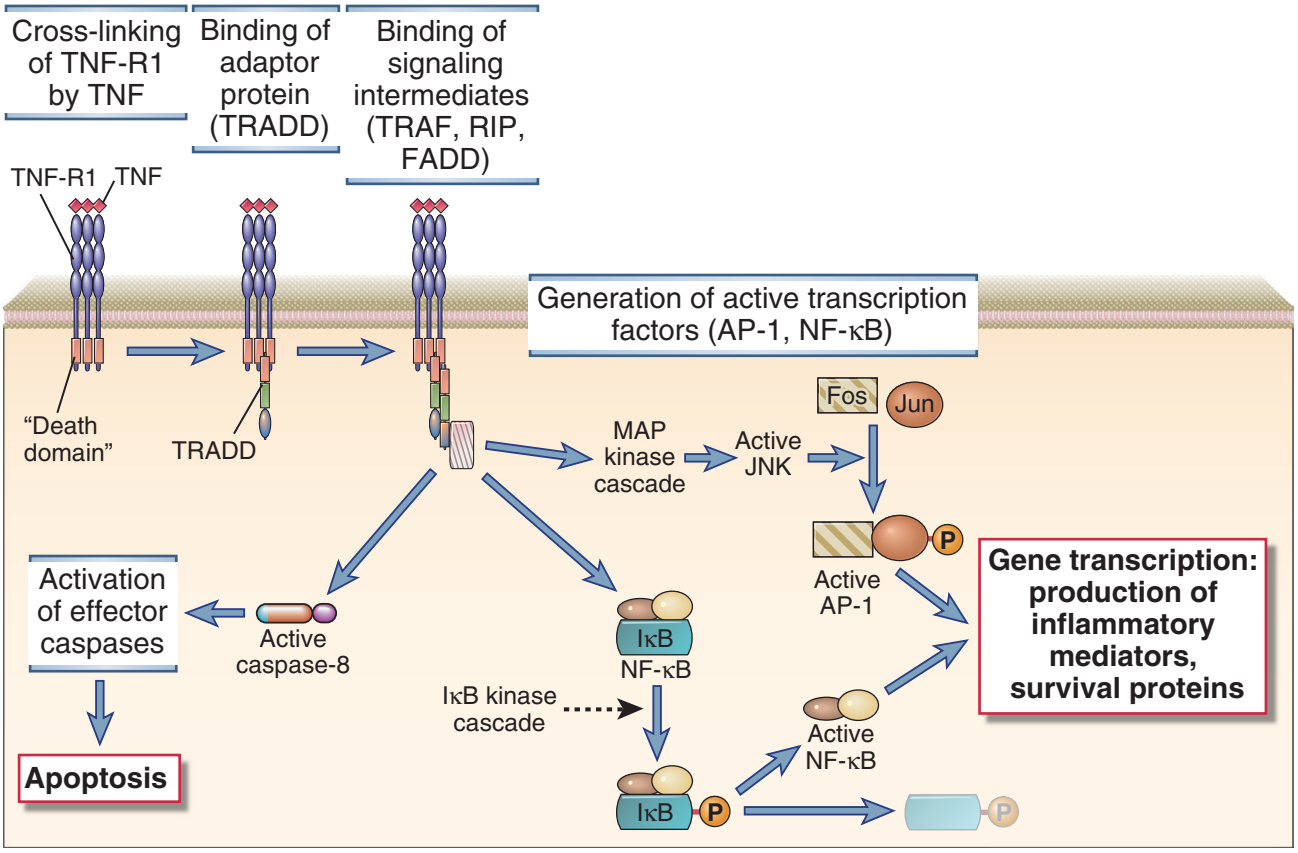


FIGURE 7–24 Signaling through the TNF receptor can result in NF-κB and MAP kinase activation or in the induction of apoptotic death. Ligation of the type I TNF receptor results in the recruitment of an adaptor protein called TRADD, which in turn can activate TRAF molecules (E3 ubiquitin ligase) and the RIP1 kinase. Downstream consequences include the activation of the NF-κB pathway and the JNK MAP kinase pathway or the induction of apoptotic death.

IL-1/TLR Family

The receptors of this family share a conserved cytosolic sequence, called the Toll-like/IL-1 receptor (TIR) domain, and engage similar signal transduction pathways that induce new gene transcription. Toll-like receptor (TLR) signaling has been considered in Chapter 4. Briefly, engagement of the IL-1R or of TLRs results in receptor dimerization and the recruitment of one or more of four known TIR domain–containing adaptors to the TIR domain of the cytoplasmic tail of the receptor. The adaptors link TLRs to different members of the IRAK (IL-1R–associated kinase) family. IRAKs can in turn link adaptors to TRAF6, an E3 ubiquitin ligase required for NF-κB activation. Other pathways activated by TLR signaling include MAP kinase activation and the phosphorylation of IRF3 and IRF7, transcriptional inducers of type I interferons. The latter aspect of TLR signaling has been considered in the context of the antiviral state in Chapter 4. In a general sense, the MAL/MyD88 adaptor pair links TLRs to the early induction of NF-κB signaling and to MAP kinase activation, whereas the TRAM/TRIF adaptor pair leads to the delayed activation of NF-κB and the activation of IRF3. TLR4, for instance, activates MAL/Myd88 signaling initially from the cell surface and TRAM/TRIF signaling subsequently after receptor endocytosis. The mechanisms connecting IL-1R/TLR signaling and NF-κB activation are discussed below.

JAK-STAT Signaling

Cytokine receptors of the type I and type II receptor families engage signal transduction pathways that involve non-receptor tyrosine kinases called Janus kinases or JAKs and transcription factors called signal transducers and activators of transcription (STATs). The discovery of the JAK-STAT pathways came from biochemical and genetic analyses of interferon signaling. There are four known Janus kinases (JAK1-3 and TYK2) and seven STATs (STAT1-4, 5a, 5b, and 6).

The sequence of events in the JAK-STAT signaling pathways is now well defined (Fig. 7-25). Inactive JAK enzymes are noncovalently attached to the cytoplasmic domains of type I and type II cytokine receptors. When two receptor molecules are brought together by binding

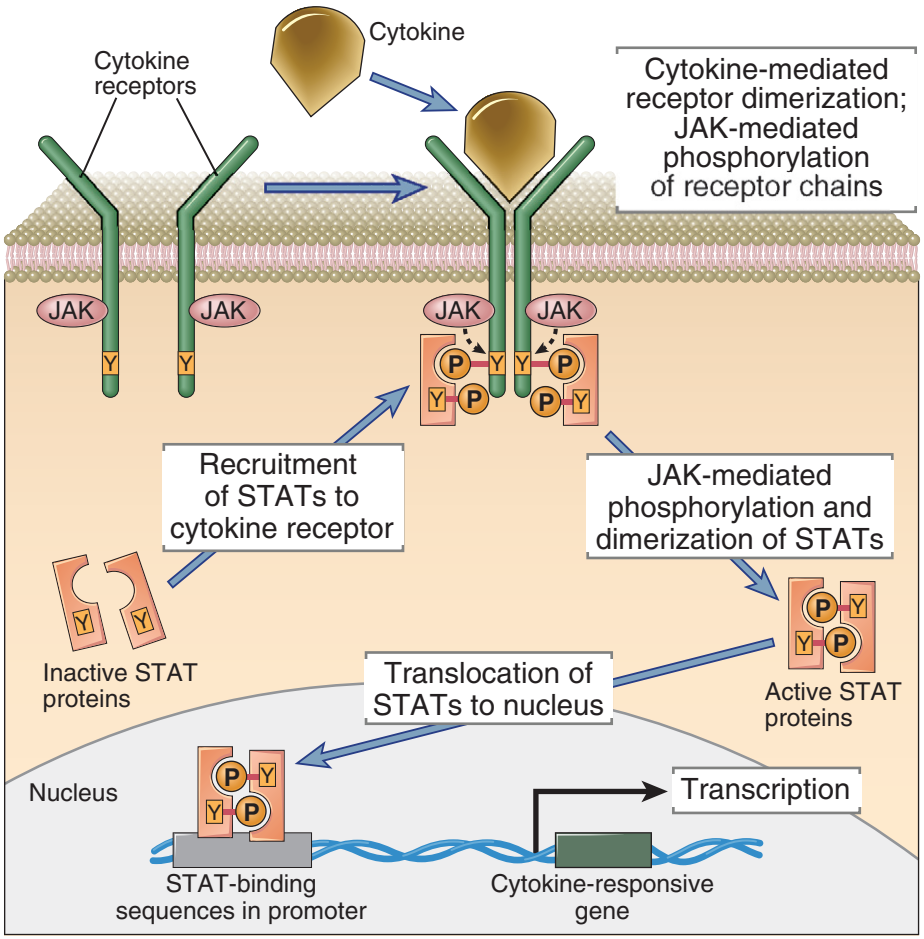


FIGURE 7–25 Type I and type II cytokines induce JAK-STAT signaling. Ligation of receptors for type I and type II cytokines results in the activation of an associated JAK tyrosine kinase, the phosphorylation of the receptor tail, and the recruitment of an SH2 domain–containing activator of transcription (STAT) to the receptor. The recruited STAT is activated by JAK phosphorylation, dimerizes, enters the nucleus, and turns on the expression of cytokine target genes.

of a cytokine molecule, the receptor-associated JAKs are activated and phosphorylate tyrosine residues in the cytoplasmic portions of the clustered receptors. Some of these phosphotyrosine moieties of the receptors are then recognized and bind to Src homology 2 (SH2) domains of monomeric cytosolic STAT proteins. The STAT proteins are thus brought close to JAKs and are phosphorylated by the receptor-associated kinases. The SH2 domain of one STAT monomer is able to bind to a phosphotyrosine residue on an adjacent STAT protein. The STAT dimers that are generated migrate to the nucleus, where they bind to specific DNA sequences in the promoter regions of cytokine-responsive genes and activate gene transcription.

An intriguing question is how the specificity of responses to many different cytokines is achieved, given the limited numbers of JAKs and STATs used by the various cytokine receptors. The likely answer is that unique amino acid sequences in the different cytokine receptors provide the scaffolding for specifically binding, and thereby activating, different combinations of JAKs and STATs. The SH2 domains of different STAT proteins selectively bind to phosphotyrosines and flanking residues of different cytokine receptors. This is largely responsible for the activation of particular STATs by various cytokine receptors and therefore for the specificity of cytokine signaling. Several type I and type II cytokine receptors are heterodimers of two different polypeptide chains, each of which binds a different JAK. Furthermore, two different STATs may heterodimerize on phosphorylation. Therefore, there is a significant amount of combinatorial diversity in the signaling that can be generated from a limited number of JAK and STAT proteins.

In addition, cytokines activate signaling pathways and transcription factors other than STATs. For instance, the IL-2 receptor β chain activates Ras-dependent MAP kinase pathways that may be involved in gene transcription and growth stimulation. Other cytokine receptors may similarly activate other signaling pathways in concert with the JAK-STAT pathways to elicit biologic responses to the cytokines.

Several mechanisms of negative regulation of JAK-STAT pathways have been identified. Proteins called suppressors of cytokine signaling (SOCS) can be identified by the presence of an SH2 domain and a conserved 40–amino acid C-terminal region called a SOCS box. SOCS proteins serve as adaptors for multisubunit E3 ligase activity. They can bind to activated STATs and JAKs, and the tightly associated E3 ligases ubiquitinate the JAKs and STATs, thus targeting them for proteasomal degradation. SOCS protein levels can be regulated by TLR ligands, by cytokines themselves, and by other stimuli. In this way, SOCS serve as negative feedback regulators of the cytokine-mediated activation of cells. Other inhibitors of JAK-STAT signaling include tyrosine phosphatases, such as SHP-1 and SHP-2, which can dephosphorylate and therefore deactivate JAK molecules. Another family of inhibitory proteins, called protein inhibitors of activated STAT (PIAS), were originally defined as negative regulators of STATs. PIAS proteins bind phosphorylated STATs and prevent their interaction with DNA. It is now known that PIAS proteins also interact with and block the function of other transcription factors associated with cytokine signaling, including NF-κB and SMADs (transcription factors downstream of members of the TGF-β receptor family).

Pathways of NF-κB Activation

NF-κB is a transcription factor that plays a central role in inflammation, lymphocyte activation, cell survival, and the formation of secondary lymphoid organs. It is also an important player in lymphocyte development and in the pathogenesis of many cancers, including malignant neoplasms derived from activated lymphocytes. NF-κB is activated by many cytokine and TLR stimuli and by antigen recognition and is discussed here as the prototype of a transcription factor with fundamental roles in innate and adaptive immunity.

There are five NF-κB proteins. The domain that is common to all NF-κB proteins is a DNA-binding domain called a Rel homology domain. For a transcription factor to be active, it must both bind DNA and contain an activation domain that can facilitate transcriptional initiation. Three NF-κB proteins have both Rel homology domains and activation domains. These are p65/RelA, RelB, and c-Rel. NF-κB1/p50 and NF-κB2/p52 proteins contain a DNA-binding Rel homology domain but lack activation domains. NF-κB1 typically forms active heterodimers with p65/RelA or with c-Rel, and these heterodimers are typically considered "canonical" NF-κB heterodimers (Fig. 7-26). Canonical NF-κB heterodimers reside in the cytosol bound to an inhibitor of NF-κB called IκBα. Canonical NF-κB heterodimers are activated by a number of signaling receptors that drive inflammation or lymphocyte activation.

As we have noted earlier in this chapter, TLRs, the BCR, the TCR, and many cytokine receptors of the TNF and IL-1R family activate NF-κB, and we will examine the common pathway involved in activating canonical NF-κB signaling. This NF-κB pathway induces the tagging and degradation of IκBα, allowing the unfettered heterodimeric NF-κB transcription factor to migrate into the nucleus. Most receptors that activate NF-κB do so by inducing this pathway. Two very different types of polyubiquitination events are required for canonical NF-κB activation. There are a few common steps in the canonical pathway that apply to all upstream signal inputs.

- Upstream signaling leads to the activation of a unique type of ubiquitin E3 ligase that can add a lysine-63 type of ubiquitin chain to a protein called NEMO or IKKγ that is a noncatalytic subunit of a trimeric enzyme complex called the IκB kinase (IKK) complex. This complex contains two other subunits called IKKα and IKKβ, both of which have the potential to be catalytically active serine/threonine kinases. Ubiquitination of NEMO allows IKKβ to be activated by an upstream kinase.
- Active IKKβ phosphorylates the inhibitory protein bound to NF-κB, IκBα, on two specific serine

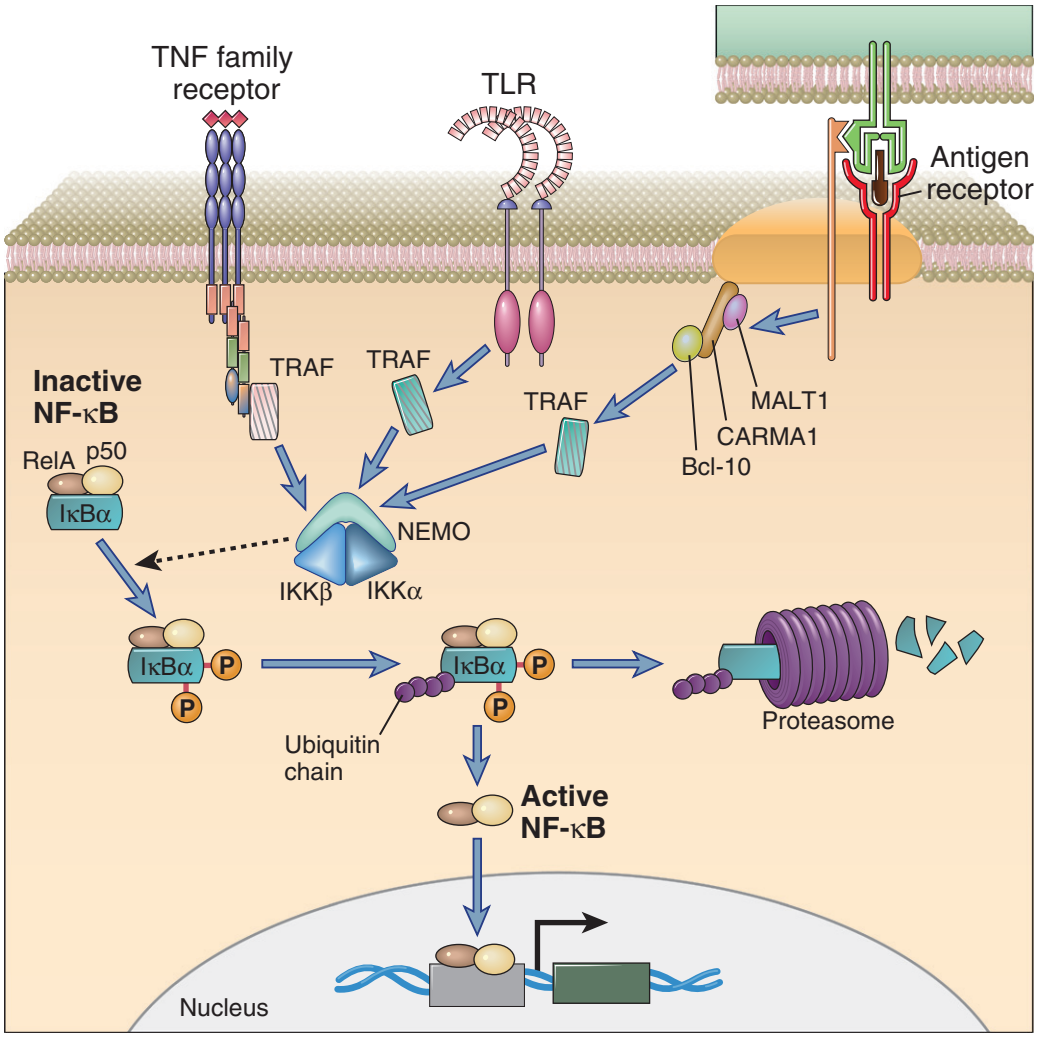


FIGURE 7–26 The canonical NF-κB pathway. Antigen receptors activate specific PKCs that activate the CARMA1/Bcl-10/MALT1 complex, which in turn contributes to the induction of a TRAF E3 ligase that can polyubiquitinate NEMO/IKKγ, a component of the IκB kinase (IKK) complex, forming lysine-63–linked ubiquitin chains. This leads to the phosphorylation and activation of IKKβ by an upstream kinase. IKKβ phosphorylates the inhibitor of NF-κB (IκBα) and targets it for lysine-48 polyubiquitination and proteasomal degradation. Degradation of IκBα leads to the entry of active NF-κB into the nucleus. TLRs, members of the IL-1R family, and many members of the TNF receptor family activate TRAF family members that can activate this pathway.

residues and thus tags this protein for lysine-48 ubiquitination.

- Polyubiquitinated IκBα is targeted for degradation in the proteasome, and the canonical NF-κB heterodimer is then free to enter the nucleus (see Fig. 7-26).

We have discussed earlier how TCR and BCR signaling contributes to the activation of PKC-θ and PKC-β, respectively. These PKCs can phosphorylate a protein called CARMA1 that forms a complex with two proteins called Bcl-10 and MALT1. The CARMA1/MALT1/Bcl-10 complex can contribute to the activation of a lysine-63 type of ubiquitin E3 ligase called TRAF6. Active TRAF6 can activate TAK1 and also add a lysine-63 ubiquitin chain to NEMO, thus facilitating the activation of IKKβ. TLRs and the IL-1R also activate TRAF6 to initiate IKK activation. Many members of the TNF receptor family, including the TNF receptor and CD40, can activate canonical NF-κB signaling through the activation of other TRAF proteins such as TRAF2, TRAF3, and TRAF5.

Heterodimers of NF-κB2 and RelB make up a "non-canonical" form of NF-κB, and these heterodimers are activated by a separate signaling pathway that is particularly important for lymphoid organ biogenesis and the survival of naive B lymphocytes. The two key receptors that induce the non-canonical or alternative NF-κB pathway, the LTβR (lymphotoxin β receptor) and the BAFFR (BAFF receptor), activate an IKK-like complex that contains IKKα homodimers. This leads to ubiquitination and degradation of a part of the NF-κB2–RelB dimer and release of the active protein.

SUMMARY

- Signaling receptors, typically located on the cell surface, generally initiate signaling in the cytosol, followed by a nuclear phase.
- Many different types of signaling receptors contribute to innate and adaptive immunity, the most prominent category being immune receptors that belong to a receptor family in which non-receptor tyrosine kinases phosphorylate tyrosine-containing ITAM motifs on the cytoplasmic tails of proteins in the receptor complex.
- Some of the other types of receptors of interest in immunology include those of the receptor tyrosine kinase family, nuclear receptors, heterotrimeric G protein–coupled serpentine receptors, and receptors of the Notch family.
- Antigen receptors on T and B cells are members of the immune receptor family.
- Antigen receptors can produce widely varying outputs, depending on the affinity and valency of the antigen that can recruit different numbers of ITAMs.
- Antigen receptors use coreceptors to enhance signaling. Coreceptors bind to the same antigen complex that is being recognized by the antigen receptor.
- Signaling from antigen receptors can be attenuated by inhibitory receptors.
- The TCR complex is made up of the TCR α and β chains that contribute to antigen recognition and the ITAM-containing signaling chains CD3 γ, δ, and ε as well as the ζ homodimer. The CD3 chains each contain one ITAM, whereas each ζ chain contains three ITAMs.
- TCR ligation results in tyrosine phosphorylation of CD3 and ζ ITAMs by Src family kinases and the recruitment of ZAP-70 to the phospho-ITAMs, each SH2 domain of ZAP-70 binding to one phosphorylated tyrosine of the ITAM.
- Activated ZAP-70 phosphorylates tyrosine residues on adaptors, and downstream enzymes are recruited to the signalosome.
- Enzymes that mediate the exchange of GTP for GDP on small G proteins such as Ras and Rac help initiate MAP kinase pathways. These pathways lead to the induction or activation of transcription factors such as Jun and Fos, components of the AP-1 transcription factor.
- Activation of PLCγ1 leads to the release of IP3 from PIP2, and IP3 induces release of calcium from intracellular stores. Depletion of calcium from intracellular stores facilitates the opening of CRAC, a store-operated channel on the cell surface that maintains the raised intracellular calcium levels. Calcium binds to calmodulin and activates downstream proteins including calcineurin, a phosphatase that facilitates the entry of the NFAT transcription factor into the nucleus.
- Diacylglycerol is generated in the membrane when PLCγ1 releases IP3 from PIP2. DAG can activate PKC-θ, which among other things can contribute to NF-κB activation .
- A lipid kinase called PI3-kinase converts PIP2 to PIP3. PIP3 can recruit and activate PH domain–containing proteins to the plasma membrane. PIP3 activates Itk in T cells and Btk in B cells. It activates PDK1, a kinase that can phosphorylate a downstream kinase called Akt that mediates cell survival.
- Costimulatory receptors initiate signaling separately from antigen receptors, but signaling outputs from antigen receptors and costimulatory receptors synergize in the nucleus. The major costimulatory receptor in T cells is CD28.
- T cell signaling can be inhibited by phosphatases that may be recruited by inhibitory receptors such as CTLA-4 and PD-1.
- T cell signaling is also attenuated by ubiquitin E3 ligases that can contribute to the monoubiquitination and lysosomal degradation of activated signaling proteins.
- The B cell receptor is made up of membrane-bound immunoglobulin and an associated disulfide-linked Igα and Igβ heterodimer. Both Igα and Igβ contain ITAM motifs in their cytoplasmic tails. Signaling pathways linked to the BCR are broadly similar to signaling pathways downstream of the TCR.
- The coreceptor for the BCR is CD21, also known as CR2 (complement receptor type 2).
- Attenuation of immune receptor signaling in B cells, T cells, and NK cells, among others, is mediated by inhibitory receptors that frequently contain inhibitory tyrosine–containing motifs or ITIMs in their cytoplasmic tails.
- Another important mechanism of signal attenuation involves the ubiquitination of signaling proteins by E3 ubiquitin ligases.
- Cytokine receptors can be divided into a few broad categories based on structural considerations and mechanisms of signaling.
- Many cytokine receptors use non-receptor tyrosine kinases called JAKs to phosphorylate transcription factors called STATs.
- Some cytokine receptors such as those of the TNF receptor family activate either canonical or non-canonical NF-κB signaling.
- Canonical NF-κB signaling is activated downstream of many receptors, including TNF receptor family cytokine receptors, TLRs and IL-1R family members, and antigen receptors. The pathway involves activation of IKKβ in the IKK complex, phosphorylation of the IκBα inhibitor by activated IKKβ, ubiquitination and proteasomal degradation of IκBα, and transport of NF-κB to the nucleus.

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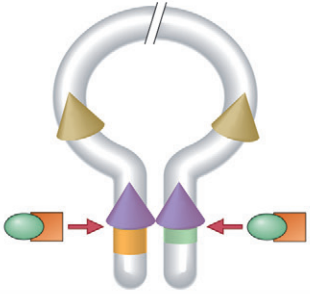
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CHAPTER

8

Lymphocyte Development and Antigen Receptor Gene Rearrangement

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Lymphocytes express highly diverse antigen receptors capable of recognizing a wide variety of foreign substances. This diversity is generated during the development of mature B and T lymphocytes from precursor cells that do not express antigen receptors and cannot recognize and respond to antigens. The process by which lymphocyte progenitors in the thymus and bone marrow differentiate into mature lymphocytes that populate peripheral lymphoid tissues is called **lymphocyte development** or **lymphocyte maturation**. The immune repertoire is made up of the collection of antigen receptors—and therefore specificities—expressed by B and T lymphocytes that are produced during the maturation of these cells. Maturation is initiated by signals from cell surface receptors that have two main roles—they promote the proliferation of progenitors and also induce the expression of transcription factors that work together to initiate the rearrangement of specific antigen receptor genes and commit developing cells to a B or a T cell fate. The rearrangement of antigen receptor genes is a key event in the commitment of a progenitor cell to a lymphoid fate.

We begin this chapter by considering the process of commitment to the B and T lymphocyte lineages and discuss some common principles and mechanisms of B and T cell development. This is followed by a description of the processes that are unique to the maturation of B cells and then of those unique to the development of cells of the T lymphocyte lineage.

OVERVIEW OF LYMPHOCYTE DEVELOPMENT

The maturation of B and T lymphocytes involves a series of events that occur in the generative lymphoid organs (Fig. 8-1). These events include the following:

- The **commitment of progenitor cells** to the B cell or T cell lineage.
- **Proliferation** of progenitors and immature committed cells at specific early stages of development, providing a large pool of cells that can generate useful lymphocytes.
- The **sequential and ordered rearrangement of antigen receptor genes** and the expression of antigen receptor proteins.

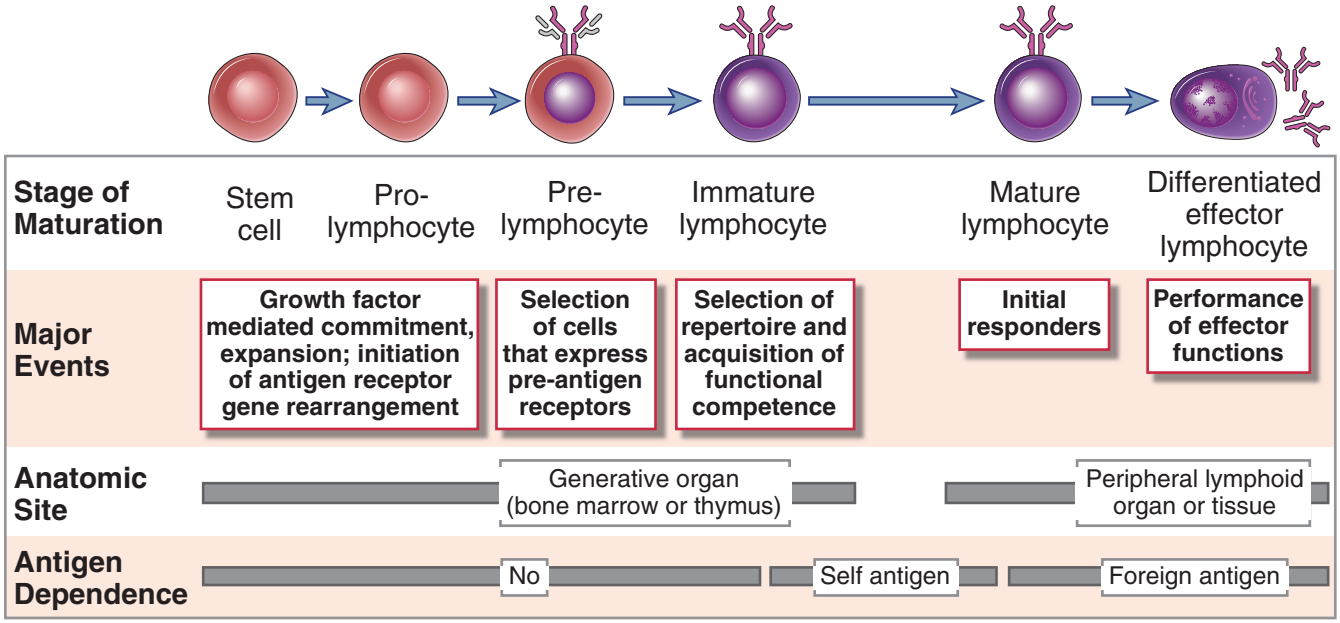


FIGURE 8–1 Stages of lymphocyte maturation. Development of both B and T lymphocytes involves the sequence of maturational stages shown. B cell maturation is illustrated, but the basic stages of T cell maturation are similar.

- **Selection events** that preserve cells that have produced correct antigen receptor proteins and eliminate potentially dangerous cells that strongly recognize self antigens. These checkpoints during development ensure that lymphocytes that express functional receptors with useful specificities will mature and enter the peripheral immune system.
- **Differentiation of B and T cells into functionally and phenotypically distinct subpopulations.** B cells develop into follicular, marginal zone, and B-1 B cells, and T cells develop into $CD4^+$ and $CD8^+$ T lymphocytes and γδ T cells. This differentiation into distinct classes provides the specialization that is an important characteristic of the adaptive immune system.

We next describe features of each of these events that are common to both B and T lymphocyte lineages.

Commitment to the B and T Cell Lineages and Proliferation of Progenitors

Pluripotent stem cells in the bone marrow (and fetal liver), referred to as hematopoietic stem cells (HSCs), give rise to all lineages of blood cells, including lymphocytes. HSCs mature into common lymphoid progenitors that can give rise to B cells, T cells, NK cells, and some dendritic cells (Fig. 8-2). The maturation of B cells from progenitors committed to this lineage occurs mostly in the bone marrow and before birth in the fetal liver. Fetal liver–derived stem cells give rise mainly to a type of B cell called a B-1 B cell (best defined in rodents), whereas bone marrow–derived HSCs give rise to the majority of circulating B cells (follicular B cells). Precursors of T lymphocytes leave the fetal liver before birth and the bone marrow later in life and circulate to the thymus, where they complete their maturation. The majority of T cells, which are αβ T cells, develop from bone marrow–derived HSCs; most γδ T cells arise from fetal liver HSCs. In general, the B and T cells that are generated early in fetal life have less diverse antigen receptors. Despite their different anatomic locations, the early maturation events of both B and T lymphocytes are fundamentally similar.

Commitment to the B or T lineage depends on instructions received from several cell surface receptors, which signal to induce specific transcriptional regulators that drive a common lymphoid progenitor to specifically assume a B cell or a T cell fate. The cell surface receptors and transcription factors that contribute to commitment function to induce the proteins involved in antigen receptor gene rearrangement and make particular antigen receptor gene loci accessible to these proteins. In the case of developing B cells, the immunoglobulin (Ig) heavy chain locus, originally in a "closed" chromatin configuration, is opened up so that it becomes accessible to the proteins that will mediate antigen receptor gene rearrangement and expression. In developing αβ T cells, the T cell receptor (TCR) β gene locus is made available first. In addition to genes involved in the process of antigen receptor gene rearrangement, genes that drive the further differentiation of T and B cells are expressed at this stage.

Different sets of transcription factors drive the development of the B and T cell lineages from uncommitted precursors (see Fig. 8-2). The Notch family of proteins are cell surface molecules that are proteolytically cleaved when they interact with specific ligands on neighboring cells. The cleaved intracellular portions of Notch proteins migrate to the nucleus and modulate the expression of specific target genes. Notch-1, a member of the Notch family, is activated in lymphoid progenitor cells and collaborates with a transcription factor called GATA-3 to commit developing lymphocytes to the T lineage. These transcriptional regulators contribute to the induction of

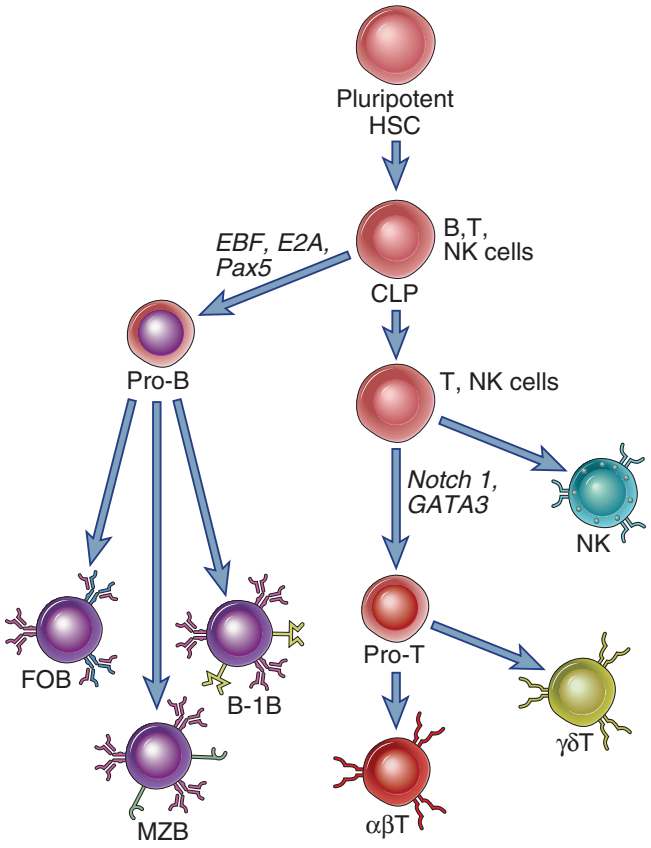


FIGURE 8–2 Pluripotent stem cells give rise to distinct B and T lineages. Hematopoietic stem cells (HSCs) give rise to distinct progenitors for various types of blood cells. One of these progenitor populations (shown here) is called a common lymphoid progenitor (CLP). CLPs give rise mainly to B and T cells but may also contribute to NK cells and some dendritic cells (not depicted here). Pro-B cells can eventually differentiate into follicular (FO) B cells, marginal zone (MZ) B cells, and B-1 B cells. Pro-T cells may commit to either the αβ or γδ T cell lineages. Commitment to the T lineage depends on signals delivered by Notch-1, whose intracellular domain mediates transcriptional activation of T lineage genes in collaboration with other transcription factors such as GATA-3. Commitment to the B lineage is mediated initially by the EBF and E2A transcription factors and subsequently by Pax-5. These transcription factors work together to induce the transcription of B cell–specific genes and of genes of the recombination machinery. (Transcription factors are indicated by italics.)

a number of genes that are required for the further development of αβ T cells. Downstream target genes include components of the pre-TCR and of the machinery for V(D)J recombination, described later. In B cells, the EBF and E2A transcription factors contribute to the induction of another transcription factor called Pax-5, and these three proteins collaborate to induce the process of commitment to the B lineage by facilitating the expression of a number of genes. These genes, which are described in more detail later, include those encoding the Rag-1 and Rag-2 proteins, surrogate light chains, and the Igα and Igβ proteins that contribute to signaling through the pre-B cell receptor and the B cell receptor. The role of these receptors in B cell development will be considered later in the chapter.

Early B and T cell development is characterized by the proliferation of committed progenitors induced by cytokine-derived signals. Proliferation ensures that a large enough pool of progenitor cells will be generated to eventually provide a highly diverse repertoire of mature, antigen-specific lymphocytes. In rodents, the cytokine interleukin-7 (IL-7) drives proliferation of both T and B cell progenitors; in humans, IL-7 is required for the proliferation of T cell progenitors but not of progenitors in the B lineage. IL-7 is produced by stromal cells in the bone marrow and by epithelial and other cells in the thymus. Mice with targeted mutations in either the IL-7 gene or the IL-7 receptor gene show defective maturation of lymphocyte precursors beyond the earliest stages and, as a result, profound deficiencies in mature T and B cells. Mutations in a chain of the IL-7 receptor, called the common γ chain because it is shared by several cytokine receptors, give rise to an immunodeficiency disorder in humans called **X-linked severe combined immunodeficiency disease (X-SCID)**. This disease is characterized by a block in T cell and NK cell development, but normal B cell development, reflecting the role of IL-7 in humans (see Chapter 20).

The proliferative activity in early lymphocyte development, driven mainly by IL-7, ceases just before gene rearrangement for one chain of the antigen receptor is completed, and subsequent proliferation occurs only in cells that have successfully rearranged and expressed the gene encoding the first antigen receptor chain in a developing B or T cell. Once this receptor chain is produced, it forms the pre-B cell receptor (pre-BCR) or pre-T cell receptor (pre-TCR), described later, that selects cells for survival, proliferation, and further differentiation but is incapable of recognizing antigen. Pre-antigen receptor–based selection of the cells that have successfully made an Ig heavy chain protein in the B cell lineage and the TCR β chain in the αβ T cell lineage supports the greatest expansion of lymphocyte progenitors during development. This is an important developmental checkpoint that cells must successfully negotiate because only cells that express the first component of antigen receptors can survive, expand, and proceed to the next stage of maturation.

MicroRNAs and Lymphocyte Development

Although gene expression during lymphocyte development is driven primarily by transcription factors, an additional level of regulation is mediated by microRNAs (miRNAs). miRNAs are small endogenous noncoding RNAs that are initially generated in the nucleus as longer primary miRNA transcripts that are processed at this site by an endoribonuclease called Drosha into shorter pre-miRNAs that have a stem loop structure and can be exported into the cytosol. In the cytosol, the pre-miRNA is processed by another endoribonuclease called Dicer into a short double-stranded miRNA about 21 to 22 base pairs in length, one strand of which can be used to pair with a complementary sequence in a number of cellular mRNAs. These mRNAs associate with miRNAs and proteins called Argonaute proteins to form complexes known as RISC (RNA-induced silencing complex). If the 6– to 8–base pair miRNA seed sequence is not perfectly complementary to the mRNA, the mRNA is prevented from

being translated efficiently. mRNAs may be targeted for degradation when complementarity is perfect. In either case, the result is a reduction in the abundance of proteins encoded by genes targeted by miRNAs. Gene ablation studies have revealed that miRNAs are involved in both B and T cell development. Numerous miRNAs have been reported to regulate lymphocyte activation as well.

Antigen Receptor Gene Rearrangement and Expression

The rearrangement of antigen receptor genes is the key event in lymphocyte development that is responsible for the generation of a diverse repertoire. Antigen receptor gene products also provide signals that ensure the selective survival of lymphocytes with useful specificities. As we discussed in Chapter 7, each clone of B or T lymphocytes produces an antigen receptor with a unique antigen-binding structure. In any individual, there may be 10^7 or more different B and T lymphocyte clones, each with a unique receptor. The ability of each individual to generate these enormously diverse lymphocyte repertoires has evolved in a way that does not require an equally large number of distinct antigen receptor genes; otherwise, a large proportion of the genome would be devoted to encoding the vast number of Ig and TCR molecules. Functional antigen receptor genes are produced in immature B cells in the bone marrow and in immature T cells in the thymus by a process of gene rearrangement, which is designed to generate a large number of variable region–encoding exons using a relatively small fraction of the genome. In any given developing lymphocyte, one of many variable region gene segments is randomly selected and joined to a downstream DNA segment. The DNA rearrangement events that lead to the production of antigen receptors are not dependent on or influenced by the presence of antigens. In other words, as the clonal selection hypothesis had proposed, diverse antigen receptors are generated and expressed before encounter with antigens (see Fig. 1-7, Chapter 1). We will discuss the molecular details of antigen receptor gene rearrangement later in the chapter.

Selection Processes That Shape the B and T Lymphocyte Repertoires

The process of lymphocyte development contains numerous intrinsic steps, called **checkpoints**, at which the developing cells are "tested" and continue to mature only if a preceding step in the process has been successfully completed. One of these checkpoints is based on the successful production of one of the polypeptide chains of the two chain antigen receptor protein and a second checkpoint requires the assembly of a complete receptor. The requirement for traversing these checkpoints ensures that only lymphocytes that have successfully completed antigen receptor gene rearrangement processes, and are therefore likely to be functional, are selected to mature. Additional selection processes operate after antigen receptors are expressed and serve to eliminate potentially harmful, self-reactive lymphocytes and to commit developing cells to particular lineages. We next summarize the general principles of these selection events.

Pre-antigen receptors and antigen receptors deliver signals to developing lymphocytes that are required for the survival of these cells and for their proliferation and continued maturation (Fig. 8-3). Antigen receptor gene rearrangement involves the opening of a particular receptor gene locus (such as a TCR gene locus in T cells and an Ig locus in B cells) and the joining of DNA segments in this locus to generate a functional antigen receptor gene. During this process, bases are randomly added or removed between the gene segments being joined together, thus maximizing variability among receptors. In developing B cells, the first antigen receptor gene to be completely rearranged is the Ig heavy chain or Ig H gene. In αβ T cells, the β chain of the TCR is rearranged first. Cells of the B lymphocyte lineage that successfully rearrange their Ig heavy chain genes express the Ig H chain protein and assemble a pre-antigen receptor known as the pre-BCR. In an analogous fashion, developing T cells that make a productive TCR β chain gene rearrangement synthesize the TCR β chain protein and assemble a pre-antigen receptor known as the pre-TCR. Only about one in three developing B and T cells that rearranges an antigen receptor gene makes an in-frame rearrangement and is therefore capable of generating a proper full-length protein. If cells make out-of-frame rearrangements at the Ig μ or TCR β chain loci, the pre-antigen receptors are not expressed, the cells do not receive necessary survival signals, and they undergo programmed cell death. The assembled pre-BCR and pre-TCR complexes provide signals for survival, for proliferation, for the phenomenon of allelic exclusion discussed later, and for the further development of early B and T lineage cells. Thus, expression of the pre-antigen receptor is the first checkpoint during lymphocyte development.

Lymphocytes that have successfully navigated this checkpoint continue to develop in the generative lymphoid organs and express the complete antigen receptor while they are still immature. At this immature stage, potentially harmful cells that strongly recognize self structures may be eliminated or induced to alter their antigen receptors, and cells that express useful antigen receptors may be preserved (Fig. 8-4). A process called **positive selection** facilitates the survival of potentially useful lymphocytes, and this developmental event is linked to lineage commitment, the process by which lymphocyte subsets are generated. In the T cell lineage, positive selection ensures the maturation of T cells whose receptors recognize self MHC molecules and also that the expression of the appropriate coreceptor on a T cell (CD8 or CD4) is matched to the recognition of the appropriate type of MHC molecule (MHC class I or MHC class II). Mature T cells whose precursors were positively selected by self MHC molecules in the thymus are able to recognize foreign peptide antigens displayed by the same self MHC molecules on antigen-presenting cells in peripheral tissues. In the B cell lineage, positive selection preserves receptor-expressing cells and is coupled to the generation of different subsets, discussed later.

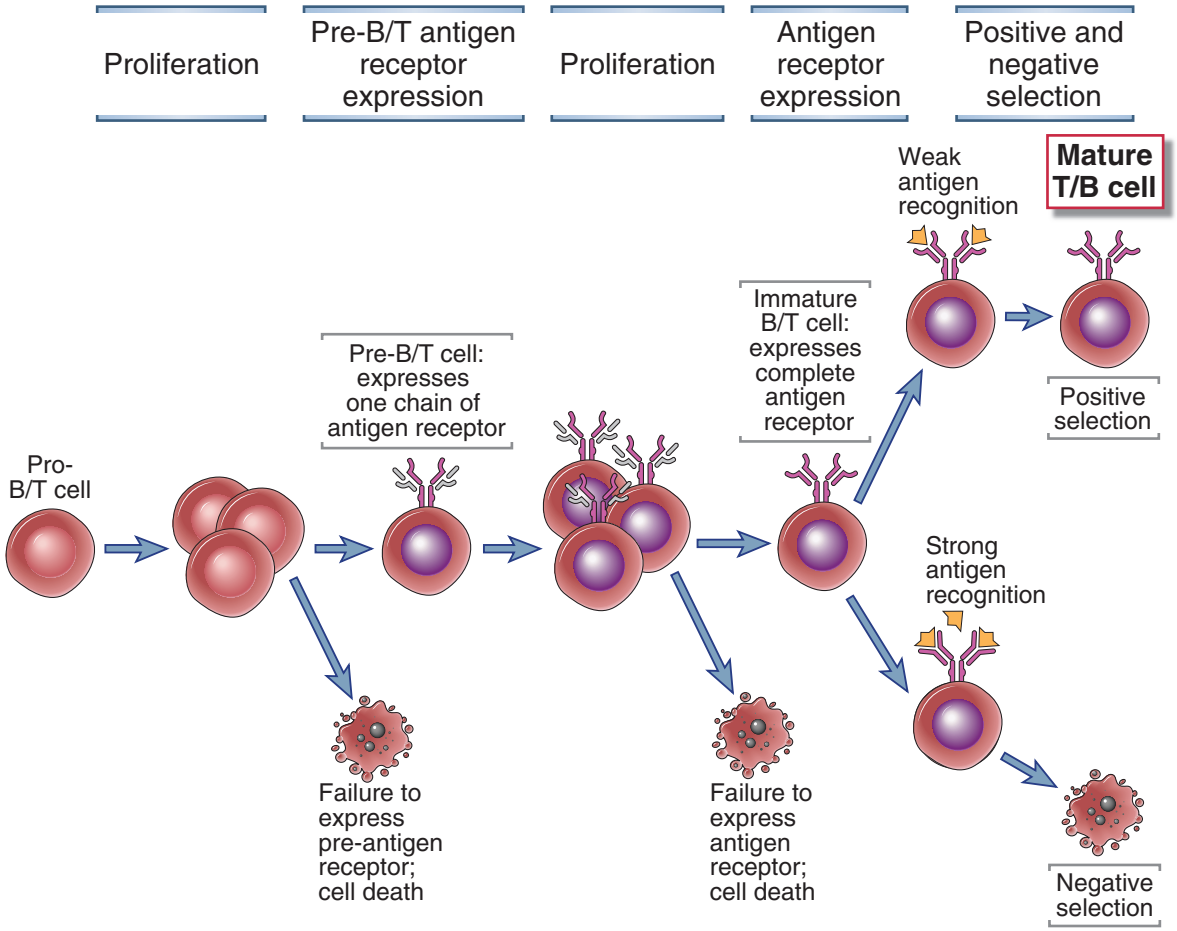


FIGURE 8–3 Checkpoints in lymphocyte maturation. During development, the lymphocytes that express receptors required for continued proliferation and maturation are selected to survive, and cells that do not express functional receptors die by apoptosis. Positive selection and negative selection further preserve cells with useful specificities. The presence of multiple checkpoints ensures that only cells with useful receptors complete their maturation.

Negative selection is the process that eliminates or alters developing lymphocytes whose antigen receptors bind strongly to self antigens present in the generative lymphoid organs. Both developing B and T cells are susceptible to negative selection during a short period after antigen receptors are first expressed. Developing T cells with a high affinity for self antigens are eliminated by apoptosis, a phenomenon known as **clonal deletion**. Strongly self-reactive immature B cells may be induced to make further Ig gene rearrangements and thus evade self-reactivity. This phenomenon is called **receptor editing**. If editing fails, the self-reactive B cells die, also called **clonal deletion**. Negative selection of immature lymphocytes is an important mechanism for maintaining tolerance to many self antigens; this is also called **central tolerance** because it develops in the central (generative) lymphoid organs (see Chapter 14).

The Generation of Lymphocyte Subsets

Another important feature of lymphocyte development that is linked to positive selection is the generation of functionally distinct subsets in both the T and the B cell lineages. Precursors that express both CD4 and CD8 differentiate into either $CD4^+$ class II MHC–restricted T cells or $CD8^+$ class I MHC–restricted T cells. The naive $CD4^+$ T cells that leave the thymus can be activated by antigens to differentiate into helper T cells whose effector functions are mediated by specific membrane proteins and by secreted cytokines. The $CD8^+$ cells can differentiate into cytotoxic T lymphocytes whose major effector function is to kill infected target cells. A similar lineage commitment process during positive selection of B cells drives the development of these cells into distinct peripheral B cell subsets. Bone marrow–derived developing B cells may differentiate into follicular B cells, which recirculate and mediate T cell–dependent immune responses in secondary lymphoid organs, or marginal zone B cells, which reside in the vicinity of the marginal sinus in the spleen and mediate largely T cell–independent responses to blood-borne antigens.

With this introduction, we proceed to a more detailed discussion of lymphocyte maturation, starting with the key event in the process, the rearrangement and expression of antigen receptor genes.

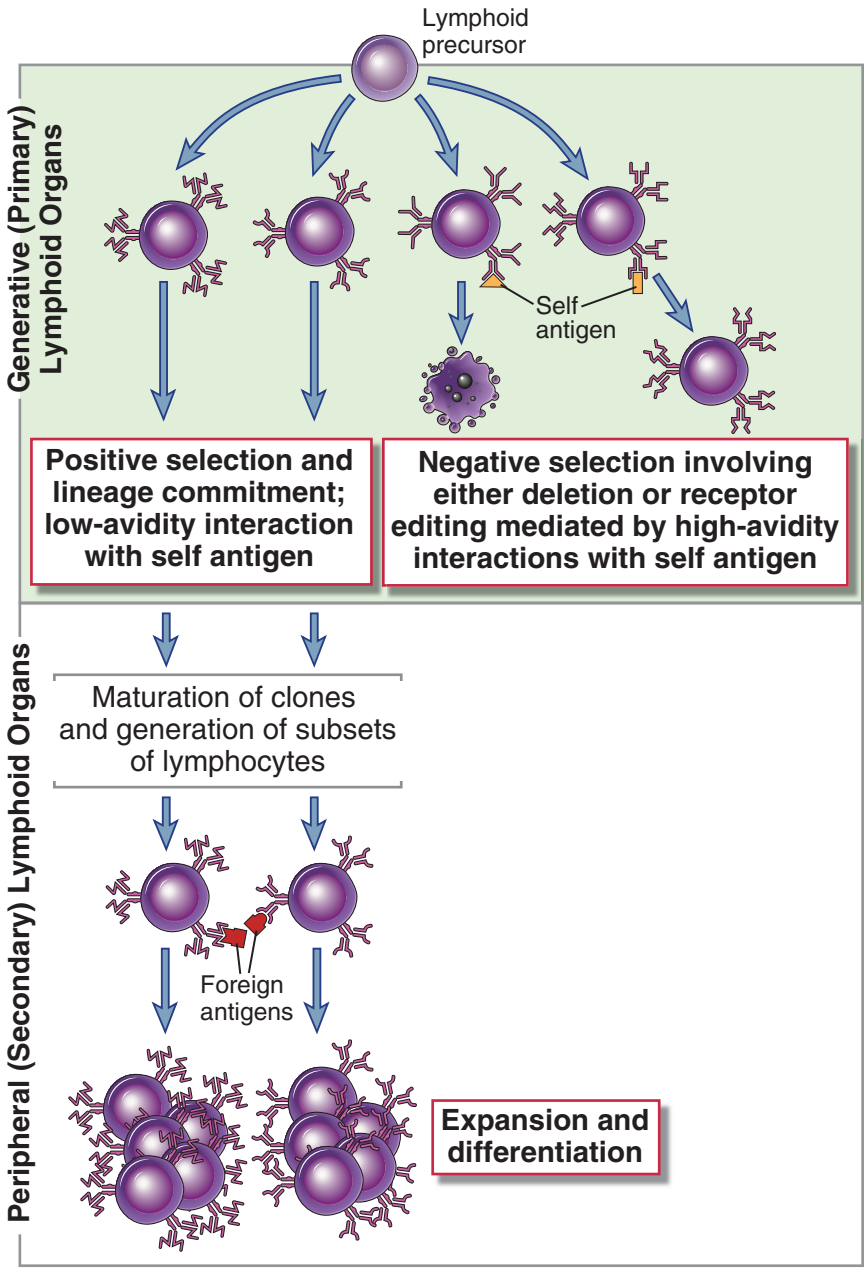


FIGURE 8–4 Positive and negative selection during lymphocyte maturation. After immature clones of lymphocytes in generative lymphoid organs express antigen receptors, they are subject to both positive and negative selection processes. In positive selection, lymphocyte precursors with antigen receptors that bind some self ligand with low avidity are selected to survive and mature further. Developing B cells receive survival signals simply because of expression of complete antigen receptors, without recognition of a self antigen. However, as in T cells, self antigen of different affinities can drive the differentiation of different B cell subsets. Positive selection in both B and T lineages is therefore tightly linked to the process of generating lymphocyte subsets, also referred to as lineage commitment. Positively selected lymphocytes enter peripheral lymphoid tissues, where they respond to foreign antigens. In negative selection, cells that bind antigens present within the generative organs, with high avidity, receive signals that either lead to cell death or induce further rearrangement of antigen receptor genes, a process known as receptor editing. As a result, the repertoire of mature lymphocytes lacks cells capable of responding to these self antigens. The diagram illustrates selection of B cells; the principles are the same for T lymphocytes, except that there is no receptor editing in the T lineage.

REARRANGEMENT OF ANTIGEN RECEPTOR GENES IN B AND T LYMPHOCYTES

The genes that encode diverse antigen receptors of B and T lymphocytes are generated by the rearrangement in individual lymphocytes of different variable (V) region gene segments with diversity (D) and joining (J) gene segments. A novel rearranged exon for each antigen receptor gene is generated by fusing a specific distant upstream V gene segment to a downstream segment on the same chromosome. This specialized process of site-specific gene rearrangement is called **V(D)J recombination**. (The terms *recombination* and *rearrangement* are used interchangeably.) Elucidation of the mechanisms of antigen receptor gene rearrangement, and therefore of the underlying basis for the generation of immune diversity, represents one of the landmark achievements of modern immunology.

The first insights into how millions of different antigen receptors could be generated from a limited amount of coding DNA in the genome came from analyses of the amino acid sequences of Ig molecules. These analyses showed that the polypeptide chains of many different antibodies of the same isotype shared identical sequences at their C-terminal ends (corresponding to the constant domains of antibody heavy and light chains) but differed considerably in the sequences at their N-terminal ends that correspond to the variable domains of immunoglobulins (see Chapter 5). Contrary to one of the central tenets of molecular genetics, enunciated as the "one gene–one polypeptide hypothesis" by Beadle and Tatum in 1941, Dreyer and Bennett postulated in 1965 that each antibody chain is actually encoded by at least two genes,

one variable and the other constant, and that the two are physically combined at the level of DNA or of messenger RNA (mRNA) to eventually give rise to functional Ig proteins. Formal proof of this hypothesis came more than a decade later when Susumu Tonegawa demonstrated that the structure of Ig genes in the cells of an antibody-producing tumor, called a myeloma or plasmacytoma, is different from that in embryonic tissues or in nonlymphoid tissues not committed to Ig production. These differences arise because DNA segments within the loci encoding Ig heavy and light chains are specifically ligated together only in developing B cells but not in other tissues or cell types. Similar rearrangements were found to occur during T cell development in the loci encoding the polypeptide chains of TCRs. Antigen receptor gene rearrangement is best understood by first describing the unrearranged, or germline, organization of Ig and TCR genes and then describing their rearrangement during lymphocyte maturation.

Germline Organization of Ig and TCR Genes

The germline organizations of Ig and TCR genetic loci are fundamentally similar and are characterized by spatial segregation of multiple sequences that encode variable and constant domains of receptor proteins; distinct variable region sequences are joined to constant region sequences in different lymphocytes. We first describe the Ig loci and then the TCR loci.

Organization of Ig Gene Loci

Three separate loci encode, respectively, all the Ig heavy chains, the Ig κ light chain, and the Ig λ light chain. Each locus is on a different chromosome. The organization of human Ig genes is illustrated in Figure 8-5, and the relationship of gene segments after rearrangement to the domains of the Ig heavy and light chain proteins is shown in Figure 8-6A. Ig genes are organized in essentially the same way in all mammals, although their chromosomal locations and the number and sequence of different gene segments in each locus may vary.

At the 5′ end of each of the Ig loci, there is a cluster of V gene segments, each V gene in the cluster being about 300 base pairs long. The numbers of V gene segments vary considerably among the different Ig loci and among different species. For example, there are about 35 V genes in the human κ light chain locus, about 30 in the λ locus, and about 100 functional V genes in the human heavy chain locus, whereas the mouse λ light chain locus has only two V genes and the mouse heavy chain locus has more than 1000 V genes. The V gene segments for each locus are spaced over large stretches of DNA, up to 2000 kilobases long. Located 5′ of each V segment is a leader exon that encodes the 20 to 30 N-terminal residues of the translated protein. These residues are moderately hydrophobic and make up the leader (or signal) peptide. Signal sequences are found in all newly synthesized secreted and transmembrane proteins and are involved in guiding nascent polypeptides being translated on membrane-bound ribosomes into the lumen of the endoplasmic reticulum. Here, the signal sequences are rapidly cleaved, and they are not present in the mature proteins. Upstream of each leader exon is a V gene promoter at which transcription can be initiated, but, as discussed later, this occurs most efficiently after rearrangement.

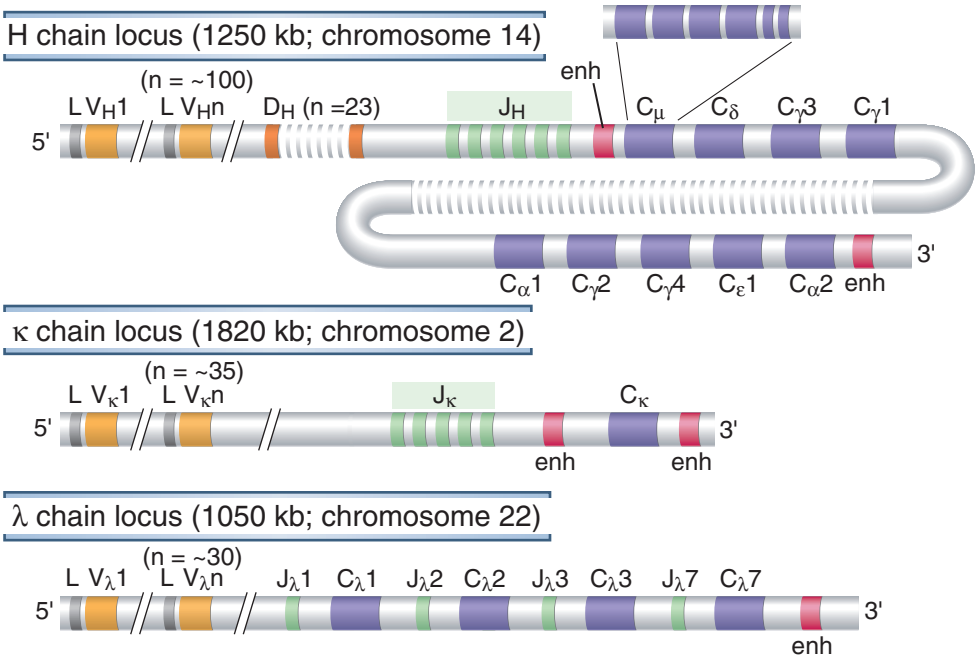


FIGURE 8–5 Germline organization of human Ig loci. The human heavy chain, κ light chain, and λ light chain loci are shown. Only functional genes are shown; pseudogenes have been omitted for simplicity. Exons and introns are not drawn to scale. Each C_H gene is shown as a single box but is composed of several exons, as illustrated for C_μ. Gene segments are indicated as follows: L, leader (often called signal sequence); V, variable; D, diversity; J, joining; C, constant; enh, enhancer.

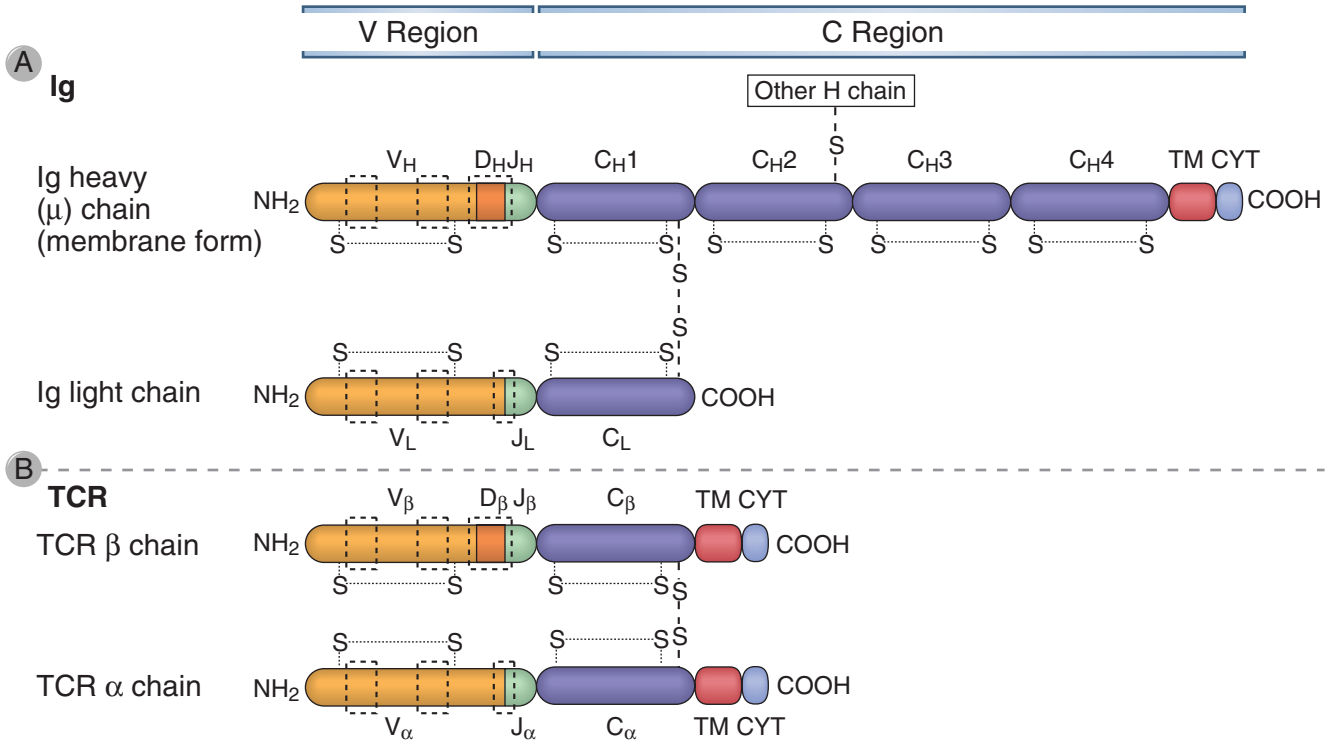


FIGURE 8–6 Domains of Ig and TCR proteins. The domains of Ig heavy and light chains are shown in **A,** and the domains of TCR α and β chains are shown in **B.** The relationships between the Ig and TCR gene segments and the domain structure of the antigen receptor polypeptide chains are indicated. The V and C regions of each polypeptide are encoded by different gene segments. The locations of intrachain and interchain disulfide bonds (S-S) are approximate. Areas in the dashed boxes are the hypervariable (complementarity-determining) regions. In the Ig μ chain and the TCR α and β chains, transmembrane (TM) and cytoplasmic (CYT) domains are encoded by separate exons.

At varying distances 3′ of the V genes are several J segments that are closely linked to downstream constant region exons. J segments are typically 30 to 50 base pairs long and are separated by noncoding sequences. Between the V and J segments in the Ig H locus, there are additional segments known as D segments. As for V genes, the numbers of J and D genes vary in different Ig loci and different species.

Each Ig locus has a distinct arrangement and number of C region genes. In humans, the Ig κ light chain locus has a single C gene (C_κ) and the λ light chain locus has four functional C genes (C_λ). The Ig heavy chain locus has nine C genes (C_H), arranged in a tandem array, that encode the C regions of the nine different Ig isotypes and subtypes (see Chapter 5). The C_κ and C_λ genes are each composed of a single exon that encodes the entire C domain of the light chains. In contrast, each C_H gene is composed of five or six exons. Three or four exons (each similar in size to a V gene segment) each encode a C_H domain of the Ig heavy chain, and two smaller exons code for the carboxyl-terminal ends of the membrane form of each Ig heavy chain, including the transmembrane and cytoplasmic domains of the heavy chains (see Fig. 8-6A).

In an Ig light chain protein (κ or λ), the V domain is encoded by the V and J gene segments; in the Ig heavy chain protein, the V domain is encoded by the V, D, and J gene segments (see Fig. 8-6A). All junctional residues between the rearranged V and D segments and the D and J segments as well as the sequences of the D and J segments themselves make up the third hypervariable region (also known as complementarity-determining region 3 or CDR3) in the case of the Ig H and TCR β V domains. The junctional sequences between the rearranged V and J segments as well as the J segment itself make up the third hypervariable region of Ig light chains. CDR1 and CDR2 are encoded in the germline V gene segment itself. The V and C domains of Ig molecules share structural features, including a tertiary structure called the Ig fold. As we discussed in Chapter 5, proteins that include this structure are members of the Ig superfamily.

Noncoding sequences in the Ig loci play important roles in recombination and gene expression. As we shall see later, sequences that dictate recombination of different gene segments are found adjacent to each coding segment in Ig genes. Also present are V gene promoters and other *cis*-acting regulatory elements, such as locus control regions, enhancers, and silencers, that regulate gene expression at the level of transcription.

Organization of TCR Gene Loci

The genes encoding the TCR α chain, the TCR β chain, and the TCR γ chain map to three separate loci on three different chromosomes, and the TCR δ chain locus is

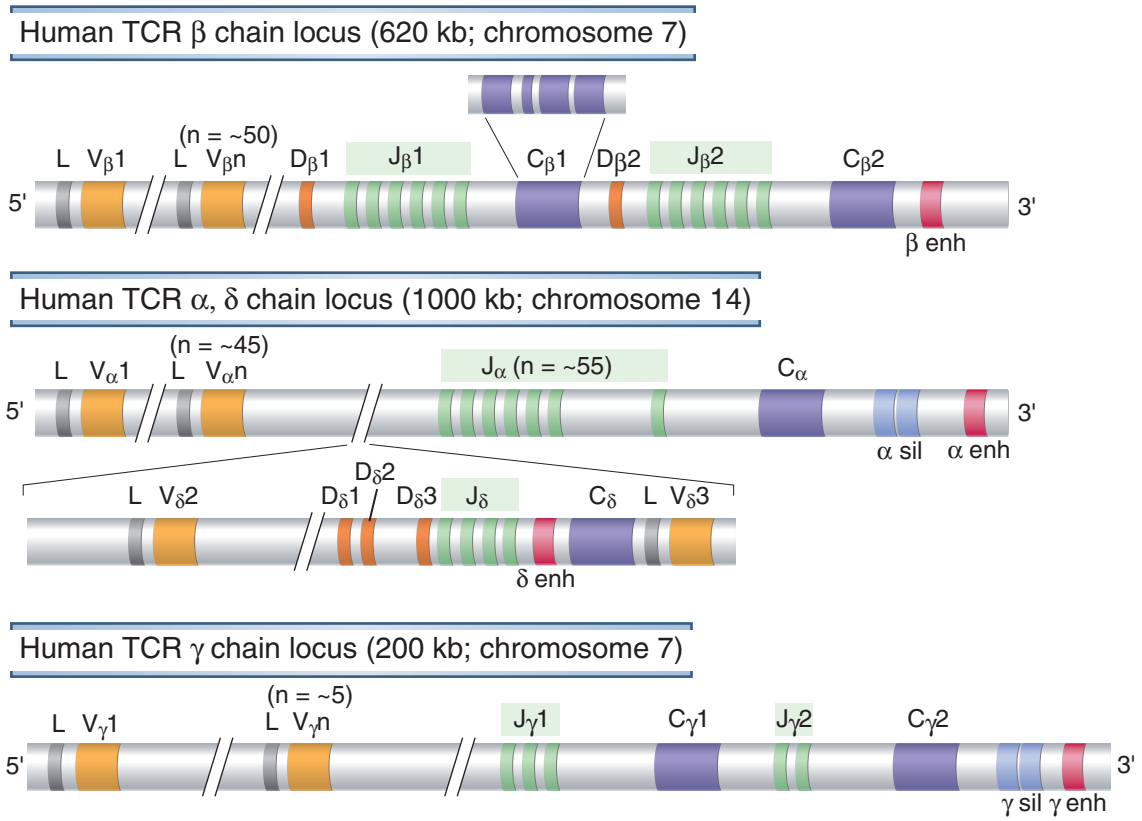


FIGURE 8–7 Germline organization of human TCR loci. The human TCR β, α, γ, and δ chain loci are shown, as indicated. Exons and introns are not drawn to scale, and nonfunctional pseudogenes are not shown. Each C gene is shown as a single box but is composed of several exons, as illustrated for $C_\beta1$. Gene segments are indicated as follows: L, leader (usually called signal sequence); V, variable; D, diversity; J, joining; C, constant; enh, enhancer; sil, silencer (sequences that regulate TCR gene transcription).

contained within the TCR α locus (Fig. 8-7). Each germline TCR locus includes V and J gene segments, the latter being just upstream of C region exons in each locus. In addition, TCR β and TCR δ loci also have D segments, like the Ig heavy chain locus. At the 5′ end of each of the TCR loci, there is a cluster of several V gene segments, arranged in a very similar way to the Ig V gene segments. Upstream of each TCR V gene is an exon that encodes a leader peptide, and upstream of each leader exon is a promoter for each V gene.

In each TCR locus, similar to the Ig loci, C region genes are located just 3′ of the J segments. There are two C genes in each of the human TCR β (C_β) and TCR γ (C_γ) loci and only one C gene each in the TCR α (C_α) and TCR δ (C_δ) loci. Each TCR C region gene is composed of four exons encoding the extracellular C region Ig-like domain, a short hinge region, the transmembrane segment, and the cytoplasmic tail. The TCR β and TCR δ chain loci resemble the Ig heavy chain locus and contain D segments between V genes and J segments. Each human TCR C gene has its own associated 5′ cluster of J segments. In the TCR α or γ chains (which are analogous to Ig light chains), the V domain is encoded by the V and J exons; and in the TCR β and δ proteins, the V domain is encoded by the V, D, and J gene segments.

The relationship of the TCR gene segments and the corresponding portions of TCR proteins that they encode is shown in Figure 8-6B. As in Ig molecules, the TCR V and C domains assume an Ig fold tertiary structure, and thus the TCR is a member of the Ig superfamily of proteins.

V(D)J Recombination

The germline organization of Ig and TCR loci described in the preceding section exists in all cell types in the body. The germline genes cannot be transcribed into mRNAs that encode functional antigen receptor proteins. Functional antigen receptor genes are created only in developing B and T lymphocytes after DNA rearrangement events that bring randomly chosen V, (D), and J gene segments into contiguity. Figure 8-8 schematically depicts a Southern blot, including an Ig light chain locus in germline configuration in a nonlymphoid cell. The V segment shown in this configuration is a considerable distance away from the J segments depicted. A hypothetical rearrangement event in a B cell clone is also shown. In this clone, a specific upstream V segment has been joined to one of the J segments during the rearrangement process.

The process of V(D)J recombination at any Ig or TCR locus involves selection of one V gene, one J segment, and one D segment (when present) in each lymphocyte and rearrangement of these gene segments together to form a single V(D)J exon that will code for the variable region of an antigen receptor protein (Fig. 8-9). In the Ig light chain and TCR α and γ loci, which lack D segments,

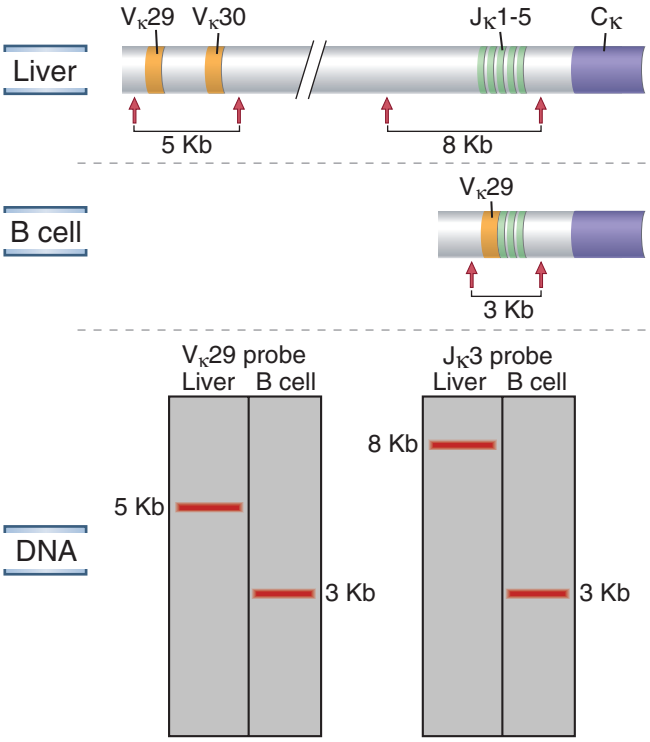


FIGURE 8–8 Antigen receptor gene rearrangements. Southern blot analysis of DNA from nonlymphoid (liver) cells and from a monoclonal population of B lymphocyte lineage origin (e.g., a B cell tumor) is shown in schematic fashion. The DNA is digested with a restriction enzyme (*Eco*RI as depicted), different-sized fragments are separated by electrophoresis, and the fragments are transferred onto a filter. The sites at which the *Eco*RI restriction enzyme cleaves the DNA are indicated by arrows. The size of the fragments containing the $J_\kappa 3$ segment of the Ig κ light chain gene or the $V_\kappa 29$ V region gene was determined by use of a radioactive probe that specifically binds to $J_\kappa 3$ segment DNA or to $V_\kappa 29$ DNA. In the hypothetical example shown, $V_\kappa 29$ is part of a 5-kb *Eco*RI fragment in liver cells but is on a 3-kb fragment in the B cell clone studied. Similarly, the $J_\kappa 3$ fragment is 8 kb in liver cells but 3 kb in the B cell clone.

a single rearrangement event joins a randomly selected V gene to an equally randomly selected J segment. The IgH and TCR β and δ loci contain D segments, and at these loci two distinct rearrangement events must be separately initiated, first joining a D to a J and then a V segment to the fused DJ segment. Each rearrangement event involves a number of sequential steps. First, the chromatin must be opened in specific regions of the antigen receptor chromosome to make gene segments accessible to the enzymes that mediate recombination. Next, two selected gene segments must be brought next to one another across a considerable chromosomal distance. Double-stranded breaks are then introduced at the coding ends of these two segments, nucleotides are added or removed at the broken ends, and finally the processed ends are ligated to produce clonally unique but diverse antigen receptor genes that can be efficiently transcribed. The C regions lie downstream of the rearranged V(D)J exon separated by the germline J-C intron. This rearranged exon is transcribed to form a primary (nuclear) RNA transcript. Subsequent RNA splicing brings together the leader exon, the V(D)J exon, and the C region exons, forming an mRNA that can be translated on membrane-bound ribosomes to produce one of the chains of the antigen receptor. The use of different combinations of V, D, and J gene segments and the addition and removal of nucleotides at the joints contribute to the tremendous diversity of antigen receptors, as we will discuss in more detail later.

Recognition Signals That Drive V(D)J Recombination

Critical lymphocyte-specific factors that mediate V(D)J recombination recognize certain DNA sequences called recombination signal sequences (RSSs), located 3′ of each V gene segment, 5′ of each J segment, and flanking each D segment on both sides (Fig. 8-10A). The RSSs consist of a highly conserved stretch of 7 nucleotides, called the heptamer, usually CACAGTG, located adjacent to the coding sequence, followed by a spacer of exactly 12 or 23 nonconserved nucleotides, followed by a highly

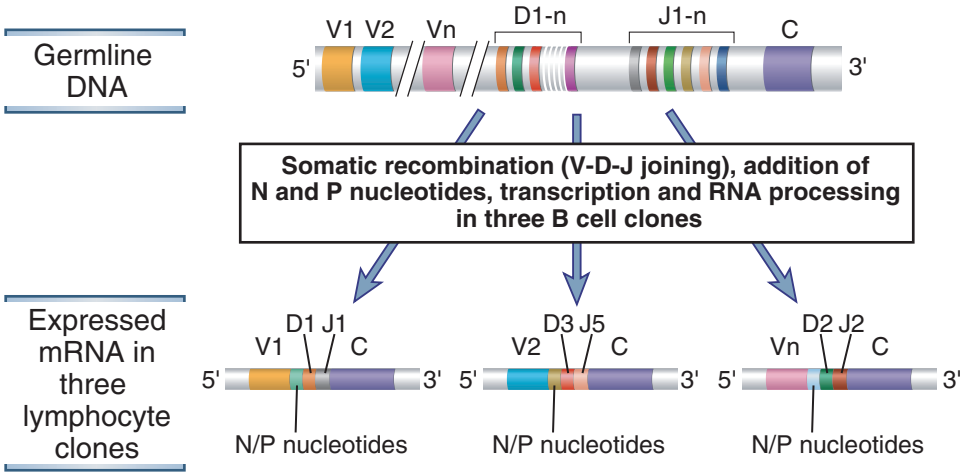


FIGURE 8–9 Diversity of antigen receptor genes. From the same germline DNA, it is possible to generate recombined DNA sequences and mRNAs that differ in their V-D-J junctions. In the example shown, three distinct antigen receptor mRNAs are produced from the same germline DNA by the use of different gene segments and the addition of nucleotides to the junctions.

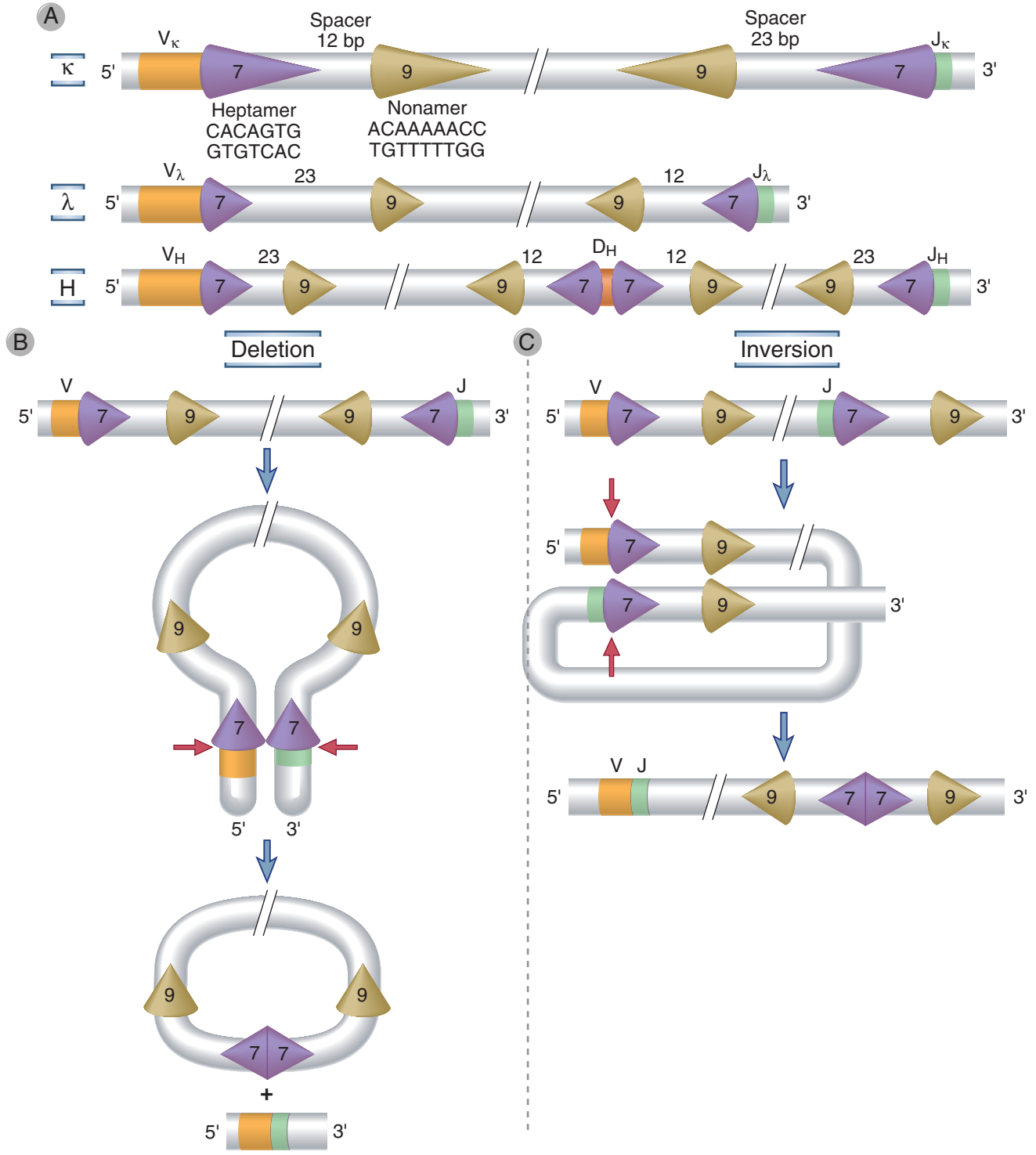


FIGURE 8–10 V(D)J recombination. The DNA sequences and mechanisms involved in recombination in the Ig gene loci are depicted. The same sequences and mechanisms apply to recombinations in the TCR loci. **A,** Conserved heptamer (7 bp) and nonamer (9 bp) sequences, separated by 12- or 23-bp spacers, are located adjacent to V and J exons (for κ and λ loci) or to V, D, and J exons (in the H chain locus). The V(D)J recombinase recognizes these recombination signal sequences and brings the exons together. **B, C,** Recombination of V and J exons may occur by deletion of intervening DNA and ligation of the V and J segments **(B)** or, if the V gene is in the opposite orientation, by inversion of the DNA followed by ligation of adjacent gene segments **(C)**. Red arrows indicate the sites where germline sequences are cleaved before their ligation to other Ig or TCR gene segments.

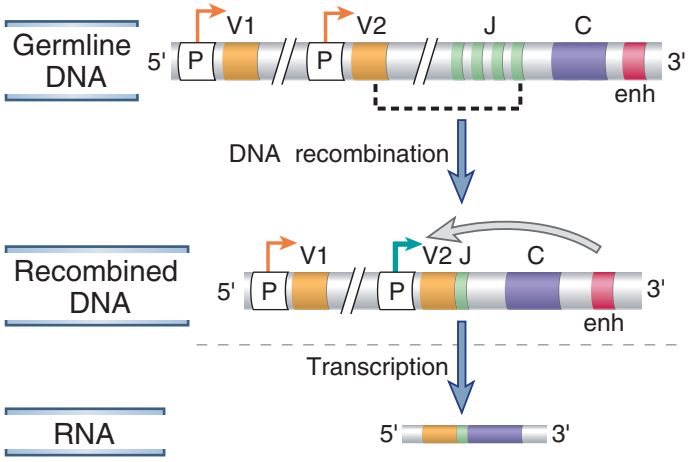


FIGURE 8–11 Transcriptional regulation of Ig genes. V-D-J recombination brings promoter sequences (shown as P) close to the enhancer (enh). The enhancer promotes transcription of the rearranged V gene (V2, whose active promoter is indicated by a bold green arrow). Many receptor genes have an enhancer in the J-C intron and another 3′ of the C region. Only the 3′ enhancer is depicted here.

conserved AT-rich stretch of 9 nucleotides, called the nonamer. The 12- and 23-nucleotide spacers roughly correspond to one or two turns of a DNA helix, respectively, and they presumably bring two distinct heptamers into positions that are simultaneously accessible to the enzymes that catalyze the recombination process.

During V(D)J recombination, double-stranded breaks are generated between the heptamer of the RSS and the adjacent V, D, or J coding sequence. In Ig light chain V-to-J recombination, for example, breaks will be made 3′ of a V segment and 5′ of a J segment. The intervening double-stranded DNA, containing signal ends (the ends that contain the heptamer and the rest of the RSS), is removed in the form of a circle, and this is accompanied by the joining of the V and J coding ends (Fig. 8-10B). Some V genes, especially in the Ig κ locus, are in the same orientation as the J segments, such that the RSSs 5′ of these V segments and 3′ of the J segments do not "face" each other. In these cases, the intervening DNA is inverted and the V and J exons are properly aligned, and the fused RSSs are not deleted but retained in the chromosome (Fig. 8-10C). Most Ig and TCR gene rearrangements occur by deletion; rearrangement by inversion occurs in up to 50% of rearrangements in the Ig κ locus. Recombination occurs between two segments only if one of the segments is flanked by a 12-nucleotide spacer and the other is flanked by a 23-nucleotide spacer; this is called the 12/23 rule. A coding segment with a "one-turn" RSS therefore always recombines with a coding segment with a "two-turn" RSS. The type of the flanking RSSs (one turn or two turn) ensures that the appropriate gene segments will recombine. For example, in the Ig heavy chain locus, the RSSs flanking both V and J segments have 23-nucleotide spacers (two turns) and therefore cannot join directly; D-to-J recombination occurs first, followed by V-to-DJ recombination, and this is possible because the D segments are flanked on both sides by 12-nucleotide spacers, allowing D-J and then V-DJ joining. The RSSs described here are unique to Ig and TCR genes. Therefore, V(D)J recombination can occur in antigen receptor genes but not in other genes.

One of the consequences of V(D)J recombination is that the process brings promoters located immediately 5′ of V genes close to downstream enhancers that are located in the J-C introns and also 3′ of the C region genes (Fig. 8-11). These enhancers maximize the transcriptional activity of the V gene promoters and are thus important for high-level transcription of rearranged V genes in lymphocytes. Because Ig and TCR genes are sites for multiple DNA recombination events in B and T cells, and because these sites become transcriptionally active after recombination, genes from other loci can be abnormally translocated to these loci and, as a result, may be aberrantly transcribed. In tumors of B and T lymphocytes, oncogenes are often translocated to Ig or TCR gene loci. Such chromosomal translocations are frequently accompanied by enhanced transcription of the oncogenes and are believed to be one of the factors causing the development of lymphoid tumors.

The Mechanism of V(D)J Recombination

Rearrangement of Ig and TCR genes represents a special kind of nonhomologous DNA recombination event, mediated by the coordinated activities of several enzymes, some of which are found only in developing lymphocytes, whereas others are ubiquitous DNA double-stranded break repair (DSBR) enzymes. Although the mechanism of V(D)J recombination is fairly well understood and will be described here, how exactly specific loci are made accessible to the machinery involved in recombination remains to be determined. It is likely that the accessibility of the Ig and TCR loci to the enzymes that mediate recombination is regulated in developing B and T cells by several mechanisms, including alterations in chromatin structure, DNA methylation, and basal transcriptional activity in the gene loci.

The process of V(D)J recombination can be divided into four distinct events that flow sequentially from one to the next (Fig. 8-12):

1. *Synapsis:* Portions of the chromosome on which the antigen receptor gene is located are made accessible to the recombination machinery. Two selected coding segments and their adjacent RSSs are brought together by a chromosomal looping event and held in position for subsequent cleavage, processing, and joining.
2. *Cleavage:* Double-stranded breaks are enzymatically generated at RSS-coding sequence junctions by

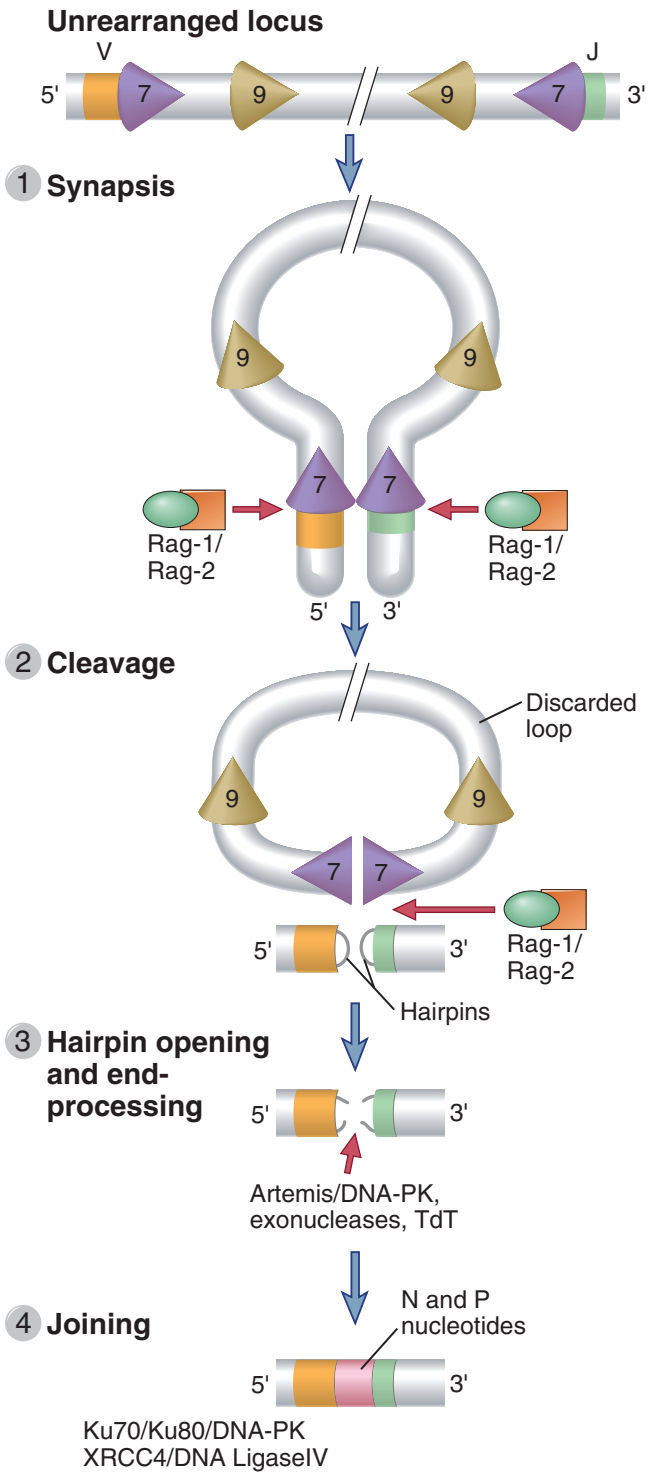


FIGURE 8–12 Sequential events during V(D)J recombination. Synapsis and cleavage of DNA at the heptamer/coding segment boundary are mediated by Rag-1 and Rag-2. The coding end hairpin is opened by the Artemis endonuclease, and broken ends are repaired by the NHEJ machinery.

machinery that is lymphoid specific. Two proteins encoded by lymphoid-specific genes, called ***recombination-activating gene 1*** and ***recombination-activating gene 2*** (*Rag-1* and *Rag-2*), form a tetrameric complex that plays an essential role in V(D)J recombination. The Rag-1/Rag-2 complex is also known as the **V(D)J recombinase**. The Rag-1 protein, in a manner similar to a restriction endonuclease, recognizes the DNA sequence at the junction between a heptamer and a coding segment and cleaves it, but it is enzymatically active only when complexed with the Rag-2 protein. The Rag-2 protein may help link the Rag-1/Rag-2 tetramer to other proteins, including accessibility factors that bring these proteins to specific "open" receptor gene loci at specific times and at defined stages of lymphocyte development. Rag-1 and Rag-2 contribute to holding together gene segments during the process of chromosomal folding or synapsis. Rag-1 then makes a nick (on one strand) between the coding end and the heptamer. The released 3′ OH of the coding end then attacks a phosphodiester bond on the other strand, forming a covalent hairpin. The signal end (including the heptamer and the rest of the RSS) does not form a hairpin and is generated as a blunt double-stranded DNA terminus that undergoes no further processing. This double-stranded break results in a closed hairpin of one coding segment being held in apposition to the closed hairpin of the other coding end and two blunt recombination signal ends being placed next to each other. Rag-1 and Rag-2, apart from generating the double-stranded breaks, also hold the hairpin ends and the blunt ends together before the modification of the coding ends and the process of ligation.

Rag genes are lymphoid specific and are expressed only in developing B and T cells. Rag proteins are expressed mainly in the G_0 and G_1 stages of the cell cycle and are inactivated in proliferating cells. It is thought that limiting DNA cleavage and recombination to these stages minimizes the risk of generating inappropriate DNA breaks during DNA replication or during mitosis.

3. *Hairpin opening and end-processing:* The broken coding ends (but not the signal/RSS ends) are modified by the addition or removal of bases, and thus greater diversity is generated. After the formation of double-stranded breaks, hairpins must be resolved (opened up) at the coding junctions, and bases may be added to or removed from the coding ends to ensure even greater diversification. *Artemis* is an endonuclease that opens up the hairpins at the coding ends. In the absence of Artemis, hairpins cannot be opened, and mature T and B cells cannot be generated. A rare immunodeficiency characterized by the absence of T and B cells is caused by mutations in *Artemis* (see Chapter 20). A lymphoid-specific enzyme, called terminal deoxynucleotidyl transferase (TdT), adds bases to broken DNA ends and will be discussed later in this chapter in the context of junctional diversity.
4. *Joining:* The broken coding ends as well as the signal ends are brought together and ligated by a double-stranded break repair process found in all cells that is called nonhomologous end joining. A number of ubiquitous factors participate in nonhomologous end joining. Ku70 and Ku80 are DNA end-binding proteins that bind to the breaks and recruit the catalytic subunit of DNA-dependent protein kinase (DNA-PK),

a double-stranded DNA repair enzyme. This enzyme is defective in mice carrying the severe combined immunodeficiency *(scid)* mutation (see Chapter 20). Like *Rag*-deficient mice, *scid* mice fail to produce mature lymphocytes. DNA-PK also phosphorylates and activates Artemis, which, as mentioned before, is involved in end processing. Ligation of the processed broken ends is mediated by DNA ligase IV and XRCC4, the latter being a noncatalytic but essential subunit of this ligase.

Generation of Diversity in B and T Cells

The enormous diversity of the B and T cell repertoires is created not only by random combinations of germline gene segments being brought together but also by random addition or deletion of sequences at the junctions between segments that have been united. Several genetic mechanisms contribute to this diversity, and the relative importance of each mechanism varies among the different antigen receptor loci (Table 8-1). Not discussed here is somatic hypermutation, a mechanism that involves point mutations and other changes in DNA in activated B cells, which will be considered in Chapter 11.

- **Combinatorial diversity.** V(D)J rearrangement brings together multiple germline gene segments that may combine randomly, and different combinations produce different antigen receptors. The maximum possible number of combinations of these gene segments is the product of the numbers of V, J, and (if present) D gene segments at each antigen receptor locus. Therefore, the amount of combinatorial diversity that can be generated at each locus reflects the number of germline V, J, and D gene segments at that locus. After synthesis of antigen receptor proteins, combinatorial diversity is further enhanced by the juxtaposition of two different, randomly generated V regions (i.e., V_H and V_L in Ig molecules and V_α and V_β in TCR molecules). Therefore, the total combinatorial diversity is theoretically the product of the combinatorial diversity of each of the two associating chains. The actual degree of combinatorial diversity in the expressed Ig and TCR repertoires in any individual is likely to be considerably less than the theoretical maximum. This is because not all recombinations of gene segments are equally likely to occur, and not all pairings of Ig heavy and light chains or TCR α and β chains may form functional antigen receptors. Importantly, because the numbers of V, D, and J segments in each locus are limited (see Table 8-1), the maximum possible numbers of combinations are on the order of thousands. This is, of course, much less than the actual diversity of antigen receptors in mature lymphocytes.
- **Junctional diversity.** The largest contribution to the diversity of antigen receptors is made by the removal or addition of nucleotides at the junctions of the V and D, D and J, or V and J segments at the time these segments are joined. One way in which this can occur is if endonucleases remove nucleotides from the germline sequences at the ends of the recombining gene segments. In addition, new nucleotide sequences, not present in the germline, may be added at junctions. As described earlier, coding segments (e.g., V and J gene segments) that are cleaved by Rag-1 form hairpin loops whose ends are often cleaved asymmetrically by the enzyme Artemis so that one DNA strand is longer than the other (Fig. 8-13). The shorter strand has to be extended with nucleotides complementary to the longer strand before the ligation of the two segments. The short lengths of added nucleotides are called P nucleotides, and their templated addition introduces new sequences at the V-D-J junctions. Another mechanism of junctional diversity is the random addition of up to 20 non–template-encoded nucleotides called N nucleotides (see Fig. 8-13). N region diversification is more common in Ig heavy chains and in TCR β and γ chains than in Ig κ or λ chains. This addition of new nucleotides is mediated by the enzyme **terminal deoxynucleotidyl transferase (TdT)**. In mice rendered deficient in TdT by gene knockout, the diversity of B and T cell repertoires is substantially less than in normal mice. The addition of P nucleotides and N nucleotides at the recombination sites may introduce

TABLE 8–1 Contributions of Different Mechanisms to the Generation of Diversity in Ig and TCR Genes

	Ig		TCR αβ		TCR γδ	
Mechanism	**Heavy Chain**	**κ**	**α**	**β**	**γ**	**δ**
Variable (V) segments	45	35	45	50	5	2
Diversity (D) segments	23	0	0	2	0	3
D segments read in all three reading frames	Rare	—	—	Often	—	Often
N region diversification	V-D, D-J	None	V-J	V-D, D-J	V-J	V-D1, D1-D2, D1-J
Joining (J) segments	6	5	55	12	5	4
Total potential repertoire with junctional diversity	$\sim 10^{11}$		$\sim 10^{16}$		$\sim 10^{18}$	

The potential number of antigen receptors with junctional diversity is much greater than the number that can be generated only by combinations of V, D, and J gene segments. Note that although the upper limit on the numbers of Ig and TCR proteins that may be expressed is very large, it is estimated that each individual contains on the order of 10^7 clones of B and T cells with distinct specificities and receptors; in other words, only a fraction of the potential repertoire may actually be expressed.

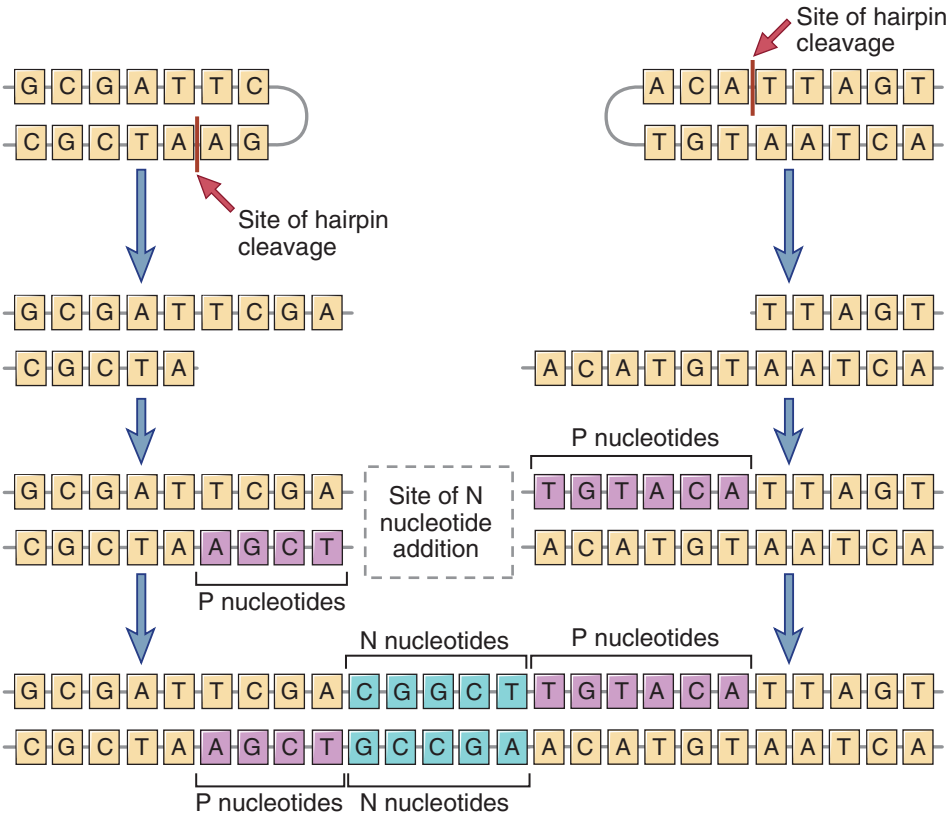


FIGURE 8–13 Junctional diversity. During the joining of different gene segments, addition or removal of nucleotides may lead to the generation of novel nucleotide and amino acid sequences at the junction. Nucleotides (P sequences) may be added to asymmetrically cleaved hairpins in a templated manner. Other nucleotides (N regions) may be added to the sites of VD, VJ, or DJ junctions in a nontemplated manner by the action of the enzyme TdT. These additions generate new sequences that are not present in the germline.

frameshifts, theoretically generating termination codons in two of every three joining events. These genes cannot produce functional proteins, but such inefficiency is the price that is paid for generating diversity.

Because of junctional diversity, antibody and TCR molecules show the greatest variability at the junctions of V and C regions, which form the third hypervariable region, or CDR3 (see Fig. 8-6). In fact, because of junctional diversity, the numbers of different amino acid sequences that are present in the CDR3 regions of Ig and TCR molecules are much greater than the numbers that can be encoded by germline gene segments. The CDR3 regions of Ig and TCR molecules are also the most important portions of these molecules for determining the specificity of antigen binding (see Chapters 5 and 7). Thus, the greatest diversity in antigen receptors is concentrated in the regions of the receptors that are the most important for antigen binding.

Although the theoretical limit of the number of Ig and TCR proteins that can be produced is enormous (see Table 8-1), the actual number of antigen receptors on B or T cells expressed in each individual is probably on the order of only 10^7. This may reflect the fact that most receptors, which are generated by random DNA recombination, do not pass the selection processes needed for maturation.

A practical application of our knowledge of junctional diversity is the determination of the clonality of lymphoid tumors that have arisen from B or T cells. This laboratory test is commonly used to distinguish monoclonal tumors from polyclonal proliferations of lymphocytes in response to some external stimulus. Because every lymphocyte clone expresses a unique antigen receptor CDR3 region, the sequence of nucleotides at the V(D)J recombination site serves as a specific marker for each clone. Thus, by measuring the length of the junctional regions of Ig or TCR genes in different B or T cell lesions by polymerase chain reaction assays, one can establish whether these lesions arose from a single clone (indicating a tumor) or independently from different clones (implying non-neoplastic proliferation of lymphocytes). The same method may be used to identify small numbers of tumor cells in the blood or tissues.

With this background, we proceed to a discussion of B lymphocyte development and then the maturation of T cells.

B LYMPHOCYTE DEVELOPMENT

The principal events during the maturation of B lymphocytes are the rearrangement and expression of Ig genes in a precise order, selection and proliferation of developing

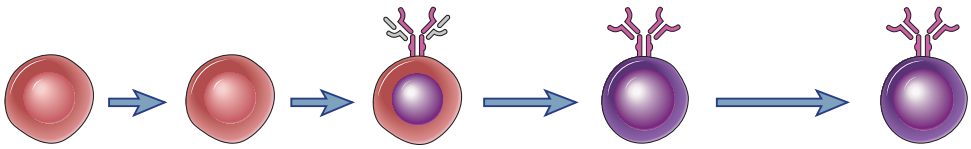
B cells at the pre-antigen receptor checkpoint, and selection of the mature B cell repertoire. Before birth, B lymphocytes develop from committed precursors in the fetal liver, and after birth, B cells are generated in the bone marrow. The majority of B lymphocytes arise from adult bone marrow progenitors that are initially Ig negative, develop into immature B cells that express membrane-bound IgM molecules, and then leave the bone marrow to mature further primarily in the spleen. In the spleen, cells that develop into follicular B cells express IgM and IgD on the cell surface and acquire the ability to recirculate and populate all peripheral lymphoid organs. These follicular B cells home to lymphoid follicles and are able to recognize foreign antigens and to respond to them. The development of a mature B cell from a lymphoid progenitor is estimated to take 2 to 3 days in humans.

Stages of B Lymphocyte Development

During their maturation, cells of the B lymphocyte lineage go through distinguishable stages, each characterized by distinct cell surface markers and a specific pattern of Ig gene expression (Fig. 8-14). The major stages and the events in each are described next.

The Pro-B and Pre-B Stages of B Cell Development

The earliest bone marrow cell committed to the B cell lineage is called a **pro-B cell**. Pro-B cells do not produce Ig, but they can be distinguished from other immature cells by the expression of B lineage–restricted surface molecules such as CD19 and CD10. Rag proteins are first expressed at this stage, and the first recombination of Ig genes occurs at the heavy chain locus. This recombination brings together one D and one J gene segment, with deletion of the intervening DNA (Fig. 8-15A). The D segments that are 5′ of the rearranged D segment and the J segments that are 3′ of the rearranged J segment are not affected by this recombination (e.g., D1 and J2 to J6 in Fig. 8-15A). After the D-J recombination event, one of the many 5′ V genes is joined to the DJ unit, giving rise to a rearranged VDJ exon. At this stage, all V and D segments between the rearranged V and D genes are also deleted. V-to-DJ recombination at the Ig H chain locus occurs only in committed B lymphocyte precursors and is a critical event in Ig expression because only the rearranged V gene is subsequently transcribed. The TdT enzyme, which catalyzes the nontemplated addition of junctional N nucleotides, is expressed most abundantly during the pro-B stage when VDJ recombination occurs at the Ig H locus and levels of TdT decrease before light chain gene V-J recombination is complete. Therefore, junctional diversity attributed to addition of N nucleotides is more prominent in rearranged heavy chain genes than in light chain genes. The heavy chain C region exons remain separated from the VDJ complex by DNA containing the distal J segments and the J-C



Stage of maturation	Stem cell	Pro-B	Pre-B	Immature B	Mature B
Proliferation					
RAG expression					
TdT expression					
Ig DNA, RNA	Unrecombined (germline) DNA	Unrecombined (germline) DNA	Recombined H chain gene (VDJ); μ mRNA	Recombined H chain gene (VDJ), κ or λ genes (VJ); μ or κ or λ mRNA	Alternative splicing of VDJ-C RNA (primary transcript), to form C_μ and C_δ mRNA
Ig expression	None	None	Cytoplasmic μ and pre-B receptor–associated μ	Membrane IgM (μ+ κ or λ light chain)	Membrane IgM and IgD
Surface markers	$CD43^+$	$CD43^+$ $CD19^+$ $CD10^+$	$B220^{lo}$ $CD43^+$	IgM^{lo} $CD43^-$	IgM^{hi}
Anatomic site	Bone marrow			Periphery	
Response to antigen	None	None	None	Negative selection (deletion), receptor editing	Activation (proliferation and differentiation)

FIGURE 8–14 Stages of B cell maturation. Events corresponding to each stage of B cell maturation from a bone marrow stem cell to a mature B lymphocyte are illustrated. Several surface markers in addition to those shown have been used to define distinct stages of B cell maturation.

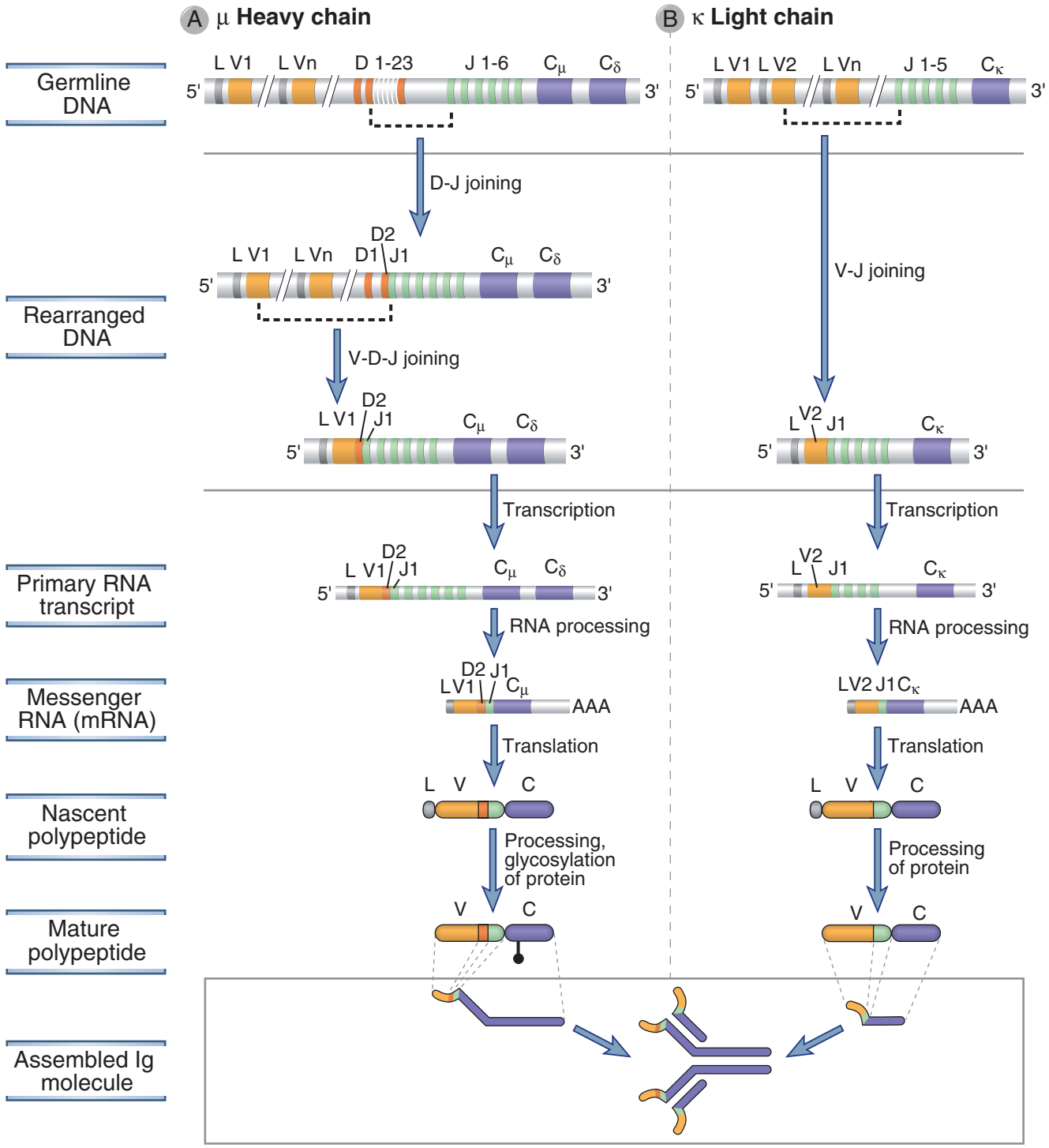


FIGURE 8–15 Ig heavy and light chain gene recombination and expression. The sequence of DNA recombination and gene expression events is shown for the Ig μ heavy chain **(A)** and the Ig κ light chain **(B)**. In the example shown in **A,** the V region of the μ heavy chain is encoded by the exons V1, D2, and J1. In the example shown in **B,** the V region of the κ chain is encoded by the exons V2 and J1.

intron. The rearranged Ig heavy chain gene is transcribed to produce a primary transcript that includes the rearranged VDJ complex and the C_μ exons. The C_μ nuclear RNA is cleaved downstream of one of two consensus polyadenylation sites, and multiple adenine nucleotides, called poly-A tails, are added to the 3′ end. This nuclear RNA undergoes splicing, an RNA processing event in which the introns are removed and exons joined together. In the case of the μ RNA, introns between the leader exon and the VDJ exon, between the VDJ exon and the first exon of the C_μ locus, and between each of the subsequent constant region exons of C_μ are removed, thus giving rise to a spliced mRNA for the μ heavy chain. If the mRNA is derived from an Ig locus

at which rearrangement was productive, translation of the rearranged μ heavy chain mRNA leads to synthesis of the μ protein. For a rearrangement to be productive (in the correct reading frame), bases must be added or removed at junctions in multiples of three. This ensures that the rearranged Ig gene will be able to correctly encode an Ig protein. Approximately half of all pro-B cells make productive rearrangements at the Ig H locus on at least one chromosome and can thus go on to synthesize the μ heavy chain protein. Only cells that make productive rearrangements survive and differentiate further.

Once a productive Ig μ rearrangement is made, a cell ceases to be called a pro-B cell and has differentiated into the pre-B stage. **Pre-B cells** are developing B lineage cells that express the Ig μ protein but have yet to rearrange their light chain loci. The pre-B cell expresses the μ heavy chain on the cell surface, in association with other proteins, as the pre-B cell receptor, which has several important roles in B cell maturation.

The Pre-B Cell Receptor

Complexes of μ, surrogate light chains, and signal-transducing proteins called Igα and Igβ form the pre-antigen receptor of the B lineage, known as the pre-B cell receptor (pre-BCR). The μ heavy chain associates with the λ5 and V pre-B proteins, also called **surrogate light chains** because they are structurally homologous to κ and λ light chains but are invariant (i.e., they are identical in all pre-B cells) and are synthesized only in pro-B and pre-B cells (Fig. 8-16A). Igα and Igβ also form part of the B cell receptor in mature B cells (see Chapter 7). Signals from the pre-BCR drive the pro-B to pre-B transition and are responsible for the largest proliferative expansion of B lineage cells in the bone marrow. It is not known what the pre-BCR recognizes; the consensus view at present is that this receptor functions in a ligand-independent manner and that it is activated by the process of assembly (i.e., the fully assembled receptor is in the "on" mode). The importance of pre-BCRs is illustrated by studies of knockout mice and rare cases of

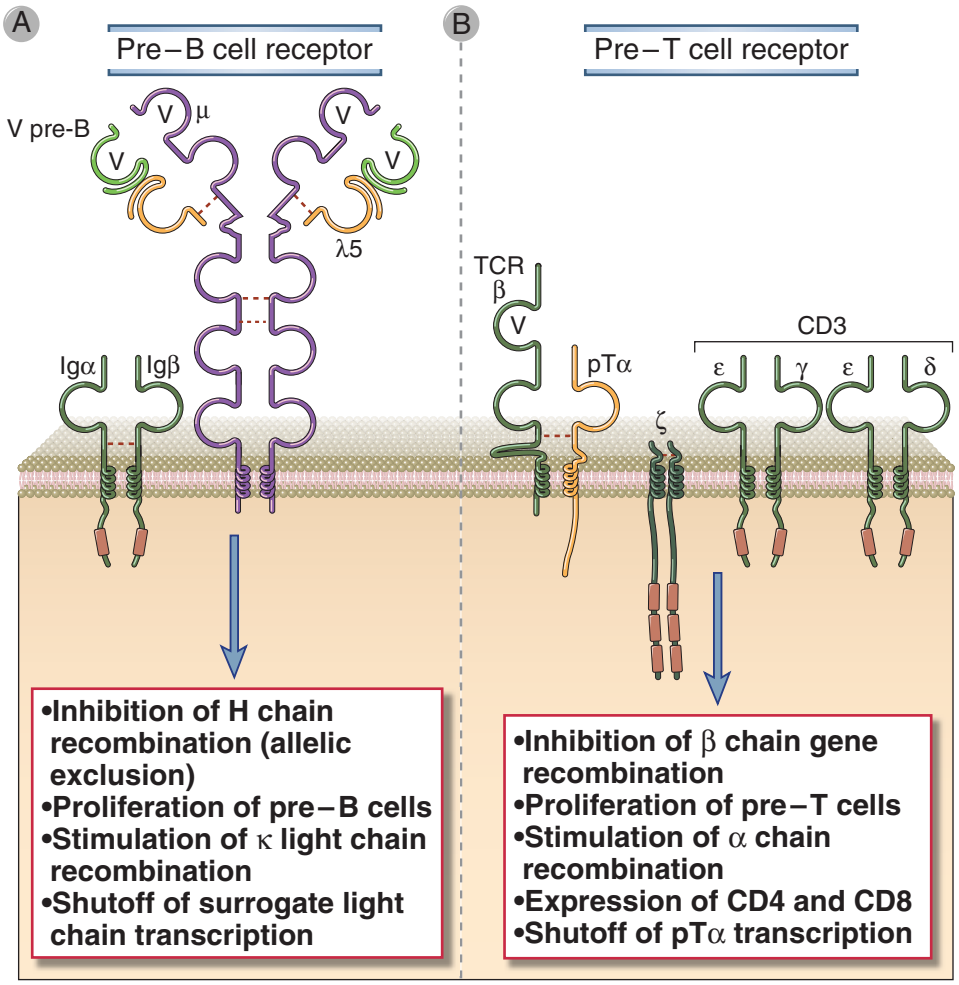


FIGURE 8–16 Pre-B cell and pre-T cell receptors. The pre-B cell receptor **(A)** and the pre-T cell receptor **(B)** are expressed during the pre-B and pre-T cell stages of maturation, respectively, and both receptors share similar structures and functions. The pre-B cell receptor is composed of the μ heavy chain and an invariant surrogate light chain. The surrogate light chain is composed of two proteins, the V pre-B protein, which is homologous to a light chain V domain, and a λ5 protein that is covalently attached to the μ heavy chain by a disulfide bond. The pre-T cell receptor is composed of the TCR β chain and the invariant pre-T α (pTα)chain. The pre-B cell receptor is associated with the Igα and Igβ signaling molecules that are part of the BCR complex in mature B cells (see Chapter 9), and the pre-T cell receptor associates with the CD3 and ζ proteins that are part of the TCR complex in mature T cells (see Chapter 7).

human deficiencies of these receptors. For instance, in mice, knockout of the gene encoding the μ chain or one of the surrogate light chains results in markedly reduced numbers of mature B cells because development is blocked at the pro-B stage.

The expression of the pre-BCR is the first checkpoint in B cell maturation. Numerous signaling molecules linked to both the pre-BCR and the BCR are required for cells to successfully negotiate the pre-BCR–mediated checkpoint at the pro-B to pre-B cell transition. A kinase called Bruton's tyrosine kinase (Btk) is activated downstream of the pre-BCR and is required for delivery of signals from this receptor that mediate survival, proliferation, and maturation at and beyond the pre-B cell stage. In humans, mutations in the *BTK* gene result in the disease called **X-linked agammaglobulinemia (XLA)**, which is characterized by a failure of B cell maturation (see Chapter 20). In mice, mutations in *btk* result in a less severe B cell defect in a mouse strain called *Xid* (for X-linked immunodeficiency). The defect is less severe than in XLA because murine pre-B cells express a second Btk-like kinase called Tec that compensates for the defective Btk.

The pre-BCR regulates further rearrangement of Ig genes in two ways. First, if a μ protein is produced from the recombined heavy chain locus on one chromosome and forms a pre-BCR, this receptor signals to irreversibly inhibit rearrangement of the Ig heavy chain locus on the other chromosome. If the first rearrangement is nonproductive, the heavy chain allele on the other chromosome can complete VDJ rearrangement at the Ig H locus. Thus, in any B cell clone, one heavy chain allele is productively rearranged and expressed, and the other is either retained in the germline configuration or nonproductively rearranged. As a result, an individual B cell can express Ig heavy chain proteins encoded by only one of the two inherited alleles. This phenomenon is called **allelic exclusion**, and it ensures that every B cell will express a single receptor, thus maintaining clonal specificity. If both alleles undergo nonproductive Ig H gene rearrangements, the developing cell cannot produce Ig heavy chains, cannot generate a pre-BCR–dependent survival signal, and thus undergoes programmed cell death. Ig heavy chain allelic exclusion involves changes in chromatin structure in the heavy chain locus that limit accessibility to the V(D)J recombinase.

The second way in which the pre-BCR regulates the production of the antigen receptor is by stimulating κ light chain gene rearrangement. However, μ chain expression is not absolutely required for light chain gene recombination, as shown by the finding that knockout mice lacking the μ gene do initiate light chain gene rearrangements in some developing B cells (which, of course, cannot express functional antigen receptors and proceed to maturity). The pre-BCR also contributes to the inactivation of surrogate light chain gene expression as pre-B cells mature.

Immature B Cells

Following the pre-B cell stage, each developing B cell initially rearranges a κ light chain gene, and if the rearrangement is in-frame, it will produce a κ light chain protein, which associates with the previously synthesized μ chain to produce a complete IgM protein. If the κ locus is not productively rearranged, the cell can rearrange the λ locus and again produce a complete IgM molecule. (Induction of λ light chain gene rearrangement occurs mainly when Ig κ-expressing B cell receptors are self-reactive, as will be discussed later). The IgM-expressing B cell is called an **immature B cell**. DNA recombination in the κ light chain locus occurs in a similar manner as in the Ig heavy chain locus (see Fig. 8-15B). There are no D segments in the light chain loci, and therefore recombination involves only the joining of one V segment to one J segment, forming a VJ exon. This VJ exon remains separated from the C region by an intron, and this separation is retained in the primary RNA transcript. Splicing of the primary transcript results in the removal of the intron between the VJ and C exons and generates an mRNA that is translated to produce the κ or λ protein. In the λ locus, alternative RNA splicing may lead to the use of any one of the four functional C_λ exons, but there is no known functional difference between the resulting types of λ light chains. Production of a κ protein prevents λ rearrangement, and, as stated before, λ rearrangement occurs only if the κ rearrangement was nonproductive or if a self-reactive rearranged κ light chain is deleted. As a result, an individual B cell clone can express only one of the two types of light chains; this phenomenon is called **light chain isotype exclusion**. As in the heavy chain locus, expression of κ or λ is allelically excluded and is initiated from only one of the two parental chromosomes at any given time. Also, as for heavy chains, if both alleles of both κ and λ chains are nonfunctional in a developing B cell, that cell fails to receive survival signals that are normally generated by the BCR and dies.

The assembled IgM molecules are expressed on the cell surface in association with Igα and Igβ, where they function as specific receptors for antigens. In cells that are not strongly self-reactive, the BCR provides ligand-independent tonic signals that keep the B cell alive and also mediate the shutoff of *Rag* gene expression, thus preventing further Ig gene rearrangement. Immature B cells do not proliferate and differentiate in response to antigens. In fact, if they recognize antigens in the bone marrow with high avidity, which may occur if the B cells express receptors for multivalent self antigens that are present in the bone marrow, the B cells may undergo receptor editing or cell death, as described later. These processes are important for the negative selection of strongly self-reactive B cells. Immature B cells that are not strongly self-reactive leave the bone marrow and complete their maturation in the spleen before migrating to other peripheral lymphoid organs.

Subsets of Mature B Cells

Distinct subsets of B cells develop from different progenitors (Fig. 8-17). Fetal liver–derived HSCs are the precursors of B-1 B cells, described later. Bone marrow–derived HSCs give rise to the majority of B cells, which are sometimes called B-2 B cells. These cells rapidly pass through two transitional stages and can commit to development either into **marginal zone B cells**, also described later, or into **follicular B cells**. The affinity of the B

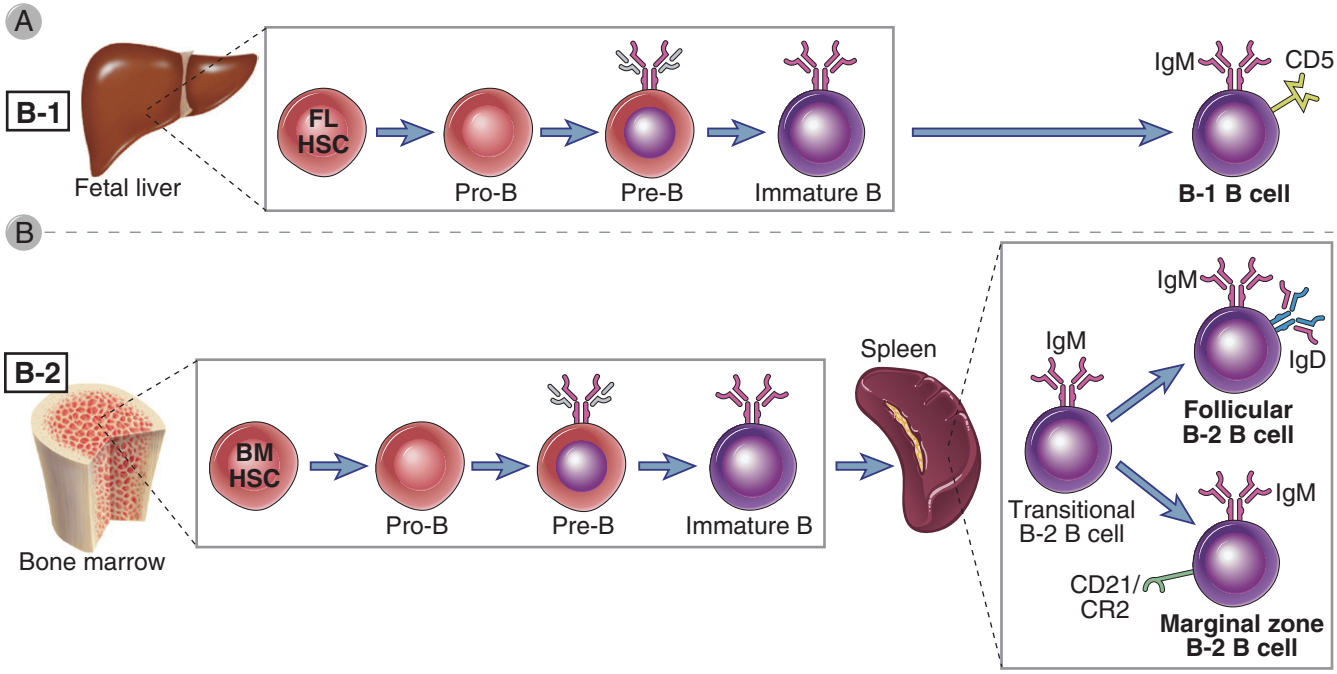


FIGURE 8–17 B lymphocyte subsets. A, Most B cells that develop from fetal liver–derived stem cells differentiate into the B-1 lineage. **B,** B lymphocytes that arise from bone marrow precursors after birth give rise to the B-2 lineage. Two major subsets of B lymphocytes are derived from B-2 B cell precursors. Follicular B cells are recirculating lymphocytes; marginal zone B cells are abundant in the spleen in rodents but can also be found in lymph nodes in humans.

cell receptor for self antigens contributes to whether a maturing B-2 B cell will differentiate into a follicular or a marginal zone B cell. This cell fate decision represents a positive selection event in B lymphocytes that is linked to lineage commitment.

Follicular B Cells

Most mature B cells belong to the follicular B cell subset and produce IgD in addition to IgM. Each of these B cells coexpresses μ and δ heavy chains using the same VDJ exon to generate the V domain and in association with the same κ or λ light chain to produce two membrane receptors with the same antigen specificity. Simultaneous expression in a single B cell of the same rearranged VDJ exon on two transcripts, one including C_μ exons and the other C_δ exons, is achieved by alternative RNA splicing (Fig. 8-18). A long primary RNA transcript is produced containing the rearranged VDJ unit as well as the C_μ and C_δ genes. If the primary transcript is cleaved and polyadenylated after the μ exons, introns are spliced out such that the VDJ exon is contiguous with C_μ exons; this results in the generation of a μ mRNA. If, however, the VDJ complex is not linked to C_μ exons but is spliced to C_δ exons, a δ mRNA is produced. Subsequent translation results in the synthesis of a complete μ or δ heavy chain protein. Thus, selective polyadenylation and alternative splicing allow a B cell to simultaneously produce mature mRNAs and proteins of two different heavy chain isotypes. The precise mechanisms that regulate the choice of polyadenylation or splice acceptor sites by which the rearranged VDJ is joined to either C_μ or C_δ are poorly understood, as are the signals that determine when and

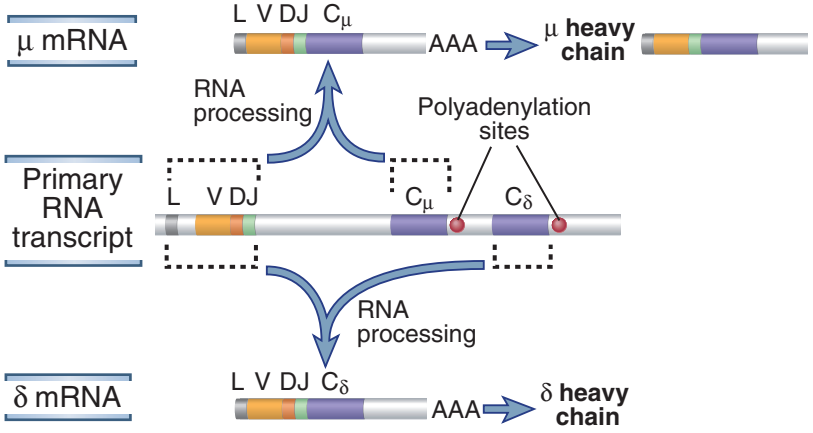


FIGURE 8–18 Coexpression of IgM and IgD. Alternative processing of a primary RNA transcript results in the formation of a μ or δ mRNA. Dashed lines indicate the H chain segments that are joined by RNA splicing.

why a B cell expresses both IgM and IgD rather than IgM alone. The coexpression of IgM and IgD is accompanied by the ability to recirculate and the acquisition of functional competence, and this is why IgM^+IgD^+ B cells are also called **mature B cells**. This correlation between expression of IgD and acquisition of functional competence has led to the suggestion that IgD is the essential activating receptor of mature B cells. However, there is no evidence for a functional difference between membrane IgM and membrane IgD. Moreover, knockout of the Ig δ gene in mice does not have a significant impact on the maturation or antigen-induced responses of B cells. Follicular B cells are also often called recirculating B cells because they migrate from one lymphoid organ to the next, residing in specialized niches known as B cell follicles (see Chapter 2). In these niches, these B cells are maintained, in part, by survival signals delivered by a cytokine of the tumor necrosis factor (TNF) family called BAFF or BlyS (see Chapter 11).

Mature, naive B cells are responsive to antigens, and unless the cells encounter antigens that they recognize with high affinity and respond to, they die in a few months. In Chapter 11, we will discuss how these cells respond to antigens and how the pattern of Ig gene expression changes during antigen-induced B cell differentiation.

B-1 and Marginal Zone B Cells

A subset of B lymphocytes, called B-1 B cells, differs from the majority of B lymphocytes and develops in a unique manner. These cells develop from fetal liver–derived HSCs and are best defined in rodents. Most murine B-1 cells express the CD5 (Ly-1) molecule. In the adult, large numbers of B-1 cells are found as a self-renewing population in the peritoneum and mucosal sites. B-1 cells develop earlier during ontogeny than conventional B cells do, express a relatively limited repertoire of V genes, and exhibit far less junctional diversity than conventional B cells do (because TdT is not expressed in the fetal liver). B-1 cells as well as marginal zone B cells spontaneously secrete IgM antibodies that often react with microbial polysaccharides and lipids. These antibodies are sometimes called natural antibodies because they are present in individuals without overt immunization, although it is possible that microbial flora in the gut are the source of antigens that stimulate their production. B-1 cells contribute to rapid antibody production against microbes in particular tissues, such as the peritoneum. At mucosal sites, as many as half the IgA-secreting cells in the lamina propria may be derived from B-1 cells. B-1 B cells are analogous to γδ T cells in that they both have antigen receptor repertoires of limited diversity, and they are both presumed to respond to commonly encountered microbial antigens early in immune responses.

The major marker used to delineate murine B-1 cells is CD5. In humans, B-1–like cells have been described but these cells do not express CD5. In humans, CD5 is found on transitional B cells and some activated B cell populations.

Marginal zone B cells are located primarily in the vicinity of the marginal sinus in the spleen and are similar to B-1 cells in terms of their limited diversity and their ability to respond to polysaccharide antigens and to generate natural antibodies. Marginal zone B cells exist in both mice and humans and express IgM and the CD21 coreceptor. In mice, marginal zone B cells exist only in the spleen, whereas in humans, they can be found in the spleen as well as in lymph nodes. Marginal zone B cells respond very rapidly to blood-borne microbes and differentiate into short-lived IgM-secreting plasma cells. Although they generally mediate T cell–independent humoral immune responses to circulating pathogens, marginal zone B cells also appear capable of mediating some T cell–dependent immune responses.

Selection of the Mature B Cell Repertoire

The repertoire of mature B cells is positively selected from the pool of immature B cells. As we shall see later, positive selection is well defined in T lymphocytes and is responsible for matching the TCRs on newly generated $CD8^+$ and $CD4^+$ T cells with their ability to recognize self MHC class I and MHC class II molecules, respectively. There is no comparable restriction for B cell antigen recognition. Nevertheless, positive selection appears to be a general phenomenon primarily geared to identification of lymphocytes that have completed their antigen receptor gene rearrangement program successfully. It is believed that only B cells that express functional membrane Ig molecules receive constitutive BCR-derived survival signals, also known as "tonic" BCR signals. Tonic BCR signals mediate the shutoff of Rag gene expression in immature B cells and activate cell survival pathways in all B cells. Self antigens may influence the strength of the BCR signal and thereby the subsequent choice of peripheral B cell lineage during B cell maturation.

Immature B cells that recognize self antigens with high avidity may be induced to change their specificities by a process called **receptor editing**. In this process, antigen recognition leads to reactivation of *Rag* genes, additional light chain V-J recombination events, and production of a new Ig light chain, allowing the cell to express a different B cell receptor that is not self-reactive. Receptor editing generally is targeted at self-reactive κ light chain genes. VJ_κ exons encoding the variable domains of autoreactive light chains are deleted and replaced by new VJ_κ exons or by newly rearranged λ light chain genes. The new VJ_κ exon may be generated by the rearrangement of a V_κ gene upstream of the original V_κ gene that produced an autoreactive light chain to a J_κ segment downstream of the originally rearranged J_κ segment.

If receptor editing fails, the immature B cells that express high-affinity receptors for self antigens and encounter these antigens in the bone marrow or the spleen may die by apoptosis. This process is also called negative selection. The antigens mediating negative selection—usually abundant or polyvalent (e.g., membrane-bound) self antigens—deliver strong signals to IgM-expressing immature B lymphocytes that happen to express receptors specific for these self antigens. Both receptor editing and deletion are responsible for maintaining B cell tolerance to self antigens that are present in the bone marrow (see Chapter 14).

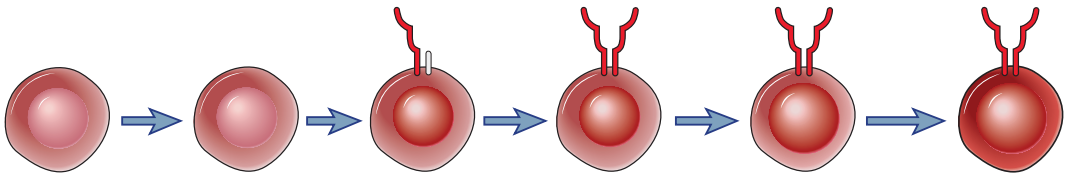
Once the transition is made to the IgD^+IgM^+ mature B cell stage, antigen recognition leads to proliferation and differentiation, not to receptor editing or apoptosis. As a result, mature B cells that recognize antigens with high affinity in peripheral lymphoid tissues are activated, and this process leads to humoral immune responses. Follicular B cells make most of the helper T cell–dependent antibody responses to protein antigens (see Chapter 11).

MATURATION OF T LYMPHOCYTES

The maturation of T lymphocytes from committed progenitors involves the sequential rearrangement and expression of TCR genes, cell proliferation, antigen-induced selection, and commitment to phenotypically and functionally distinct subsets (Fig. 8-19). In many ways, this is similar to B cell maturation. However, T cell maturation has some unique features that reflect the specificity of the majority of T lymphocytes for self MHC–associated peptide antigens and the need for a special microenvironment for selecting cells with this specificity.

Role of the Thymus in T Cell Maturation

The thymus is the major site of maturation of T cells. This function of the thymus was first suspected because of immunologic deficiencies associated with the lack of a thymus. The congenital absence of the thymus, as occurs in the DiGeorge syndrome in humans or in the nude mouse strain, is characterized by low numbers of mature T cells in the circulation and peripheral lymphoid tissues and severe deficiencies in T cell–mediated immunity (see Chapter 20). If the thymus is removed from a neonatal mouse, this animal fails to develop mature T cells. The thymic anlage develops from the endoderm of the third pharyngeal pouch and the underlying neural crest–derived mesenchyme and is subsequently populated by bone marrow–derived precursors. The thymus involutes with age and is virtually undetectable in postpubertal humans, resulting in a somewhat reduced output of mature T cells. However, maturation of T cells continues throughout adult life, as indicated by the successful reconstitution of the immune system in adult recipients of bone marrow transplants. It may be that the remnant of the involuted thymus is adequate for some T cell maturation. Because memory T cells have a long life span



Stage of maturation	Stem cell	Pro-T	Pre-T	Double positive	Single positive (immature T cell)	Naive mature T cell
Proliferation						
RAG expression						
TdT expression						
TCR DNA, RNA	Unrecombined (germline) DNA	Unrecombined (germline) DNA	Recombined β chain gene [V(D)J-C]; β chain mRNA	Recombined β, α chain genes [V(D)J-C]; β and α chain mRNA	Recombined β, α chain genes [V(D)J-C]; β and α chain mRNA	Recombined β, α chain genes [V(D)J-C]; β and α chain mRNA
TCR expression	None	None	Pre-T receptor (β chain/pre-T α)	Membrane αβ TCR	Membrane αβ TCR	Membrane αβ TCR
Surface markers	c-*kit*$^+$ $CD44^+$ $CD25^-$	c-*kit*$^+$ $CD44^+$ $CD25^+$	c-*kit*$^+$ $CD44^-$ $CD25^+$	$CD4^+CD8^+$ $TCR/CD3^{lo}$	$CD4^+CD8^-$ or $CD4^-CD8^+$ $TCR/CD3^{hi}$	$CD4^+CD8^-$ or $CD4^-CD8^+$ $TCR/CD3^{hi}$
Anatomic site	Bone marrow	Thymus				Periphery
Response to antigen	None	None	None	Positive and negative selection		Activation (proliferation and differentiation)

FIGURE 8–19 Stages of T cell maturation. Events corresponding to each stage of T cell maturation from a bone marrow stem cell to a mature T lymphocyte are illustrated. Several surface markers in addition to those shown have been used to define distinct stages of T cell maturation.

(perhaps longer than 20 years in humans) and accumulate with age, the need to generate new T cells decreases as individuals age.

T lymphocytes originate from precursors that arise in the fetal liver and adult bone marrow and seed the thymus. These precursors are multipotent progenitors that enter the thymus from the blood stream, crossing the endothelium of a postcapillary venule in the corticomedullary junction region of the thymus. In mice, immature lymphocytes are first detected in the thymus on the eleventh day of the normal 21-day gestation. This corresponds to about week 7 or 8 of gestation in humans. Developing T cells in the thymus are called thymocytes. The most immature thymocytes are found in the subcapsular sinus and outer cortical region of the thymus. From here, the thymocytes migrate into and through the cortex, where most of the subsequent maturation events occur. It is in the cortex that the thymocytes first express γδ and αβ TCRs. The αβ T cells mature into $CD4^+$ class II MHC–restricted or $CD8^+$ class I MHC–restricted T cells as they leave the cortex and enter the medulla. From the medulla, $CD4^+$ and $CD8^+$ single-positive thymocytes exit the thymus through the circulation. In the following sections, we discuss the maturation of αβ T cells; γδ T cells are discussed later in the chapter.

The thymic environment provides stimuli that are required for the proliferation and maturation of thymocytes. Many of these stimuli come from thymic cells other than the maturing T cells. Within the cortex, thymic cortical epithelial cells form a meshwork of long cytoplasmic processes, around which thymocytes must pass to reach the medulla. Epithelial cells of a distinct type known as thymic medullary epithelial cells are also present in the medulla. Bone marrow–derived dendritic cells are present at the corticomedullary junction and within the medulla, and macrophages are present primarily within the medulla. The migration of thymocytes through this anatomic arrangement allows physical interactions between the thymocytes and these other cells that are necessary for the maturation and selection of the T lymphocytes.

Two types of molecules produced by the nonlymphoid thymic cells are important for T cell maturation. The first are class I and class II MHC molecules, which are expressed on epithelial cells and dendritic cells in the thymus. The interactions of maturing thymocytes with these MHC molecules within the thymus are essential for the selection of the mature T cell repertoire, as we will discuss later. Second, thymic stromal cells, including epithelial cells, secrete cytokines and chemokines, which respectively stimulate the proliferation of immature T cells and orchestrate the cortical to medullary transit of developing αβ lineage thymocytes. The best defined of these cytokines is IL-7, which was mentioned earlier as a critical lymphopoietic growth factor. The movement of cells into and through the thymus is driven by chemokines. The progenitors of thymocytes express the chemokine receptor CCR9, which binds to the chemokine CCL25, which is produced in the thymic cortex. Entry of precursors into the thymus is dependent on CCL25 and CCR9. Chemokines such as CCL21 and CCL19, which are recognized by the CCR7 chemokine receptor on thymocytes, mediate the guided movement of developing T cells from the cortex to the medulla.

The rates of cell proliferation and apoptotic death are extremely high in cortical thymocytes. A single precursor gives rise to many progeny, and 95% of these cells die by apoptosis before reaching the medulla. The cell death is due to a combination of factors including a failure to productively rearrange the TCR β chain gene and thus to negotiate the pre-TCR/β selection checkpoint described later, a failure to be positively selected by MHC molecules in the thymus, and self antigen–induced negative selection (see Figs. 8-3 and 8-4). Cortical thymocytes are also sensitive to irradiation and glucocorticoids. In vivo, high doses of glucocorticoids induce apoptotic death of immature cortical thymocytes.

Stages of T Cell Maturation

During T cell maturation, there is a precise order in which TCR genes are rearranged and in which the TCR and CD4 and CD8 coreceptors are expressed (Fig. 8-20; see also Fig. 8-19). In the mouse, surface expression of the γδ TCR occurs first, 3 to 4 days after precursor cells first arrive in the thymus, and the αβ TCR is expressed 2 or 3 days later. In human fetal thymuses, γδ TCR expression begins at about 9 weeks of gestation, followed by expression of the αβ TCR at 10 weeks.

Double-Negative Thymocytes

The most immature cortical thymocytes, which are recent arrivals from the bone marrow, contain TCR genes in their germline configuration and do not express TCR, CD3, ζ chains, CD4, or CD8; these cells are called **double-negative thymocytes**. Thymocytes at this stage are also considered to be at the pro-T cell stage of maturation. The majority (>90%) of the double-negative thymocytes that survive thymic selection processes will ultimately give rise to αβ TCR–expressing, MHC-restricted $CD4^+$ and $CD8^+$ T cells, and the remainder of these thymocytes will give rise to γδ T cells. Rag-1 and Rag-2 proteins are first expressed at the pro-T stage of development and are required for the rearrangement of TCR genes. D_β-to-J_β rearrangements at the TCR β chain locus occur first; these involve either joining of the $D_\beta1$ gene segment to one of the six $J_\beta1$ segments or joining of the $D_\beta2$ segment to one of the six $J_\beta2$ segments (Fig. 8-21A). V_β-to-DJ_β rearrangements occur at the transition between the pro-T stage and the subsequent pre-T stage during αβ T cell development. The DNA sequences between the segments undergoing rearrangement, including D, J, and possibly $C_\beta1$ genes (if $D_\beta2$ and $J_\beta2$ segments are used), are deleted during this rearrangement process. The primary nuclear transcripts of the TCR β genes contain the intron between the recombined VDJ_β exon and the relevant C_β gene. Poly-A tails are added after cleavage of the primary transcript downstream of consensus polyadenylation sites located 3′ of the C_β region, and the sequences between the VDJ exon and C_β are spliced out to form a mature mRNA in which VDJ segments are juxtaposed to either of the two C_β genes (depending on which J segment was selected during the rearrangement process). Translation of this mRNA gives rise to a full-length TCR β chain

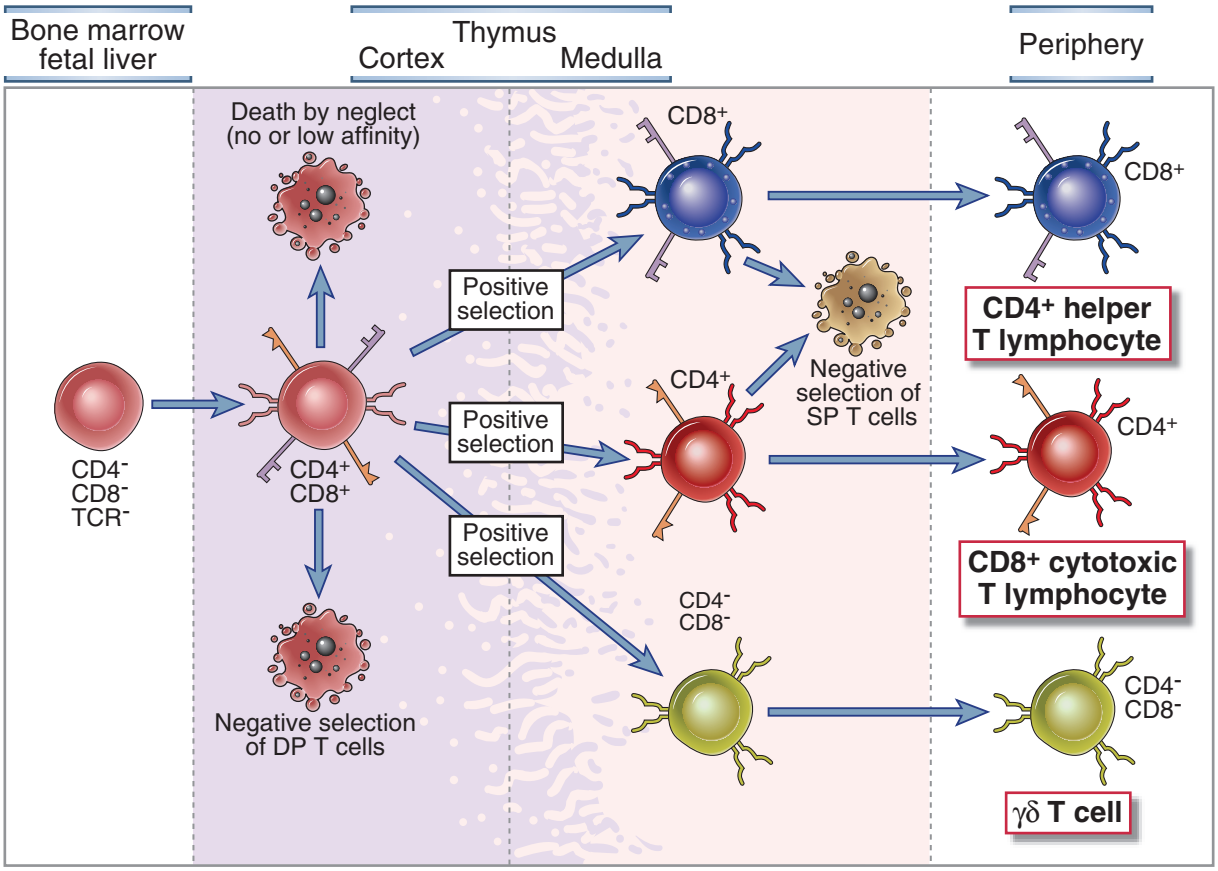


FIGURE 8–20 Maturation of T cells in the thymus. Precursors of T cells travel from the bone marrow through the blood to the thymus. In the thymic cortex, progenitors of αβ T cells express TCRs and CD4 and CD8 coreceptors. Selection processes eliminate self-reactive T cells in the cortex at the double-positive (DP) stage and also single-positive (SP) medullary thymocytes. They promote survival of thymocytes whose TCRs bind self MHC molecules with low affinity. Functional and phenotypic differentiation into $CD4^+CD8^-$ or $CD8^+CD4^-$ T cells occurs in the medulla, and mature T cells are released into the circulation.

protein. The two C_β genes appear to be functionally interchangeable, and there is no evidence that an individual T cell ever switches from one C gene to another. Furthermore, the use of either C_β gene segment does not influence the function or specificity of the TCR. The promoters in the 5′ flanking regions of V_β genes function together with a powerful enhancer that is located 3′ of the $C_\beta 2$ gene once V genes are brought close to the C gene by VDJ recombination. This proximity of the promoter to the enhancer is responsible for high-level T cell–specific transcription of the rearranged TCR β chain gene.

Pre-T Cell Receptor

If a productive (i.e., in-frame) rearrangement of the TCR β chain gene occurs in a given pro-T cell, the TCR β chain protein is expressed on the cell surface in association with an invariant protein called pre-Tα and with CD3 and ζ proteins to form the pre-T cell receptor (pre-TCR) (see Fig. 8-16B). The pre-TCR mediates the selection of the developing pre-T cells that productively rearrange the β chain of the TCR. Roughly half of all developing pre-T cells add or remove bases at rearrangement junctions that are multiples of three (on at least one TCR β chromosome), and therefore approximately half of all developing pre-T cells fail to express a TCR β protein. The function of the pre-TCR complex in T cell development is similar to that of the surrogate light chain–containing pre-BCR in B cell development. Signals from the pre-TCR mediate the survival of pre-T cells and contribute to the largest proliferative expansion during T cell development. Pre-TCR signals also initiate recombination at the TCR α chain locus and drive the transition from the double-negative to the double-positive stage of thymocyte development (discussed later). These signals also inhibit further rearrangement of the TCR β chain locus largely by limiting accessibility of the other allele to the recombination machinery. This results in β chain allelic exclusion (i.e., mature T cells express only one of the two inherited β chain alleles). As in pre-B cells, it is not known what, if any, ligand the pre-TCR recognizes. Pre-TCR signaling, like pre-BCR signaling, is generally believed to be initiated in a ligand-independent manner, dependent on the successful assembly of the pre-TCR complex. Pre-TCR signaling is mediated by a number of cytosolic kinases and adaptor proteins that are known also to be linked to TCR signaling (see Chapter 7). The essential function of the pre-TCR in T cell maturation has been demonstrated by numerous studies with genetically mutated mice, in which lack of any component of the pre-TCR complex (i.e., the TCR β chain, pre-Tα, CD3, ζ, or Lck) results in a block in the maturation of T cells at the double-negative stage.

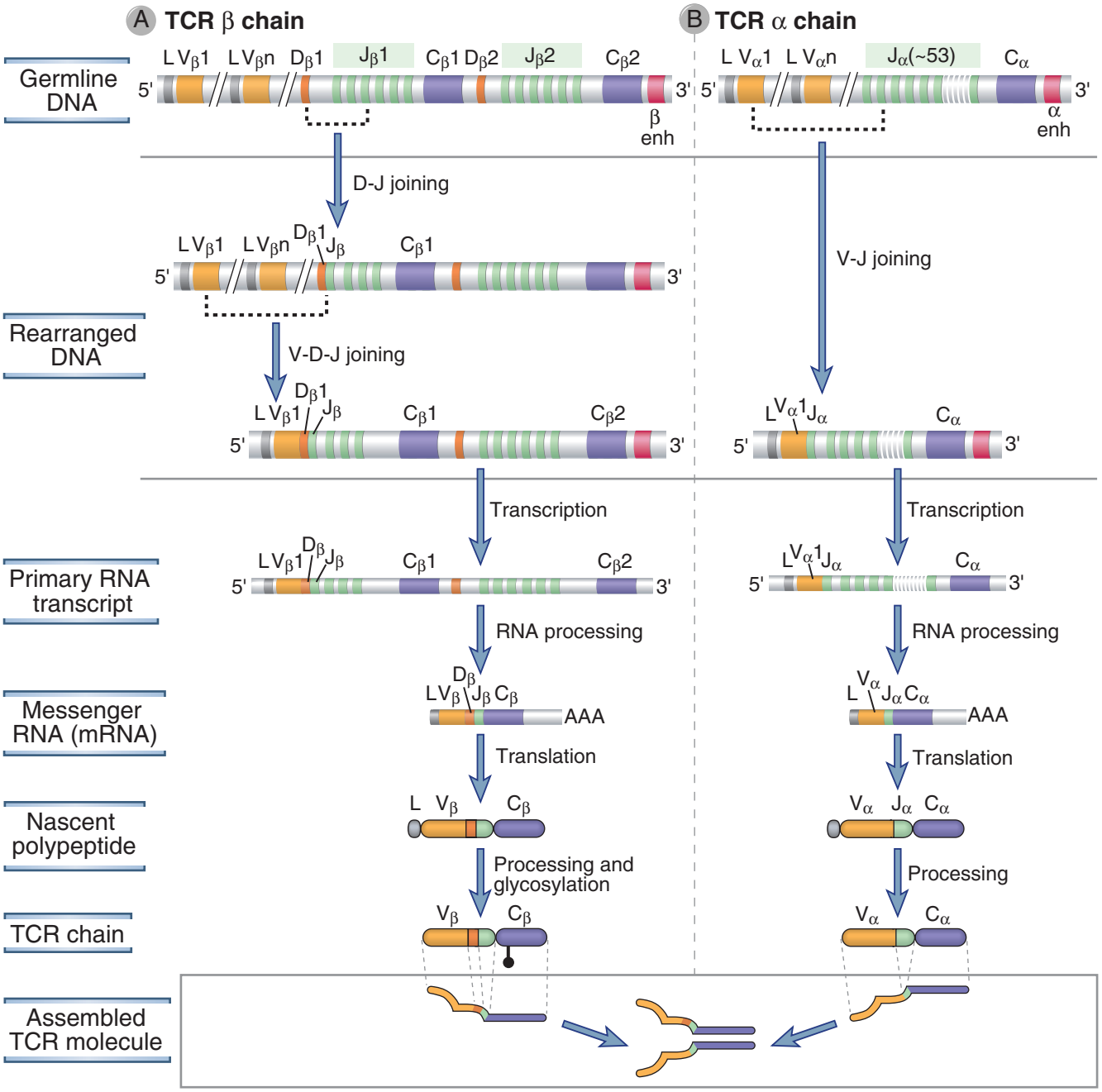


FIGURE 8–21 TCR α and β chain gene recombination and expression. The sequence of recombination and gene expression events is shown for the TCR β chain **(A)** and the TCR α chain **(B)**. In the example shown in **A,** the variable (V) region of the rearranged TCR β chain includes the $V_\beta1$ and $D_\beta1$ gene segments and the third J segment in the $J_\beta1$ cluster. The constant (C) region is encoded by the $C_\beta1$ exon. Note that at the TCR β chain locus, rearrangement begins with D-to-J joining followed by V-to-DJ joining. In humans, 14 J_β segments have been identified, and not all are shown in the figure. In the example shown in **B,** the V region of the TCR α chain includes the $V_\alpha1$ gene and the second J segment in the J_α cluster (this cluster is made up of at least 61 J_α segments in humans; not all are shown here).

Double-Positive Thymocytes

At the next stage of T cell maturation, thymocytes express both CD4 and CD8 and are called double-positive thymocytes. The expression of CD4 and CD8 is essential for subsequent selection events, discussed later. The rearrangement of the TCR α chain genes and the expression of TCR αβ heterodimers occur in the $CD4^+CD8^+$ double-positive population soon after cells cross the pre-TCR checkpoint (see Figs. 8-19 and 8-20). A second wave of *Rag* gene expression late in the pre-T stage promotes TCR α gene recombination. Because there are no D segments in the TCR α locus, rearrangement consists solely of the joining of V and J segments (see Fig. 8-21B). The large number of J_α segments permits multiple attempts at productive V-J joining on each chromosome, thereby increasing the probability that a functional αβ TCR will be produced. In contrast to the TCR β chain locus, where production of the protein and formation of the pre-TCR

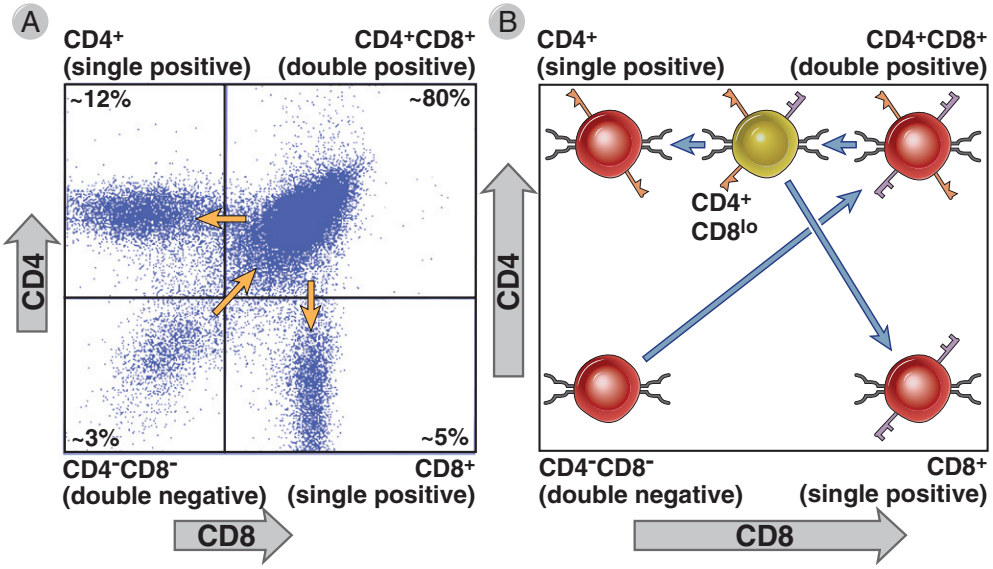


FIGURE 8–22 CD4 and CD8 expression on thymocytes and positive selection of T cells in the thymus. A, The maturation of thymocytes can be followed by changes in expression of the CD4 and CD8 coreceptors. A two-color flow cytometric analysis of thymocytes using anti-CD4 and anti-CD8 antibodies, each tagged with a different fluorochrome, is illustrated. The percentages of all thymocytes contributed by each major population are shown in the four quadrants. The least mature subset is the $CD4^-CD8^-$ (double-negative) cells. Arrows indicate the sequence of maturation. **B,** Positive selection of T cells. Double-positive T cells differentiate into a $CD4^+CD8^{low}$ stage and are instructed to become $CD4^+$ cells if the TCR on a double-positive T cell recognizes self class II MHC with moderate avidity and therefore receives adequate coreceptor signals. A $CD4^+CD8^{low}$ T cell whose TCR recognizes MHC class I molecules fails to receive strong coreceptor signals and differentiates into a $CD8^+$ T cell, silencing CD4 expression.

suppress further rearrangement, there is little or no allelic exclusion in the α chain locus. Therefore, productive TCR α rearrangements may occur on both chromosomes, and if this happens, the T cell will express two α chains. In fact, up to 30% of mature peripheral T cells do express two different TCRs, with different α chains but the same β chain. It is possible that only one of the two α chains participates in the formation of the functional antigen-specific TCR. Transcriptional regulation of the α chain gene occurs in a similar manner to that of the β chain. There are promoters 5′ of each V_α gene that have low-level activity and are responsible for high-level T cell–specific transcription when brought close to an α chain enhancer located 3′ of the C_α gene. The inability to successfully rearrange the TCR α chain on either chromosome leads to a failure of positive selection (discussed later). Thymocytes that fail to make a productive rearrangement of the TCR α chain gene will die by apoptosis.

TCR α gene expression in the double-positive stage leads to the formation of the complete αβ TCR, which is expressed on the cell surface in association with CD3 and ζ proteins. The coordinate expression of CD3 and ζ proteins and the assembly of intact TCR complexes are required for surface expression. Rearrangement of the TCR α gene results in deletion of the TCR δ locus that lies between V segments (common to both α and δ loci) and J_α segments (see Fig. 8-7). As a result, this T cell is no longer capable of becoming a γδ T cell and is completely committed to the αβ T cell lineage. The expression of *Rag* genes and further TCR gene recombination cease after this stage of maturation.

Double-positive cells that successfully undergo selection processes go on to mature into $CD4^+$ or $CD8^+$ T cells, which are called single-positive thymocytes. Thus, the stages of T cell maturation in the thymus can readily be distinguished by the expression of CD4 and CD8 (Fig. 8-22A). This phenotypic maturation is accompanied by functional maturation. $CD4^+$ cells acquire the ability to produce cytokines in response to subsequent antigen stimulation and to express effector molecules (such as CD40 ligand) that "help" B lymphocytes, dendritic cells, and macrophages, whereas $CD8^+$ cells become capable of producing molecules that kill other cells. Mature single-positive thymocytes enter the thymic medulla and then leave the thymus to populate peripheral lymphoid tissues.

Selection Processes in the Maturation of MHC-Restricted αβ T Cells

The selection of developing T cells is dependent on recognition of antigen (peptide-MHC complexes) in the thymus and is responsible for preserving useful cells and eliminating potentially harmful ones. The immature, or unselected, repertoire of T lymphocytes consists of cells whose receptors may recognize any peptide antigen (self or foreign) displayed by any MHC molecule (also self or foreign). In addition, receptors may theoretically be expressed that do not recognize any peptide–MHC molecule complex. In every individual, the only useful T cells are the ones specific for foreign peptides presented by that individual's MHC molecules, that is, self MHC molecules. When double-positive thymocytes first express αβ TCRs, these receptors encounter self peptides (the only peptides normally present in the thymus) displayed by self MHC molecules (the only MHC molecules available

to display peptides) mainly on thymic epithelial cells in the cortex. The outcome of this recognition is determined primarily by the strength of the encounter between TCRs and self antigen–MHC complexes. **Positive selection** is the process that preserves T cells that recognize self MHC (with self peptides) with low avidity. This recognition preserves cells that can see antigens displayed by that individual's MHC molecules. At the same time, the cells become committed to the CD4 or CD8 lineage based on whether the TCR on an individual cell respectively recognizes MHC class II or MHC class I molecules. Also, in every individual, T cells that recognize self antigens with high avidity are potentially dangerous because such recognition may trigger autoimmunity. **Negative selection** is the process in which thymocytes whose TCRs bind strongly to self peptide antigens in association with self MHC molecules are deleted (see Fig. 8-20). The net result of these selection processes is that the repertoire of mature T cells that leaves the thymus is self MHC restricted and tolerant to many self antigens, and only the useful cells complete their maturation. In the following sections, we discuss the details of positive and negative selection.

Positive Selection of Thymocytes: Development of the Self MHC–Restricted T Cell Repertoire

Positive selection is the process in which thymocytes whose TCRs bind with low avidity (i.e., weakly) to self peptide–self MHC complexes are stimulated to survive (see Figs. 8-20 and 8-22). Double-positive thymocytes are produced without antigenic stimulation and begin to express αβ TCRs with randomly generated specificities, which may be biased toward recognition of MHC-like structures. In the thymic cortex, these immature cells encounter epithelial cells that are displaying a variety of self peptides bound to class I and class II MHC molecules. Weak recognition of these self peptide–self MHC complexes promotes the survival of the T cells. Thymocytes whose receptors do not recognize self MHC molecules are permitted to die by a default pathway of apoptosis; this phenomenon is called death by neglect (see Fig. 8-20). Thus, positive selection ensures that T cells are self MHC restricted.

During the transition from double-positive to single-positive cells, thymocytes with class I–restricted TCRs become $CD8^+CD4^-$, and cells with class II–restricted TCRs become $CD4^+CD8^-$. Immature, double-positive T cells express TCRs that may recognize either self MHC class I or self MHC class II. Two models have been proposed to explain the process of lineage commitment, as a result of which coreceptors are correctly matched with the TCRs that recognize a specific class of MHC molecules. The "stochastic" or "probabilistic" model suggests that the commitment of immature T cells toward either lineage depends on the random probability of a double-positive cell differentiating into a $CD4^+$ or a $CD8^+$ T cell. In this model, a cell that recognizes self MHC class I may randomly differentiate into a $CD8^+$ T cell (with the appropriate coreceptor) and survive or into a $CD4^+$ T cell (with the "wrong" coreceptor) that may fail to receive survival signals. In this process of random differentiation into single-positive cells, the coreceptor would fail to be matched with recognition of the right class of MHC molecules approximately half the time. A more widely accepted view is that the process of lineage commitment linked to positive selection is not a random process but is "instructional." Instructional models suggest that class I– and class II–restricted TCRs deliver different signals that actively induce expression of the correct coreceptor and shut off expression of the other coreceptor. It is known that double-positive cells go through a stage at which they express high CD4 and low CD8. If the TCR on such a cell is MHC class I restricted, when it sees the appropriate MHC class I and self peptide, it will receive a weak signal because levels of the CD8 coreceptor are low and, in addition, CD8 associates less well with the Lck tyrosine kinase than CD4. These weak signals activate transcription factors (such as Runx3) that stimulate CD8 expression, generating a class I–restricted $CD8^+$ T cell. Conversely, if the TCR on the cell is class II restricted, when it sees class II, it will receive a stronger signal because CD4 levels are high and CD4 associates relatively well with Lck. These strong signals activate a different set of transcription factors (including ThPok), which stimulate CD4 expression and shut off CD8.

Peptides bound to MHC molecules on thymic epithelial cells play an essential role in positive selection. In Chapter 6, we described how cell surface MHC class I and class II molecules always contain bound peptides. These MHC-associated peptides on thymic antigen-presenting cells probably serve two roles in positive selection—first, they promote stable cell surface expression of MHC molecules, and second, they may influence the specificities of the T cells that are selected. It is also clear from a variety of experimental studies that some peptides are better than others in supporting positive selection, and different peptides differ in the repertoires of T cells they select. These results suggest that specific antigen recognition, and not just MHC recognition, has some role in positive selection. One consequence of self peptide–induced positive selection is that the T cells that mature have the capacity to recognize self peptides. We mentioned in Chapter 2 that the survival of naive lymphocytes before encounter with foreign antigens requires survival signals that are apparently generated by recognition of self antigens in peripheral lymphoid organs. The same self peptides that mediate positive selection of double-positive thymocytes in the thymus may be involved in keeping naive, mature (single-positive) T cells alive in peripheral organs, such as the lymph nodes and spleen.

The model of positive selection based on weak recognition of self antigens raises a fundamental question: How does positive selection driven by self antigens produce a repertoire of mature T cells specific for foreign antigens? The likely answer is that positive selection allows many different T cell clones to survive and differentiate, and many of these T cells that recognize self peptides with low affinity will, after maturing, fortuitously recognize foreign peptides with a high enough affinity to be activated and to generate immune responses.

Negative Selection of Thymocytes: Central Tolerance

Thymocytes whose receptors recognize peptide-MHC complexes in the thymus with high avidity undergo apoptosis

(called negative selection) or differentiate into regulatory T cells (see Fig. 8-20). Among the double-positive T cells that are generated in the thymus, some may express TCRs that recognize self antigens with high affinity. The peptides present in the thymus are self peptides derived from widely expressed protein antigens as well as from some proteins believed to be restricted to particular tissues. (Recall that microbes that enter through the common routes, i.e., epithelia, are captured and transported to lymph nodes and tend to not enter the thymus.) In immature T cells, a major consequence of high-avidity antigen recognition is the triggering of apoptosis, leading to death, or deletion, of the cells. Therefore, many of the immature thymocytes that express high-affinity receptors for self antigens in the thymus are eliminated, resulting in negative selection of the T cell repertoire. This process eliminates the potentially most harmful self-reactive T cells and is one of the mechanisms ensuring that the immune system does not respond to many self antigens, called self-tolerance. Tolerance induced in immature lymphocytes by recognition of self antigens in the generative (or central) lymphoid organs is also called central tolerance, to be contrasted with peripheral tolerance induced in mature lymphocytes by self antigens in peripheral tissues. We will discuss the mechanisms and physiologic importance of immunologic tolerance in more detail in Chapter 14.

The deletion of immature self-reactive T cells may occur both at the double-positive stage in the cortex and in newly generated single-positive T cells in the medulla. The thymic antigen-presenting cells that mediate negative selection are primarily bone marrow–derived dendritic cells and macrophages, both abundant in the medulla, and thymic medullary epithelial cells, whereas cortical epithelial cells are especially (and perhaps uniquely) effective at inducing positive selection. Double-positive T cells are drawn to the thymic medulla by the CCR7-specific chemokines CCL21 and CCL19. In the medulla, thymic medullary epithelial cells express a nuclear protein called **AIRE (autoimmune regulator)** that induces the expression of a number of tissue-specific genes in the thymus. These genes are otherwise normally expressed only in specific peripheral organs, such as the pancreas and the thyroid. Their AIRE-dependent expression in the thymus makes many tissue-specific peptides available for presentation to developing T cells, facilitating the deletion (negative selection) of these cells. A mutation in the gene that encodes AIRE results in an autoimmune polyendocrine syndrome, underscoring the importance of AIRE in mediating central tolerance to tissue-specific antigens (see Chapter 14).

The mechanism of negative selection in the thymus is the induction of death by apoptosis. Unlike the phenomenon of death by neglect, which occurs in the absence of positive selection, in negative selection, active death-promoting signals are generated when the TCR of immature thymocytes binds with high affinity to antigen. The induction by TCR signaling of a proapoptotic protein called Bim probably plays a crucial role in the induction of mitochondrial leakiness and thymocyte apoptosis during negative selection (see Chapter 14). It is also clear that high-avidity antigen recognition by immature T cells triggers apoptosis, but the same recognition by mature lymphocytes (in concert with other signals, see Chapter 9) initiates T cell responses. The biochemical basis of this fundamental difference is not defined.

Recognition of self antigens in the thymus can generate a population of regulatory T cells that function to prevent autoimmune reactions (see Chapter 14). It is not clear what factors determine the choice between the two alternative fates of immature T cells that recognize self antigens with high avidity, namely, deletion of immature T cells and the development of regulatory T cells. It is possible that slightly lower avidity interactions than those required for deletion may lead to the development of natural regulatory T cells, but clear evidence for this kind of fine discrimination still needs to be obtained.

γδ T Lymphocytes

TCR αβ- and γδ-expressing thymocytes are separate lineages with a common precursor. In fetal thymuses, the first TCR gene rearrangements involve the γ and δ loci. Recombination of TCR γ and δ loci proceeds in a fashion similar to that of other antigen receptor gene rearrangements, although the order of rearrangement appears to be less rigid than in other loci. In a developing double-negative T cell, rearrangement of TCR β, γ, or δ loci is initially possible. If a cell succeeds in productively rearranging its TCR γ as well as its TCR δ loci before it makes a productive TCR β rearrangement, it is selected into the γδ T cell lineage. This happens in about 10% of developing double-negative T cells. About 90% of the time, a productive TCR β gene rearrangement is made first. In this situation, pre-TCR signaling selects these cells to mature into the αβ T cell lineage, and eventual deletion of TCR δ when TCR α is rearranged (the TCR δ locus is embedded in the TCR α locus) results in irreversible commitment to the αβ lineage.

The diversity of the γδ T cell repertoire is theoretically even greater than that of the αβ T cell repertoire, in part because the heptamer-nonamer recognition sequences adjacent to D segments permit D-to-D joining. Paradoxically, however, the actual diversity of expressed γδ TCRs is limited because only a few of the available V, D, and J segments are used in mature γδ T cells, for unknown reasons. This limited diversity is reminiscent of the limited diversity of the B-1 subset of B lymphocytes and is in keeping with the concept that γδ T cells serve as an early defense against a limited number of commonly encountered microbes at epithelial barriers.

NKT Cells

NKT cells are not MHC restricted and do not recognize peptides displayed by antigen-presenting cells. These cells express αβ TCRs that are CD1 restricted and also bear a surface marker found on NK cells, hence their name. The TCRs of NKT cells recognize lipid antigens presented by CD1 molecules. CD1 molecules are class I MHC–like molecules made up of a heavy chain and β_2-microglobulin. The heavy chain has a groove made up of hydrophobic residues that can bind and present lipid antigens. These lipid antigens may be derived from endocytosed microbes

or they may be self lipids (see Chapter 6). In the thymic cortex, double positive αβ T cells that express T cell receptors that recognize CD1 molecules expressed on neighboring double positive thymocytes are induced to differentiate into NKT cells. A large number of CD1-restricted NKT cells have an "invariant" TCR resulting from a unique and stereotypic TCR α chain gene rearrangement event. NKT cells secrete cytokines and participate in host defense and may also serve to regulate a variety of immune responses. The functions of NKT cells are described in Chapter 10.

SUMMARY

- B and T lymphocytes arise from a common bone marrow–derived precursor that becomes committed to the lymphocyte lineage. B cell maturation proceeds in the bone marrow, whereas early T cell progenitors migrate to and complete their maturation in the thymus. Early maturation is characterized by cell proliferation induced by cytokines, mainly IL-7, leading to an expansion in the numbers of lymphocytes that have just committed to individual lineages.
- Extracellular signals induce the activation of transcription factors that induce the expression of lineage-specific genes and open up specific antigen receptor gene loci at the level of chromatin accessibility.
- B and T cell development involves the somatic rearrangement of antigen receptor gene segments and the initial expression of the Ig heavy chain μ protein in B cell precursors and TCR β molecules in T cell precursors. The initial expression of pre-antigen receptors and the subsequent expression of antigen receptors are essential for the survival, expansion, and maturation of developing lymphocytes and for selection processes that lead to a diverse repertoire of useful antigen specificities.
- The antigen receptors of B and T cells are encoded by receptor genes made up of a limited number of gene segments that are spatially segregated in the germline antigen receptor loci but are somatically recombined in developing B and T cells.
- Separate loci encode the Ig heavy chain, Ig κ light chain, Ig λ light chain, TCR β chain, TCR α and δ chains, and TCR γ chain. These loci contain V, J, and, in the Ig heavy chain and TCR β and δ loci only, D gene segments. The J segments lie immediately upstream of exons encoding constant domains, and V segments lie a large distance upstream of the J segments. When present, D segments lie between the V and J clusters. Somatic rearrangement of both Ig and TCR loci involves the joining of D and J segments in the loci that contain D segments, followed by the joining of the V segment to the recombined DJ segments in these loci or direct V-to-J joining in the other loci.
- This process of somatic gene recombination is mediated by a recombinase enzyme complex that includes the lymphocyte-specific components Rag-1 and Rag-2.
- The diversity of the antibody and TCR repertoires is generated by the combinatorial associations of multiple germline V, D, and J genes and junctional diversity generated by the addition or removal of random nucleotides at the sites of recombination. These mechanisms generate the most diversity at the junctions of the segments that form the third hypervariable regions of both antibody and TCR polypeptides.
- B cell maturation occurs in stages characterized by different patterns of Ig gene rearrangement and expression. In the earliest B cell precursors, called pro-B cells, Ig genes are initially in the germline configuration, and D to J rearrangement occurs at the Ig heavy chain locus.
- At the pro-B to pre-B cell transition, V-D-J recombination is completed at the Ig H chain locus. A primary RNA transcript containing the VDJ exon and Ig C gene exons is produced, and the VDJ exon is spliced to the μ C region exons of the heavy chain RNA to generate a mature mRNA that is translated into the μ heavy chain protein. The pre-BCR is formed by pairing of the μ chain with surrogate light chains and by association with the signaling molecules Igα and Igβ. This receptor delivers survival and proliferation signals and also signals to inhibit rearrangement on the other heavy chain allele (allelic exclusion).
- As cells differentiate into immature B cells, V-J recombination occurs initially at the Ig κ locus, and light chain proteins are expressed. Heavy and light chains are then assembled into intact IgM molecules and expressed on the cell surface. Immature B cells leave the bone marrow to populate peripheral lymphoid tissues, where they complete their maturation. At the mature B cell stage, synthesis of μ and δ heavy chains occurs in parallel mediated by alternative splicing of primary heavy chain RNA transcripts, and membrane IgM and IgD are expressed.
- During B lymphocyte maturation, immature B cells that express high-affinity antigen receptors specific for self antigens present in the bone marrow are induced to edit their receptor genes or these cells are eliminated. Receptor editing can involve further rearrangement at the Ig κ locus and eventually also involve Ig λ light chain gene rearrangement. B cells that express λ light chains are frequently cells that have undergone receptor editing.
- T cell maturation in the thymus also progresses in stages distinguished by the pattern of expression of antigen receptor genes, CD4 and CD8 coreceptor molecules, and location in the thymus. The earliest T lineage immigrants to the thymus do not express TCRs or CD4 or CD8 molecules. The developing T cells within the thymus, called thymocytes, initially populate the outer cortex, where they undergo proliferation, rearrangement of TCR genes, and surface expression of CD3, TCR, CD4,

and CD8 molecules. As the cells mature, they migrate from the cortex to the medulla.

- The least mature thymocytes, called pro-T cells, are $CD4^-CD8^-$ (double-negative), and the TCR genes are initially in the germline configuration at this stage. Rearrangement of the TCR β, δ, and γ chain genes occurs at this stage.
- At the pre-T stage, thymocytes remain double-negative, but V-D-J recombination is completed at the TCR β chain locus. Primary β chain transcripts are expressed and processed to bring a VDJ exon adjacent to a C_β segment, and TCR β chain polypeptides are produced. The TCR β chain associates with the invariant pre-Tα protein to form a pre-TCR. The pre-TCR transduces signals that inhibit rearrangement on the other β chain allele (allelic exclusion) and promotes differentiation to the stage of dual CD4 and CD8 expression and further proliferation of immature thymocytes. At the $CD4^+CD8^+$ (double-positive) stage of T cell development, V-J recombination occurs at the TCR α locus, α chain polypeptides are produced, and low levels of the TCR are expressed on the cell surface.
- Selection processes drive maturation of TCR-expressing, double-positive thymocytes and shape the T cell repertoire toward self MHC restriction and self-tolerance.
- Positive selection of $CD4^+CD8^+$ TCR αβ thymocytes requires low-avidity recognition of peptide-MHC complexes on thymic epithelial cells, leading to a rescue of the cells from programmed death. As TCR αβ thymocytes mature, they move into the medulla and become either $CD4^+CD8^-$ or $CD8^+CD4^-$. Lineage commitment accompanies positive selection. It results in the matching of TCRs that recognize MHC class I with CD8 expression and the silencing of CD4; TCRs that recognize MHC class II molecules are matched with CD4 expression and the loss of CD8 expression.
- Negative selection of $CD4^+CD8^+$ TCR αβ double-positive thymocytes occurs when these cells recognize, with high avidity, antigens that are present in the thymus. This process is responsible for tolerance to many self-antigens. Medullary thymocytes continue to be negatively selected, and cells that are not clonally deleted acquire the ability to differentiate into either naive $CD4^+$ or $CD8^+$ T cells and finally emigrate to peripheral lymphoid tissues.

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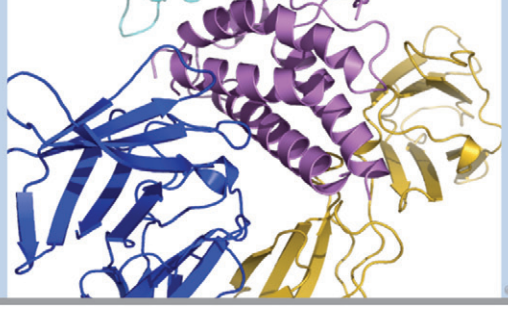
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CHAPTER

9

Activation of T Lymphocytes

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The goal of T cell activation is to generate, from a small pool of naive lymphocytes with predetermined receptors for any antigen, a large number of functional effector cells that can eliminate that antigen and a population of memory cells that remain for long periods to rapidly react against the antigen in case it is reintroduced. A fundamental characteristic of the T cell response, like all adaptive immune responses, is that it is highly specific for the antigen that elicits the response. Both the initial activation of naive T cells and the effector phases of T cell–mediated adaptive immune responses are triggered by recognition of the antigen by antigen receptors of T lymphocytes. In Chapter 6, we described the specificity of T cells for peptide fragments, derived from protein antigens, that are bound to and displayed by self major histocompatibility complex (MHC) molecules. In Chapter 7, we described the antigen receptors and other molecules of T cells that are involved in the activation of T cells by antigens. In this chapter, we describe the biology of T cell activation. We begin with a brief overview of T cell activation, discuss the role of costimulators and other signals provided by antigen-presenting cells (APCs) in T cell activation, and describe the sequence of proliferation and differentiation that occurs in the responses of $CD4^+$ and $CD8^+$ T cells to foreign antigens. The functions of differentiated effector cells in host defense are described in Chapter 10. Thus, Chapters 9 and 10 together cover the biology of T lymphocytes.

OVERVIEW OF T LYMPHOCYTE ACTIVATION

The initial activation of naive T lymphocytes occurs mainly in secondary lymphoid organs, through which these cells normally circulate and where they may encounter antigens presented by mature dendritic cells (Fig. 9-1). The immune system is designed to perform its functions of eliminating antigens only when needed, that is, when the system encounters pathogens. T lymphocytes with multiple specificities are generated in the thymus before antigen exposure. Naive T lymphocytes, which have not recognized and responded to antigens, circulate throughout the body in a resting state, and they acquire powerful functional capabilities only after they are activated. This activation of naive T lymphocytes occurs in specialized lymphoid organs, where the naive lymphocytes and APCs are brought together (see Chapter 2). Protein antigens that cross epithelial barriers or are produced in tissues are captured by dendritic cells and transported to lymph nodes. Antigens that enter the circulation may be captured by dendritic cells in the spleen. If these antigens are produced by microbes or administered with adjuvants (as in vaccines), the resulting innate immune response leads to the activation of dendritic cells and the expression of costimulators such as B7 proteins (described later in this chapter). Dendritic cells that have encountered microbes and internalized their antigens begin to mature and migrate to the T cell zones of draining lymph nodes. As discussed in Chapter 6, both naive T cells and mature dendritic cells are drawn to the T cell zones of secondary

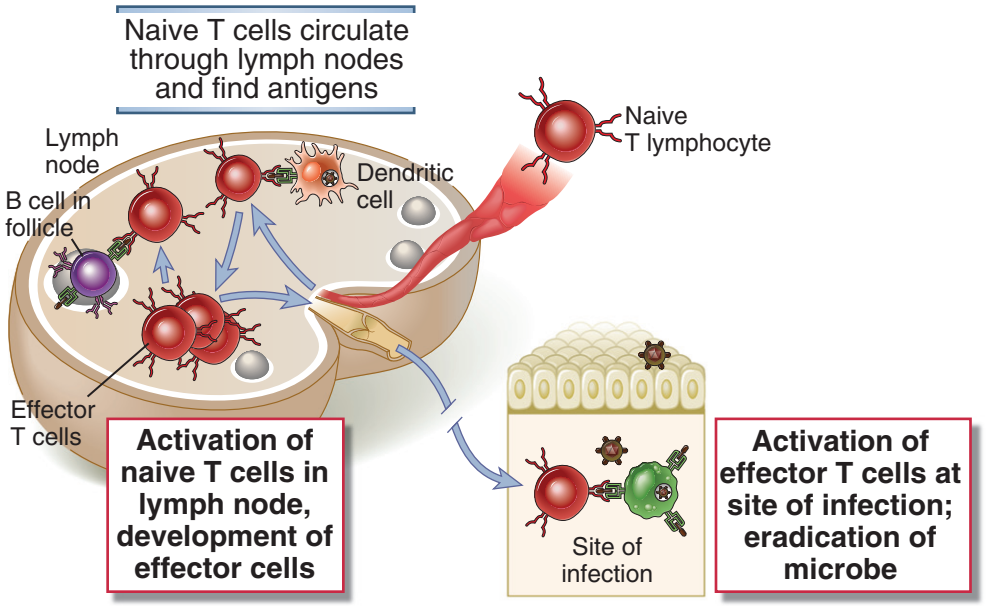


FIGURE 9–1 Activation of naive and effector T cells by antigen. Antigens that are transported by dendritic cells to lymph nodes are recognized by naive T lymphocytes that recirculate through these lymph nodes. The T cells are activated to differentiate into effector and memory cells, which may remain in the lymphoid organs or migrate to nonlymphoid tissues. At sites of infection, the effector cells are again activated by antigens and perform their various functions, such as macrophage activation.

lymphoid organs by chemokines produced in these areas that engage the CCR7 chemokine receptor on the cells. By the time the mature dendritic cells reach the T cell areas, they display peptides derived from protein antigens on MHC molecules and also express costimulators. When a naive T cell of the correct specificity recognizes the peptide-MHC complexes and receives concomitant costimulatory signals from the dendritic cells, that naive lymphocyte is activated.

Antigen recognition and other activating stimuli induce several responses: cytokine secretion from the T cells; proliferation of the antigen-specific lymphocytes, leading to an increase in the numbers of cells in the antigen-specific clones (called clonal expansion); and differentiation of the naive cells into effector and memory lymphocytes (Fig. 9-2). In addition, the process of T cell activation is associated with characteristic changes in surface molecules, many of which play important roles in promoting the responses and in limiting them. Clonal expansion and differentiation proceed rapidly because of several positive feedback amplification mechanisms. For example, cytokines made by the activated T cells stimulate both T cell proliferation and differentiation into effector cells. In addition, activated T cells deliver signals back to the APCs, further enhancing their ability to activate T cells. At the same time, some surface molecules expressed on activated T cells as well as cytokines secreted by these cells have regulatory functions that serve to establish safe limits to the response. The steps in T cell responses and the nature of the positive and negative feedback loops are described later in the chapter.

Effector T cells recognize antigens in lymphoid organs or in peripheral nonlymphoid tissues and are activated to perform functions that are responsible for the elimination of microbes and, in disease states, for inflammation and tissue damage. Whereas naive cells are activated mainly in lymphoid organs, differentiated effector cells may function in any tissue (see Fig. 9-1). The process of differentiation from naive to effector cells is associated with acquisition of the capacity to perform these specialized functions and the ability to migrate to any site of infection or inflammation. At these sites, the effector cells again encounter the antigen for which they are specific and respond in ways that serve to eliminate the source of the antigen. Effector T cells of the $CD4^+$ helper lineage are classified into several subsets on the basis of their cytokine profiles and functions. Some of these differentiated helper cells express membrane molecules and secrete cytokines that activate (help) macrophages to kill phagocytosed microbes; others secrete cytokines that recruit leukocytes and thus stimulate inflammation; others enhance mucosal barrier functions; and yet others remain in lymphoid organs and help B cells to differentiate into cells that secrete antibodies. $CD8^+$ cytotoxic T lymphocytes (CTLs), the effector cells of the $CD8^+$ lineage, kill infected cells and tumor cells that display class I MHC–associated antigens.

Memory T cells that are generated by T cell activation are long-lived cells with an enhanced ability to react against the antigen. These cells are present in the recirculating lymphocyte pool and are abundant in mucosal tissues and the skin as well as in lymphoid organs. After a T cell response wanes, there are many more memory cells of the responding clone that persist than there were naive T cells before the response. These memory cells respond rapidly to subsequent encounter with the antigen and generate new effector cells that eliminate the antigen.

T cell responses decline after the antigen is eliminated by effector cells. This process of contraction is important

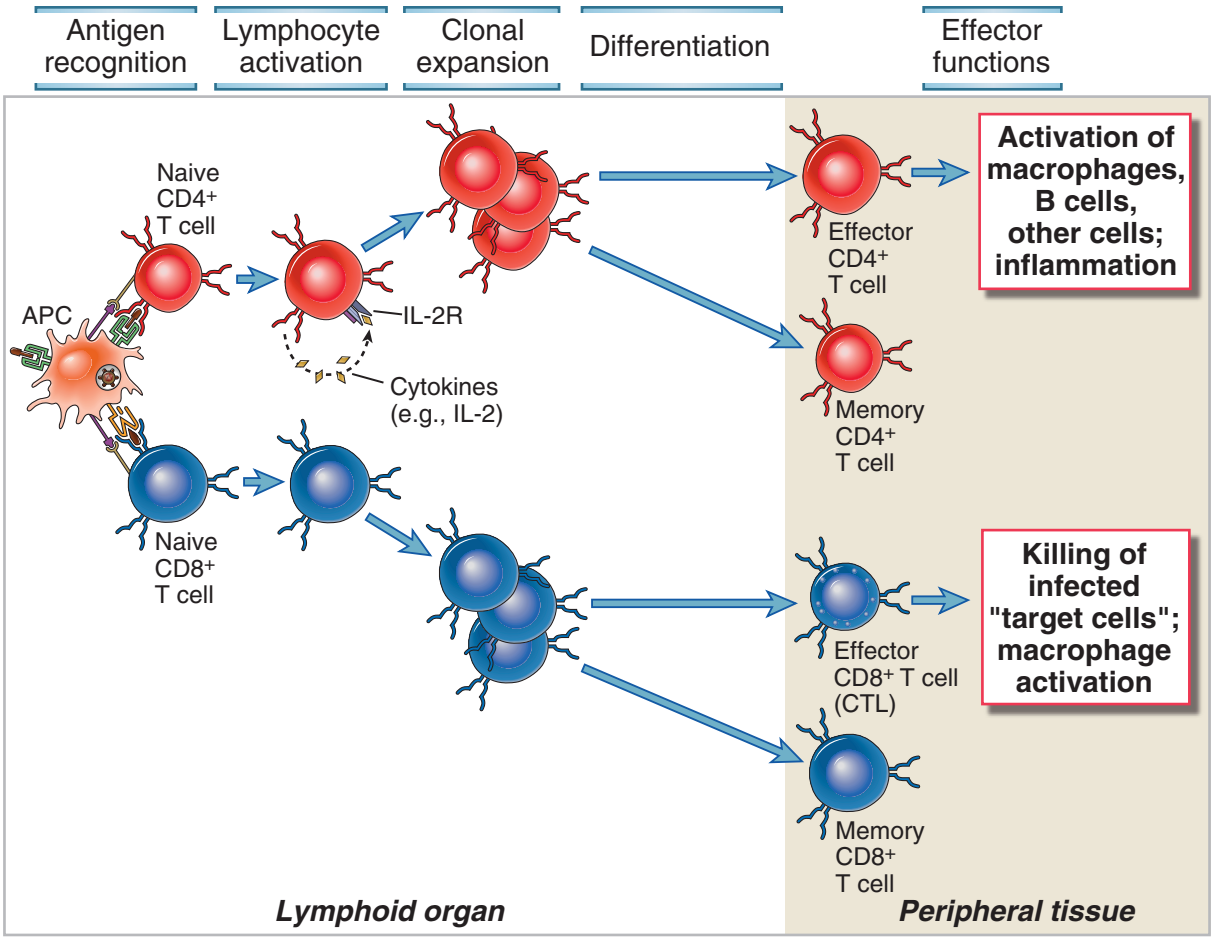


FIGURE 9–2 Phases of T cell responses. Antigen recognition by T cells induces cytokine (e.g., IL-2) secretion, particularly in $CD4^+$ T cells, clonal expansion as a result of cell proliferation, and differentiation of the T cells into effector cells or memory cells. In the effector phase of the response, the effector $CD4^+$ T cells respond to antigen by producing cytokines that have several actions, such as the recruitment and activation of leukocytes and activation of B lymphocytes, and $CD8^+$ CTLs respond by killing other cells.

for returning the immune system to a state of equilibrium, or homeostasis. It occurs mainly because the majority of antigen-activated effector T cells die by apoptosis. One reason for this is that as the antigen is eliminated, lymphocytes are deprived of survival stimuli that are normally provided by the antigen and by the costimulators and cytokines produced during inflammatory reactions to the antigen. It is estimated that more than 90% of the antigen-specific T cells that arise by clonal expansion die by apoptosis as the antigen is cleared.

With this overview, we proceed to a discussion of the signals required for T cell activation and the steps that are common to $CD4^+$ and $CD8^+$ T cells. We then describe effector and memory cells in the $CD4^+$ and $CD8^+$ lineages, with emphasis on subsets of $CD4^+$ helper T cells and the cytokines they produce. We conclude with a discussion of the decline of immune responses.

SIGNALS FOR T LYMPHOCYTE ACTIVATION

The proliferation of T lymphocytes and their differentiation into effector and memory cells require antigen recognition, costimulation, and cytokines that are produced by the T cells themselves and by APCs and other cells at the site of antigen recognition. In this section, we will summarize the nature of antigens recognized by T cells and discuss specific costimulators and their receptors that contribute to T cell activation. Cytokines are discussed later in the chapter.

Recognition of Antigen

Antigen is always the necessary first signal for the activation of lymphocytes, ensuring that the resultant immune response remains specific for the antigen. Because $CD4^+$ and $CD8^+$ T lymphocytes recognize peptide-MHC complexes displayed by APCs, they can respond only to protein antigens or chemicals attached to proteins. In addition to the TCR recognizing peptides displayed by MHC molecules, several other T cell surface proteins participate in the process of T cell activation (see Fig. 7-9, Chapter 7). These include adhesion molecules, which stabilize the interaction of the T cells with APCs, and costimulators, which are described later. The nature of the biochemical signals delivered by antigen receptors and the role of these signals in the functional responses of the T cells are discussed in Chapter 7.

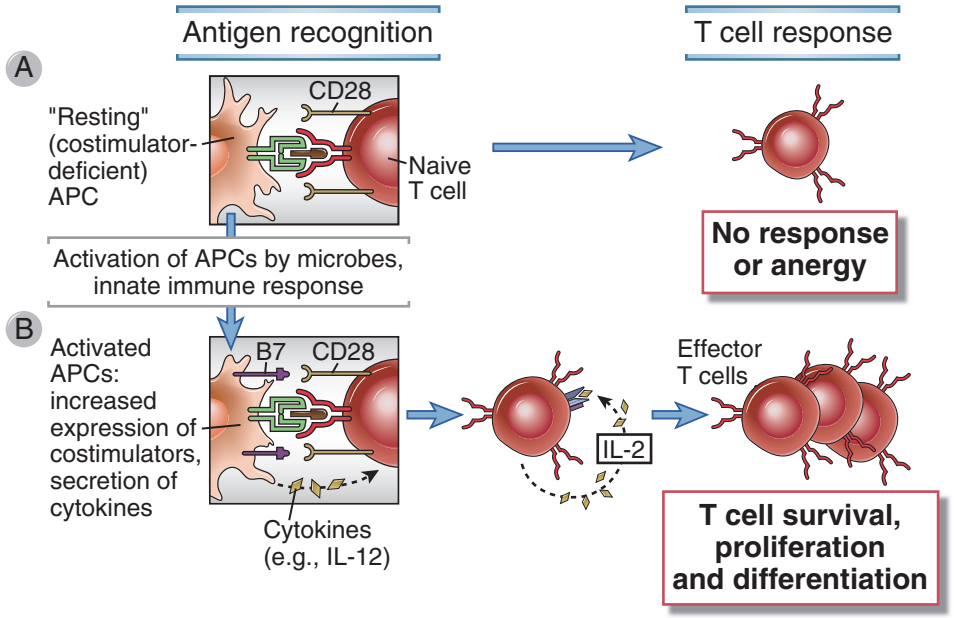


FIGURE 9–3 Functions of costimulators in T cell activation. A, The resting APC expresses few or no costimulators and fails to activate naive T cells. (Antigen recognition without costimulation may make T cells anergic; this phenomenon will be discussed in Chapter 14.) **B,** Microbes and cytokines produced during innate immune responses activate APCs to express costimulators, such as B7 molecules. The APCs then become capable of activating naive T cells. Activated APCs also produce cytokines such as IL-12, which stimulate the differentiation of naive T cells into effector cells.

Activation of naive T cells requires recognition of antigen presented by dendritic cells. The reasons that dendritic cells are the most efficient APCs for initiation of T cell responses were discussed in Chapter 6. In lymphoid organs, dendritic cells present peptides derived from endocytosed protein antigens in association with class II MHC molecules to naive $CD4^+$ T cells and peptides derived from cytosolic proteins displayed by class I MHC molecules to $CD8^+$ T cells. $CD4^+$ T cell–mediated immune reactions are elicited by protein antigens of microbes that are ingested by dendritic cells or by soluble protein antigens that are administered with adjuvants, in the case of vaccinations, and taken up by dendritic cells. These microbial or soluble antigens are internalized into vesicles by the dendritic cells, processed, and presented in association with class II MHC molecules. $CD8^+$ T cell responses are induced by antigens that either are produced in the cytoplasm of dendritic cells (e.g., by viruses that infect these cells) or are ingested by dendritic cells, processed, and "cross-presented" on class I MHC molecules. Some chemicals introduced through the skin also elicit T cell reactions, called contact sensitivity reactions. Contact-sensitizing chemicals may tightly bind to or covalently modify self proteins, creating novel peptide determinants that are presented to $CD4^+$ or $CD8^+$ T cells.

Differentiated effector T cells can respond to antigens presented by cells other than dendritic cells. In humoral immune responses, B cells present antigens to helper T cells and are the recipients of activating signals from the helper cells (see Chapter 11); in cell-mediated immune responses, macrophages present antigens to and respond to T cells (see Chapter 10); and virtually any nucleated cell can present antigen to and be killed by $CD8^+$ CTLs.

Role of Costimulators in T Cell Activation

The proliferation and differentiation of naive T cells require signals provided by molecules on APCs, called costimulators, in addition to antigen-induced signals (Fig. 9-3). The requirement for costimulatory signals was first suggested by the experimental finding that T cell antigen receptor engagement alone (e.g., with cross-linking anti-CD3 antibodies) resulted in much lower responses than those seen with antigens presented by activated APCs. This result indicated that APCs must express molecules in addition to antigen that are required for T cell activation. These molecules are called **costimulators**, and the "second signal" for T cell activation is called **costimulation** because it functions together with antigen ("signal 1") to stimulate T cells. In the absence of costimulation, T cells that encounter antigens either fail to respond and die by apoptosis or enter a state of unresponsiveness called anergy (see Chapter 14).

The B7:CD28 Family of Costimulators

The best characterized costimulatory pathway in T cell activation involves the T cell surface receptor CD28, which binds the costimulatory molecules B7-1 (CD80) and B7-2 (CD86) expressed on activated APCs. CD28 was discovered when activating antibodies against human T cell surface molecules were screened for their ability to enhance T cell responses when added to the cells together with an activating anti-CD3 antibody (which was used as a mimic of antigen). The ligands for CD28 were discovered by screening DNA expression libraries for molecules that bound to CD28. The cloning of the genes encoding B7-1 and CD28 opened the way for a variety

of experiments in mice that have clarified the role of these molecules and led to the identification of additional homologous proteins involved in T cell costimulation. For example, residual costimulatory activity of APCs from B7-1 knockout mice suggested the existence of additional costimulatory molecules, and homology-based cloning strategies led to the identification of the B7-2 molecule. The essential role of CD28 and B7-1 and B7-2 in T cell activation has been established not only by experiments with cross-linking antibodies but also by the severe T cell immune deficiency caused by knockout of these molecules in mice and by the ability of agents that bind to and block B7 to inhibit a variety of T cell responses. The development of therapeutic agents based on these principles is described later.

B7-1 and B7-2 are structurally similar integral membrane single-chain glycoproteins, each with two extracellular immunoglobulin (Ig)–like domains, although on the cell surface, B7-1 exists as a dimer and B7-2 as a monomer. CD28 is a disulfide-linked homodimer, each subunit of which has a single extracellular Ig domain. It is expressed on more than 90% of $CD4^+$ T cells and on 50% of $CD8^+$ T cells in humans (and on all naive T cells in mice).

The expression of B7 costimulators is regulated and ensures that T lymphocyte responses are initiated at the correct time and place. The B7 molecules are expressed mainly on APCs, including dendritic cells, macrophages, and B lymphocytes. They are absent or expressed at low levels on resting APCs and are induced by various stimuli, including microbial products that engage Toll-like receptors and cytokines such as interferon-γ (IFN-γ) produced during innate immune reactions to microbes. The induction of costimulators by microbes and by the cytokines of innate immunity promotes T cell responses to microbial antigens. This is an excellent illustration of the role of innate immune responses in enhancing adaptive immunity (see Chapter 4). In addition, activated T cells express CD40 ligand on their surface, which binds to CD40 expressed on APCs and delivers signals that enhance the expression of B7 costimulators on the APCs. This feedback loop serves to amplify T cell responses (described later). Of all potential APCs, mature dendritic cells express the highest levels of costimulators and, as a result, are the most potent stimulators of naive T cells. In Chapter 6, we mentioned the essential role of **adjuvants** in inducing primary T cell responses to protein antigens such as vaccines. Many adjuvants are products of microbes, or mimic microbes, and one of their major functions in T cell activation is to stimulate the expression of costimulators on APCs. Unactivated, or "resting," APCs in normal tissues are capable of presenting self antigens to T cells, but because these tissue APCs express only low levels of costimulators, potentially self-reactive T cells that see the self antigens are not activated and may be rendered anergic (see Chapter 14). Regulatory T cells (see Chapter 14) are also dependent on B7:CD28-mediated costimulation for their generation and maintenance. It is possible that the low levels of B7 costimulators that are constitutively expressed by resting APCs are necessary to maintain regulatory T cells, which are important for tolerance to self antigens. The temporal patterns of expression of B7-1 and B7-2 differ; B7-2 is expressed constitutively at low levels and induced early after activation of APCs, whereas B7-1 is not expressed constitutively and is induced hours or days later.

CD28 signals work in cooperation with antigen recognition to initiate the responses of naive T cells. CD28 engagement leads to the activation of several signaling pathways, some of which may amplify signals from the TCR complex, and others may be independent of but parallel to TCR-induced signals (Fig. 9-4). The cytoplasmic tail of CD28 includes a tyrosine-containing motif that after phosphorylation can recruit the regulatory subunit of phosphatidylinositol 3-kinase (PI3-kinase). The CD28 tail also contains two proline-rich motifs, one of which can bind the Tec family tyrosine kinase Itk, and the other binds to the Src family kinase Lck. CD28 ligated by its B7 ligands can activate PI3-kinase and the Akt kinase and also facilitates the activation of the Ras/ERK MAP kinase pathway. PI3-kinase, as discussed in Chapter 7, creates phosphatidylinositol trisphosphate (PIP3) moieties on the inner leaflet of the plasma membrane that can contribute to the recruitment and activation of the Itk tyrosine kinase, the phospholipase PLCγ, and another kinase called PDK1. PDK1 phosphorylates and activates Akt. Akt in turn phosphorylates a number of targets, inactivating proapoptotic proteins and activating antiapoptotic factors, thus contributing to increased cell survival. CD28 also provides an independent pathway for the activation of the Vav exchange factor and the subsequent activation of the Rac/JNK MAP kinase pathway. In addition, CD28 signals have been shown to induce NF-κB binding to a site in the IL-2 gene promoter, called the CD28 response element, that is not activated by TCR-mediated signals. The net result of these signaling pathways is the increased expression of antiapoptotic proteins such as Bcl-2 and Bcl-X_L, which promote survival of T cells; increased metabolic activity of T cells; enhanced proliferation of the T cells; production of cytokines such as IL-2; and differentiation of the naive T cells into effector and memory cells (see Fig. 9-4). Previously activated effector and memory T cells are less dependent on costimulation by the B7:CD28 pathway than are naive cells. This property of effector and memory cells enables them to respond to antigens presented by various APCs that may reside in nonlymphoid tissues and may express no or low levels of B7. For instance, the differentiation of $CD8^+$ T cells into effector CTLs requires costimulation, but effector CTLs can kill other cells that do not express costimulators.

Numerous receptors homologous to CD28 and their ligands homologous to B7 have been identified, and these proteins regulate T cell responses both positively and negatively (Fig. 9-5). Following the demonstration of the importance of B7 and CD28, several other proteins structurally related to B7-1 and B7-2 or to CD28 were identified by homology-based gene cloning. A surprising conclusion has emerged that some of the members of the B7:CD28 families are involved in T cell activation (and are thus costimulators) and others are critical inhibitors of T cells (and have sometimes been called coinhibitors). The costimulatory receptor other than CD28 whose function is best understood is **ICOS** (inducible costimulator, CD278). Its ligand, called ICOS-L (CD275), is expressed

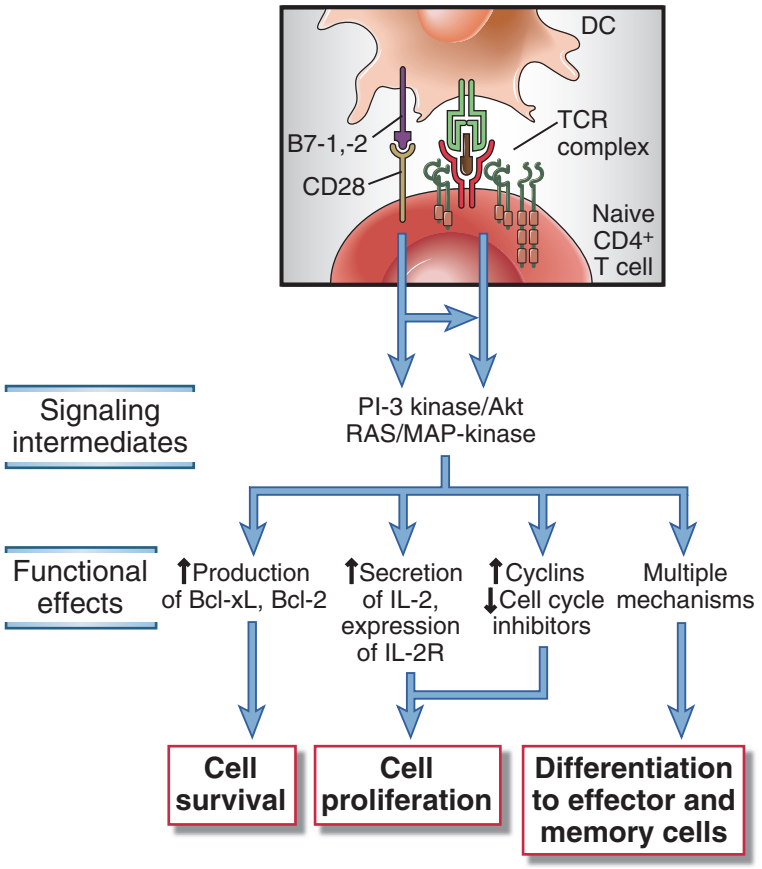


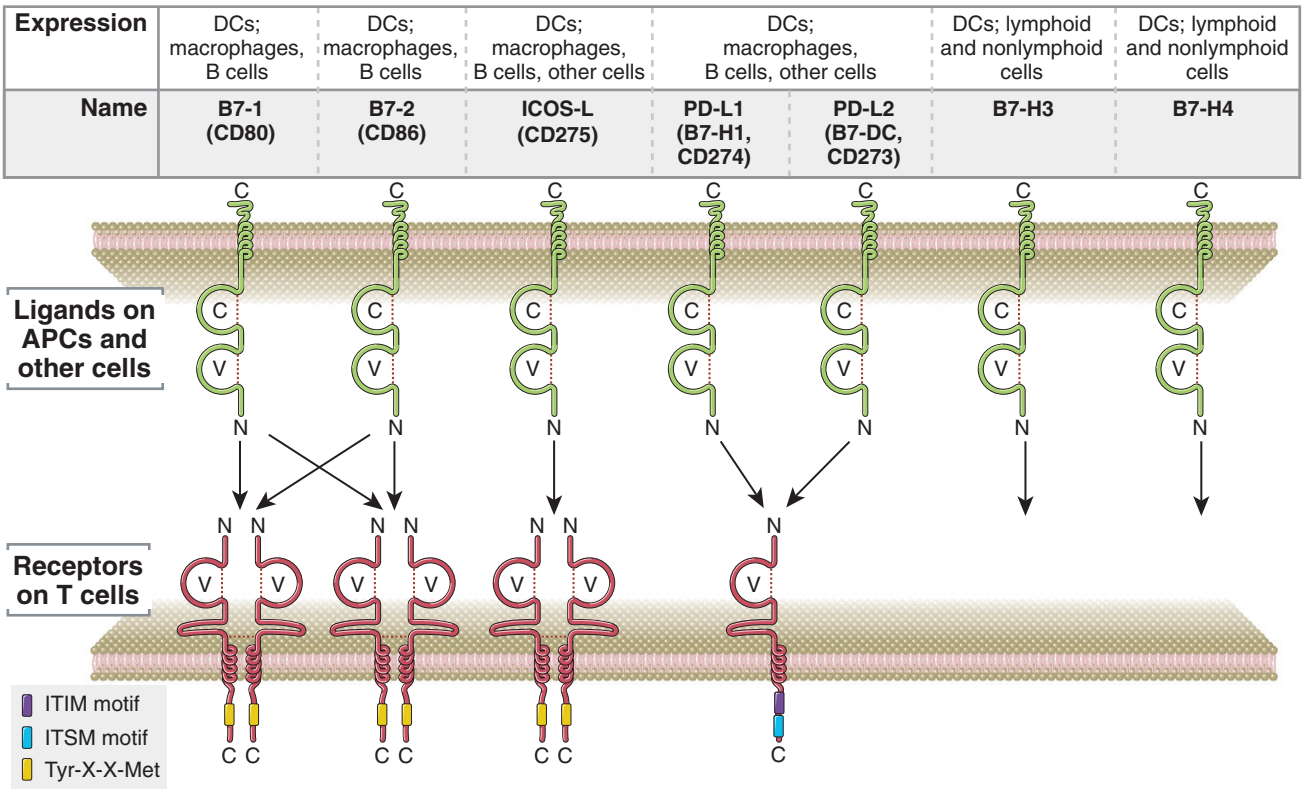
FIGURE 9–4 Mechanisms of T cell stimulation by CD28. CD28 engagement induces signaling pathways that enhance or work together with TCR signals to stimulate the expression of survival proteins, cytokines, and cytokine receptors, to promote cell proliferation, and to induce differentiation toward effector and memory cells. These differentiation events may be secondary to the increased clonal expansion and may also involve increased production of various transcription factors.

on dendritic cells, B cells, and other cell populations. ICOS plays an essential role in T cell–dependent antibody responses, particularly in the germinal center reaction. It is required for the development and activation of follicular helper T cells, which provide critical activating signals to B cells in germinal centers (see Chapter 11).

The outcome of T cell activation is influenced by a balance between engagement of activating and inhibitory receptors of the CD28 family. The inhibitory receptors of the CD28 family are **CTLA-4** (cytotoxic T lymphocyte antigen 4, so called because this molecule was the fourth protein identified in a search for molecules expressed in CTLs) and **PD-1** (programmed death 1). (The names of these two proteins do not accurately reflect their distribution or function.) The concept that a balance between activating and inhibitory receptors controls the magnitude of responses in the immune system was mentioned in Chapter 4 in the context of natural killer (NK) cells (see Fig. 4-6, Chapter 4). A similar idea is applicable to responses of T and B lymphocytes, although the receptors involved are quite different. Because the inhibitory receptors CTLA-4 and PD-1 are involved in the phenomenon of tolerance, and abnormalities in their expression or function cause autoimmune diseases, we will discuss them in more detail in Chapter 14 in the context of tolerance and autoimmunity. Suffice it to say here that CD28 and CTLA-4 provide an illustrative example of two receptors that recognize the same ligands (the B7 molecules) but have opposite functional effects on T cell activation. CTLA-4 is a high-affinity receptor for B7, and it has been postulated that it is engaged when B7 levels on APCs are low (as on resting APCs displaying self antigens or APCs that are no longer exposed to microbes, after the microbes are cleared and the innate immune response subsides). CD28 has a 20- to 50-fold lower affinity for B7, and it may be engaged when B7 levels are relatively high (e.g., on exposure to microbes and innate immune responses). According to this model, the level of B7 expression on APCs influences the relative engagement of CD28 or CTLA-4, and this in turn determines if responses are initiated or terminated. Once engaged, CTLA-4 may competitively inhibit access of CD28 to B7 molecules on APCs, remove B7 from the surface of APCs, or deliver inhibitory signals that block activating signals from the TCR and CD28.

Other Costimulatory Pathways

Many other T cell surface molecules, including CD2 and integrins, have been shown to deliver costimulatory signals in vitro, but their physiologic role in promoting T cell activation is less clear than that of the CD28 family. We have discussed the functions of CD2 family proteins in Chapter 7 and of integrins in Chapter 3. Several other receptors that belong to the large tumor necrosis factor (TNF) receptor (TNFR) superfamily and their ligands, which are homologous to TNF, have been shown to stimulate and to inhibit T cells under various experimental conditions. The roles of these proteins in controlling normal and pathologic immune responses remain areas of active investigation.



Expression	DCs; macrophages, B cells	DCs; macrophages, B cells	DCs; macrophages, B cells, other cells	DCs; macrophages, B cells, other cells		DCs; lymphoid and nonlymphoid cells	DCs; lymphoid and nonlymphoid cells
Name	**B7-1 (CD80)**	**B7-2 (CD86)**	**ICOS-L (CD275)**	**PD-L1 (B7-H1, CD274)**	**PD-L2 (B7-DC, CD273)**	**B7-H3**	**B7-H4**

Name	**CD28**	**CTLA-4 (CD152)**	**ICOS (CD278)**	**PD-1 (CD279)**	**?**	**?**
Expression	T cells; constitutive	T cells; inducible	T cells; inducible	T cells, B cells, myeloid cells; inducible		
Major function	Costimulation of naive T cells; generation of regulatory T cells	Negative regulation of immune responses; self-tolerance	Costimulation of effector and regulatory T cells; generation of follicular helper T cells	Negative regulation of T cells	Costimulation or negative regulation of T cells	Negative regulation of T cells

FIGURE 9–5 The B7 and CD28 families. The known B7 family ligands expressed on APCs and CD28 family receptors expressed on T cells are shown, with their expression patterns and likely major functions. Other inhibitory receptors have been defined, such as BTLA, but these are not homologous to CD28 and are therefore not shown here.

The interaction of CD40L on T cells with CD40 on APCs enhances T cell responses by activating the APCs. CD40 ligand (CD40L) is a TNF superfamily membrane protein that is expressed primarily on activated T cells, and CD40 is a member of the TNF receptor superfamily expressed on B cells, macrophages, and dendritic cells. The functions of CD40 in activating macrophages in cell-mediated immunity and activating B cells in humoral immune responses are described in Chapters 10 and 11, respectively. Activated helper T cells express CD40L, which engages CD40 on the APCs and activates the APCs to make them more potent by enhancing their expression of B7 molecules and secretion of cytokines such as IL-12 that promote T cell differentiation (Fig. 9-6). This phenomenon is sometimes called licensing because activated T cells license APCs to become more powerful stimulators of immune responses. Thus, the CD40 pathway indirectly amplifies T cell responses by inducing costimulators on APCs, but CD40L does not function by itself as a costimulator for T cells.

Therapeutic Costimulatory Blockade

New therapeutic agents are being developed for suppression of injurious immune responses on the basis of the understanding of these costimulatory pathways (Fig. 9-7). CTLA-4–Ig, a fusion protein consisting of the extracellular domain of CTLA-4 and the Fc portion of human IgG, binds to B7-1 and B7-2 and blocks the B7:CD28 interaction. The reason for use of the extracellular domain of CTLA-4 rather than of CD28 to block B7 molecules is that CTLA-4 binds to B7 with a 20- to 50-fold greater affinity, as mentioned before. Attachment of the Fc portion of IgG increases the in vivo half-life of the protein. CTLA-4-Ig is an approved therapy for rheumatoid arthritis, and clinical trials are currently assessing its efficacy in the treatment of transplant rejection, psoriasis, and Crohn's disease. Antibodies that block the inhibitory receptors CTLA-4 and PD-1 are in clinical trials for the immunotherapy of tumors. As one might predict from the role of CTLA-4 in maintaining self-tolerance, blocking of this inhibitory receptor induces autoimmune

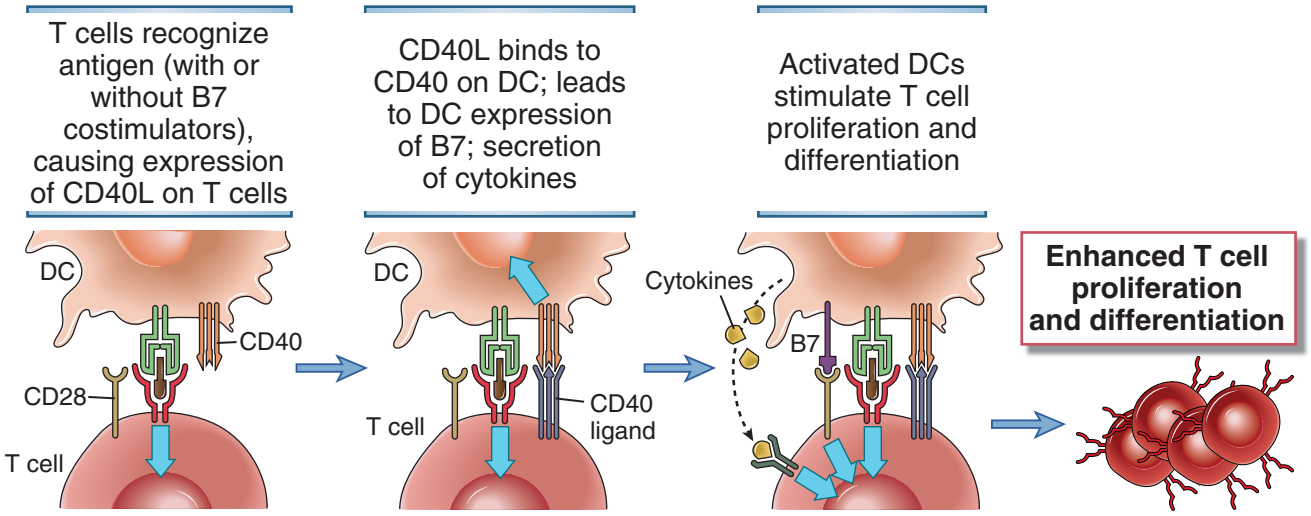


FIGURE 9–6 Role of CD40 in T cell activation. Naive T cells are activated by peptide-MHC complexes on activated APCs. Antigen recognition by T cells together with costimulation (not shown in the figure) induces the expression of CD40 ligand (CD40L) on the activated T cells. CD40L engages CD40 on APCs and may stimulate the expression of B7 molecules and the secretion of cytokines that activate T cells. Thus, CD40L on the T cells makes the APCs "better" APCs, and thus promotes and amplifies T cell activation.

reactions in some patients. Inhibitors of the CD40L:CD40 pathway are also in clinical trials for transplant rejection and chronic inflammatory autoimmune diseases.

FUNCTIONAL RESPONSES OF T LYMPHOCYTES

The earliest responses of antigen-stimulated T cells include changes in the expression of various surface molecules as well as the secretion of cytokines and the expression of cytokine receptors. These are followed by proliferation of the antigen-specific cells, driven in part by the secreted cytokines, and then by differentiation of the activated cells into effector and memory cells. In the remainder of this chapter, we will describe each of these steps, their underlying mechanisms, and their functional consequences.

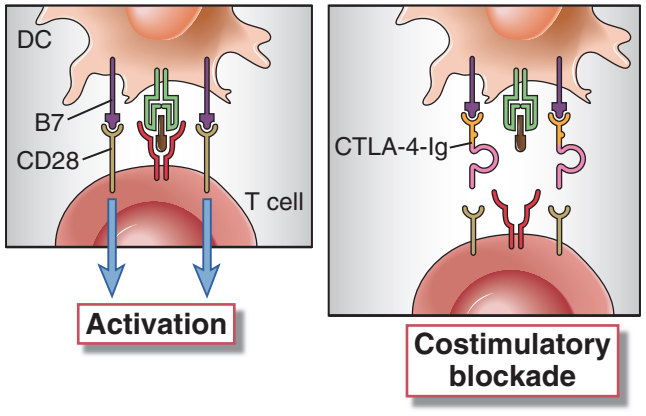


FIGURE 9–7 The mechanism of therapeutic costimulatory blockade. A fusion protein of the extracellular portion of CTLA-4 and the Fc tail of an IgG molecule is used to bind to and block B7 molecules, thus preventing their interaction with the activating receptor CD28 and inhibiting T cell activation.

Changes in Surface Molecules During T Cell Activation

After activation, there are characteristic changes in the expression of various surface molecules in T cells, which are best defined in $CD4^+$ helper cells (Fig. 9-8). Many of the molecules that are expressed in activated T cells are also involved in the functional responses of the T cells. Some of the functionally important molecules induced on activation are the following.

- ***CD69.*** Within a few hours after activation, T cells increase their expression of CD69, a plasma membrane protein. This protein binds to and reduces surface expression of the sphingosine 1-phosphate receptor S1PR1, which we described in Chapter 3 as a receptor that mediates egress of T cells from lymphoid organs. The consequence of decreased S1PR1 expression is that activated T cells are retained in lymphoid organs long enough to receive the signals that initiate their proliferation and differentiation into effector and memory cells. After cell division, CD69 expression decreases, the activated T cells re-express high levels of S1PR1, and therefore effector and memory cells can exit the lymphoid organs (see Chapter 3).
- ***CD25 (IL-2Rα).*** The expression of this cytokine receptor enables activated T cells to respond to the growth-promoting cytokine IL-2. This process is described later.
- ***CD40 ligand (CD40L, CD154).*** Within 24 to 48 hours after activation, T cells express high levels of the ligand for CD40. The expression of CD40L enables activated

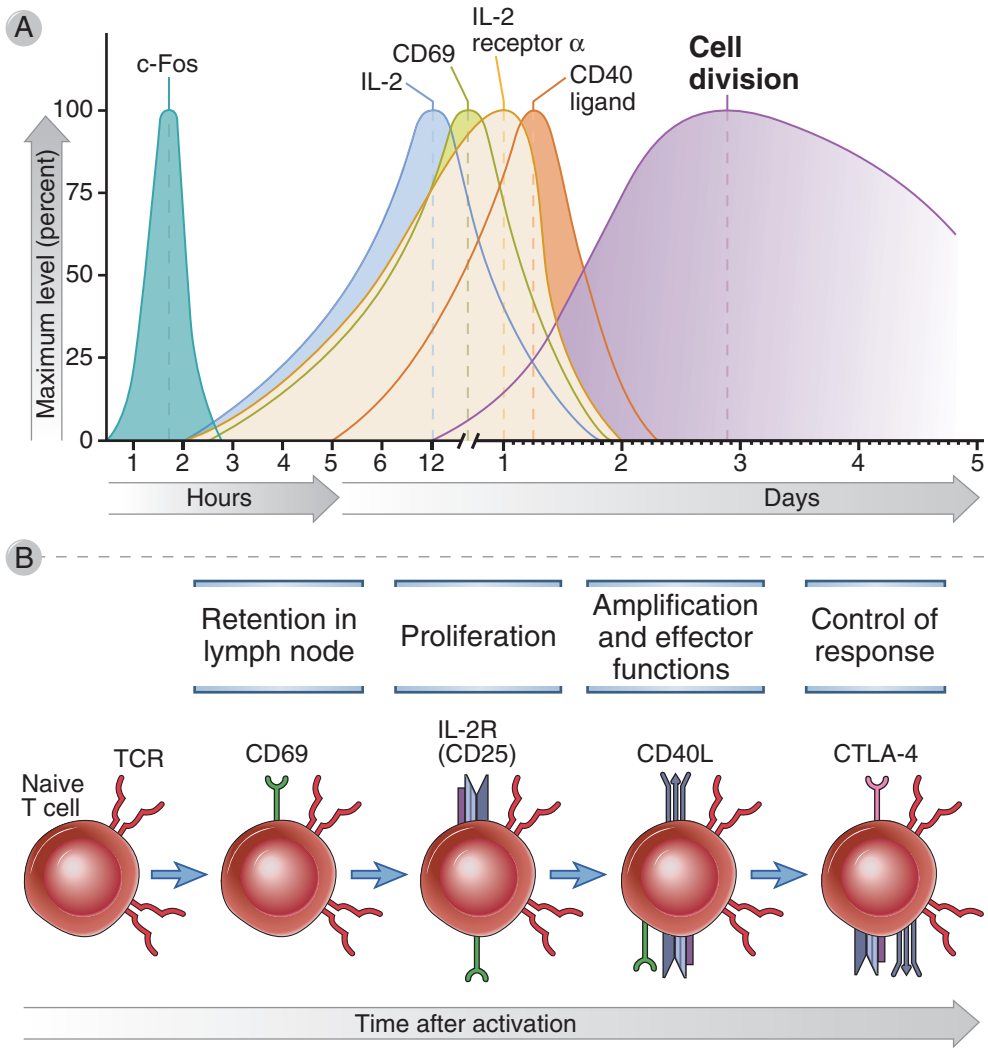


FIGURE 9–8 Changes in surface molecules after T cell activation. A, The approximate kinetics of expression of selected molecules after activation of T cells by antigens and costimulators are shown. The illustrative examples include a transcription factor (c-Fos), a cytokine (IL-2), and surface proteins. These proteins are typically expressed at low levels in naive T cells and are induced by activating signals. CTLA-4 (not shown) is induced 1 to 2 days after activation. The kinetics are estimates and will vary with the nature of the antigen, its dose and persistence, and the type of adjuvant. **B,** The major functions of selected surface molecules are shown and described in the text.

T cells to mediate their key effector functions, which are to help macrophages and B cells. In addition, as discussed earlier, CD40L on the T cells activates dendritic cells to become better APCs, thus providing a positive feedback loop mechanism for amplifying T cell responses.

- ***CTLA-4 (CD152).*** The expression of CTLA-4 on T cells also increases within 24 to 48 hours after activation. We have mentioned CTLA-4 earlier as a member of the CD28 family that functions as an inhibitor of T cell activation and thus as a regulator of the response. The mechanism of action of CTLA-4 is described in Chapter 14 (see Fig. 14-5).
- ***Adhesion molecules and chemokine receptors.*** After activation, T cells reduce expression of molecules that bring them to the lymphoid organs (such as L-selectin and the chemokine receptor CCR7) and increase the expression of molecules that are involved in their migration to peripheral sites of infection and tissue injury (such as the integrins LFA-1 and VLA-4, the ligands for E- and P-selectins, and various chemokine receptors). These molecules and their roles in T cell migration were described in Chapter 3. Activation also increases the expression of CD44, a receptor for the extracellular matrix molecule hyaluronan. Binding of CD44 to its ligand helps to retain effector T cells in the tissues at sites of infection and tissue damage (see Chapter 10).

IL-2 Secretion and IL-2 Receptor Expression

Cytokines play critical roles in adaptive immune responses; in such responses, the major sources of cytokines are T cells, especially (but not exclusively) $CD4^+$

helper T cells. The most important cytokine produced by T cells early after activation, often within 2 to 4 hours after recognition of antigen and costimulators, is interleukin-2 (IL-2), and this is described here. The cytokines secreted by effector cells are described in Chapter 10, when we discuss the functions of effector $CD4^+$ T cells.

IL-2 is a growth, survival, and differentiation factor for T lymphocytes and plays a major role in the regulation of T cell responses by virtue of its crucial role in the maintenance of regulatory T cells. Because of its ability to support proliferation of antigen-stimulated T cells, IL-2 was originally called T cell growth factor (TCGF). It acts on the same cells that produce it or on adjacent cells (i.e., it functions as an autocrine or paracrine cytokine).

IL-2 is produced mainly by $CD4^+$ T lymphocytes. Activation of T cells by antigens and costimulators stimulates transcription of the IL-2 gene and synthesis and secretion of the protein. IL-2 production is rapid and transient, starting within 2 to 3 hours of T cell activation, peaking at about 8 to 12 hours, and declining by 24 hours. $CD4^+$ T cells secrete IL-2 into the immunologic synapse formed between the T cell and APC (see Chapter 7). IL-2 receptors on T cells also tend to localize to the synapse, so that the cytokine and its receptor reach sufficiently high local concentrations to initiate cellular responses.

Secreted IL-2 is a 14- to 17-kD glycoprotein that folds into a globular protein containing four α helices (Fig. 9-9). It is the prototype of the four–α-helical cytokines that interact with type I cytokine receptors (see Chapter 7).

Functional IL-2 receptors are transiently expressed on activation of naive and effector T cells; regulatory T cells always express IL-2 receptors. The IL-2 receptor (IL-2R) consists of three noncovalently associated proteins including IL-2Rα (CD25), IL-2/15Rβ (CD122), and γ_c (CD132). Of the three chains, only IL-2Rα is unique to the IL-2R. IL-2 binds to the α chain alone with low affinity, and this does not lead to any detectable cytoplasmic signaling or biologic response. IL-2/15Rβ, which is also part of the IL-15 receptor, contributes to IL-2 binding and engages JAK3-STAT5–dependent signal transduction pathways (see Chapter 7). The γ chain is shared with a number of cytokine receptors, including those for IL-4, IL-7, IL-9, IL-15, and IL-21, and is therefore called the common γ chain (γ_c). Even though the γ_c is not directly involved in binding IL-2, its association with the receptor complex is required for high-affinity IL-2 binding and for full activation of signal transduction pathways. The IL-2Rβγ_c complexes are expressed at low levels on resting T cells (and on NK cells) and bind IL-2 with a K_d of approximately 10^{-9} M (Fig. 9-10). Expression of IL-2Rα and, to a lesser extent, of IL-2Rβ is increased on activation of naive $CD4^+$ and $CD8^+$ T cells. Cells that express IL-2Rα and form IL-2Rαβγ_c complexes can bind IL-2 more tightly, with a K_d of approximately 10^{-11} M, and growth stimulation of such cells occurs at a similarly low IL-2 concentration. IL-2, produced in response to antigen stimulation, is a stimulus for induction of IL-2Rα, providing a mechanism by which T cell responses amplify themselves. $CD4^+$ regulatory T cells (see Chapter 14) express the full IL-2R complex and are thus poised to respond to the cytokine. Chronic T cell stimulation leads to shedding of IL-2Rα, and an increased level of shed IL-2Rα in the serum is used clinically as a marker of strong antigenic stimulation (e.g., acute rejection of a transplanted organ).

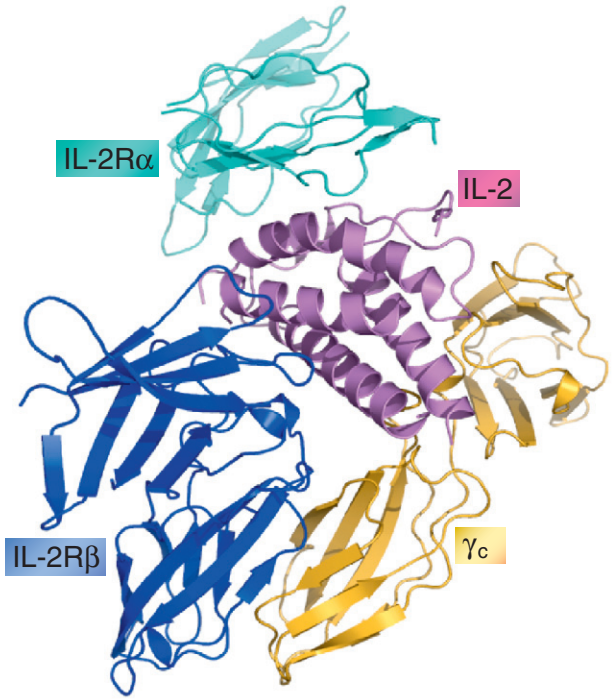


FIGURE 9–9 Structure of IL-2 and its receptor. The crystal structure of IL-2 and its trimeric receptor shows how the cytokine interacts with the three chains of to the receptor. *(Reproduced from Wang X, M Rickert, and KC Garcia. Structure of the quaternary complex of interleukin-2 with its α, β and γc receptors. Science 310:1159-1163, 2005, with the permission of the publishers. Courtesy of Drs. Patrick Lupardus and K. Christopher Garcia, Stanford University School of Medicine, Palo Alto, California.)*

Functions of IL-2

The biology of IL-2 is fascinating because it plays critical roles in both promoting and controlling T cell responses and functions (Fig. 9-11).

- ***IL-2 stimulates the survival, proliferation, and differentiation of antigen-activated T cells.*** IL-2 promotes survival of cells by inducing the antiapoptotic protein Bcl-2. It stimulates cell cycle progression through the synthesis of cyclins and relieves a block in cell cycle progression through p27 degradation. In addition, IL-2 increases production of effector cytokines, such as IFN-γ and IL-4, by the T cells.
- ***IL-2 is required for the survival and function of regulatory T cells,*** which suppress immune responses against self and other antigens. In fact, knockout mice lacking IL-2 or IL-2 receptors develop uncontrolled T and B cell proliferation and autoimmune disease because of a defect in regulatory T cells. These studies indicate other growth factors can replace IL-2 for expansion of effector T cells, but that no other cytokine can replace IL-2 for the maintenance of functional regulatory T cells. We will discuss this role of IL-2 in more detail in Chapter 14, when we describe

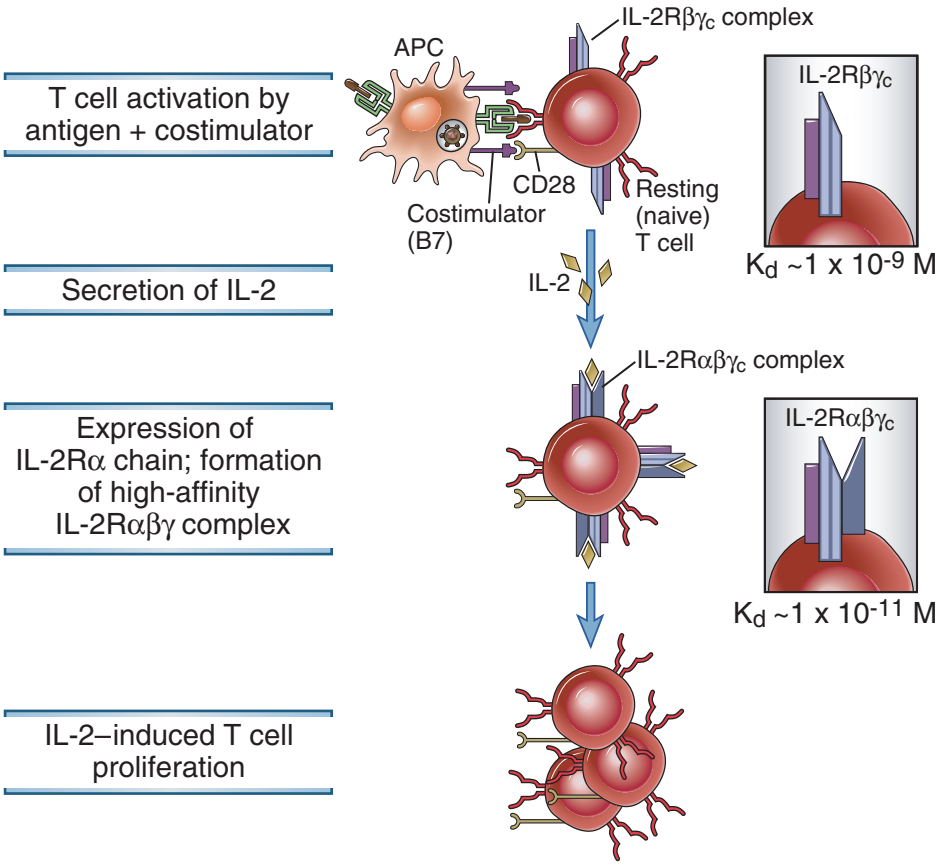


FIGURE 9–10 Regulation of IL-2 receptor expression. Resting (naive) T lymphocytes express the IL-2Rβγ complex, which has a moderate affinity for IL-2. Activation of the T cells by antigen, costimulators, and IL-2 itself leads to expression of the IL-2Rα chain and high levels of the high-affinity IL-2Rαβγ complex.

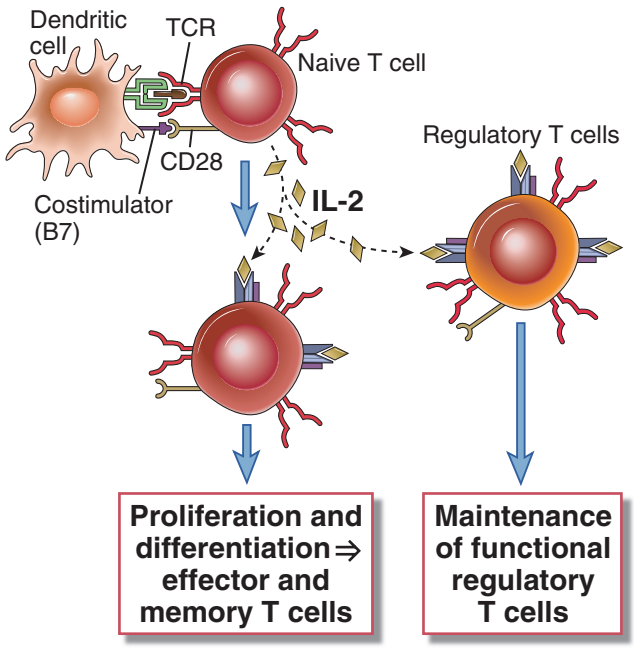


FIGURE 9–11 Biologic actions of IL-2. IL-2 stimulates the survival and proliferation of T lymphocytes, acting as an autocrine growth factor. IL-2 also maintains functional regulatory T cells and thus controls immune responses (e.g., against self antigens).

the properties and generation of regulatory T cells. An interesting feature of this function of IL-2 is that regulatory T cells do not produce significant amounts of the cytokine, implying that they depend for their survival on IL-2 made by other T cells responding to foreign antigens.

- *IL-2 has also been shown to stimulate the proliferation and differentiation of NK cells and B cells in vitro.* The physiologic importance of these actions is not established.

Clonal Expansion of T Cells

T cell proliferation in response to antigen recognition is mediated primarily by a combination of signals from the antigen receptor, costimulators, and autocrine growth factors, primarily IL-2. The cells that recognize antigen produce IL-2 and also preferentially respond to it, ensuring that the antigen-specific T cells are the ones that proliferate the most. The result of this proliferation is **clonal expansion**, which generates the large number of cells required to eliminate the antigen from a small pool of naive antigen-specific lymphocytes. Before antigen exposure, the frequency of naive T cells specific for any antigen is 1 in 10^5 to 10^6 lymphocytes. After microbial antigen exposure, the frequency of all $CD8^+$ T cells specific for that microbe may increase to about 1 in 3 to 1 in 10, representing a >50,000-fold expansion of

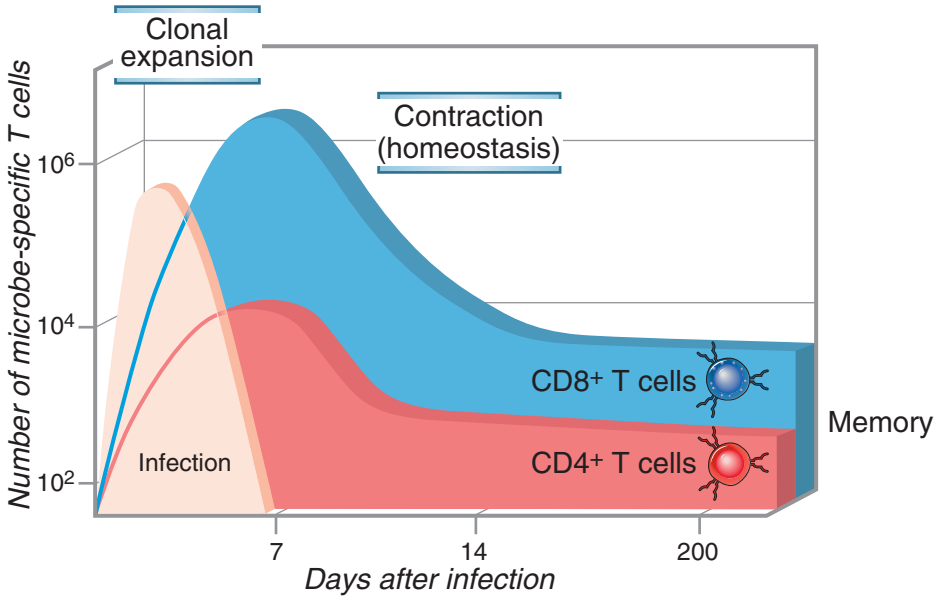


FIGURE 9–12 Clonal expansion of T cells. The numbers of $CD4^+$ and $CD8^+$ T cells specific for microbial antigens and the expansion and decline of the cells during immune responses are illustrated. The numbers are approximations based on studies of model microbial and other antigens in inbred mice.

antigen-specific $CD8^+$ T cells, and the number of specific $CD4^+$ cells increases to 1 in 100 to about 1 in 1000 lymphocytes (Fig. 9-12). Studies in mice first showed this tremendous expansion of the antigen-specific population in some acute viral infections and, remarkably, it occurred within as little as 1 week after infection. Equally remarkable was the finding that during this massive antigen-specific clonal expansion, "bystander" T cells not specific for the virus did not proliferate. The expansion of T cells specific for Epstein-Barr virus and human immunodeficiency virus (HIV) in acutely infected humans is also on this order of magnitude. This conclusion has been reached by analyses of antigen-specific T cell responses in humans, using either fluorescent multimers of MHC molecules loaded with particular peptides or intracellular cytokine stains of T cells stimulated with peptides derived from these viruses (see Appendix IV).

Many of the progeny of the antigen-stimulated cells differentiate into effector cells. Because there are important differences in effector cells of the $CD4^+$ and $CD8^+$ lineages, these are described separately below. Effector cells are short-lived, and the numbers of antigen-specific T cells rapidly decline as the antigen is eliminated. After the immune response subsides, the surviving memory cells specific for the antigen number on the order of 1 in 10^4.

Differentiation of $CD4^+$ T Cells into T_H1, T_H2, and T_H17 Effector Cells

Effector cells of the $CD4^+$ lineage are characterized by their ability to express surface molecules and to secrete cytokines that activate other cells (B lymphocytes, macrophages, and dendritic cells). Whereas naive $CD4^+$ T cells produce mostly IL-2 on activation, effector $CD4^+$ T cells are capable of producing a large number and variety of cytokines that have diverse biologic activities.

There are three distinct subsets of $CD4^+$ T cells, called T_H1, T_H2, and T_H17, that function in host defense against different types of infectious pathogens and are involved in different types of tissue injury in immunologic diseases (Fig. 9-13). A fourth population, called follicular helper T cells, is important in antibody responses and is described in Chapter 11. Regulatory T cells are another distinct population of $CD4^+$ T cells. Their function is to control immune reactions to self and foreign antigens, and they are described in Chapter 14 in the context of immunologic tolerance. Although these subsets are identifiable in immune reactions (and can often be generated in cell culture), many effector $CD4^+$ T cells produce various combinations of cytokines or only some of the cytokines characteristic of a particular subset and are not readily classifiable into separable populations. Whether these populations with mixed or limited cytokine patterns are intermediates in the development of the polarized effector cells or are themselves fixed populations is not known. It is also clear that some of these differentiated T cells may convert from one population into another by changes in activation conditions. The extent and significance of such "plasticity" are topics of active research.

Properties of T_H1, T_H2, and T_H17 Subsets

Elucidation of the development, properties, and functions of subsets of effector $CD4^+$ T cells has been one of the most impressive accomplishments of immunology research. It was appreciated many years ago that host responses to different infections varied greatly, as did the reactions in different immunologic diseases. For instance, the immune reaction to intracellular bacteria like *Mycobacterium tuberculosis* is dominated by activated macrophages, whereas the reaction to helminthic parasites

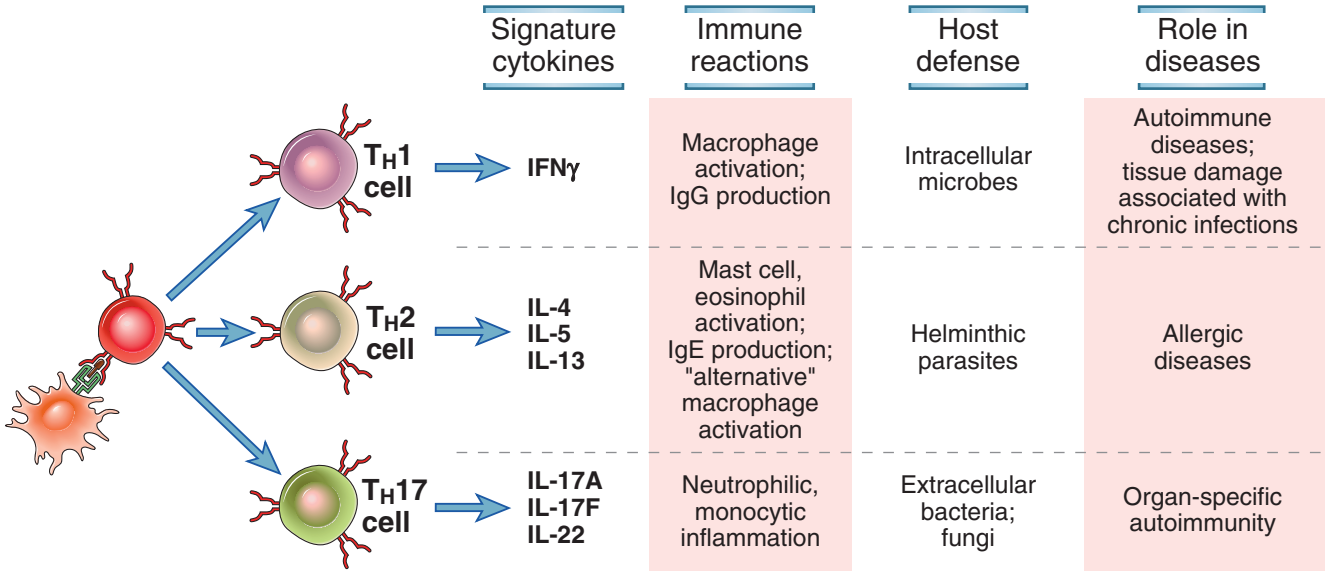


FIGURE 9–13 Properties of T_H1, T_H2, and T_H17 subsets of $CD4^+$ helper T cells. Naive $CD4^+$ T cells may differentiate into distinct subsets of effector cells in response to antigen, costimulators, and cytokines. The columns to the right list the major differences between the best-defined subsets.

consists of IgE antibody production and the activation of eosinophils. Along the same lines, in many chronic autoimmune diseases, tissue damage is caused by inflammation with accumulation of neutrophils, macrophages, and T cells, whereas in allergic disorders, the lesions contain abundant eosinophils along with other leukocytes. The realization that all these phenotypically diverse immunologic reactions are dependent on $CD4^+$ T cells raised an obvious question: How can the same $CD4^+$ cells elicit such different responses? The answer, as we now know, is that $CD4^+$ T cells consist of subsets of effector cells that produce distinct sets of cytokines, elicit quite different reactions, and are involved in host defense against different microbes as well as in distinct types of immunologic diseases. The first subsets that were discovered were called T_H1 and T_H2 (so named because they were the first two subsets identified). It was subsequently found that some inflammatory diseases that were thought to be caused by T_H1-mediated reactions were clearly not dependent on this type of T cell, and this realization led to the discovery of T_H17 cells (called T_H17 because their characteristic cytokine is IL-17). In the next section, we describe the properties of these subsets and how they develop from naive T cells. We will return to their cytokine products, effector functions, and roles in cell-mediated immunity in Chapter 10.

The defining characteristics of differentiated subsets of effector cells are the cytokines they produce, the transcription factors they express, and epigenetic changes in cytokine gene loci. These characteristics of T_H1, T_H2, and T_H17 cells are described below.

The signature cytokines produced by the major $CD4^+$ T cell subsets are IFN-γ for T_H1 cells; IL-4, IL-5, and IL-13 for T_H2 cells; and IL-17 and IL-22 for T_H17 cells (see Fig. 9-13). The cytokines produced by these T cell subsets determine their effector functions and roles in diseases. The cytokines also participate in the development and expansion of the respective subsets (described later). In addition, these subsets of T cells differ in the expression of adhesion molecules and receptors for chemokines and other cytokines, which are involved in the migration of distinct subsets to different tissues (see Chapter 10).

Development of T_H1, T_H2, and T_H17 Subsets

Differentiated T_H1, T_H2, and T_H17 cells all develop from naive $CD4^+$ T lymphocytes, mainly in response to cytokines present early during immune responses, and differentiation involves transcriptional activation and epigenetic modification of cytokine genes. The process of differentiation, which is sometimes referred to as **polarization** of T cells, can be divided into induction, stable commitment, and amplification (Fig. 9-14). Cytokines act on antigen-stimulated T cells to induce the transcription of cytokine genes that are characteristic of differentiation toward each subset. With continued activation, epigenetic changes occur so that the genes encoding that subset's cytokines are more accessible for activation, and genes that encode cytokines not produced by that subset are rendered inaccessible. Because of these changes, the differentiating T cell becomes progressively committed to one specific pathway. Cytokines produced by any given subset promote the development of this subset and inhibit differentiation toward other $CD4^+$ subpopulations. Thus, positive and negative feedback loops contribute to the generation of an increasingly polarized population of effector cells.

There are several important general features of T cell subset differentiation.

- ***The cytokines that drive the development of $CD4^+$ T cell subsets are produced by APCs (primarily dendritic cells and macrophages) and other immune cells (such as NK cells and basophils or mast cells) present at the site of the immune response.*** Dendritic cells that encounter

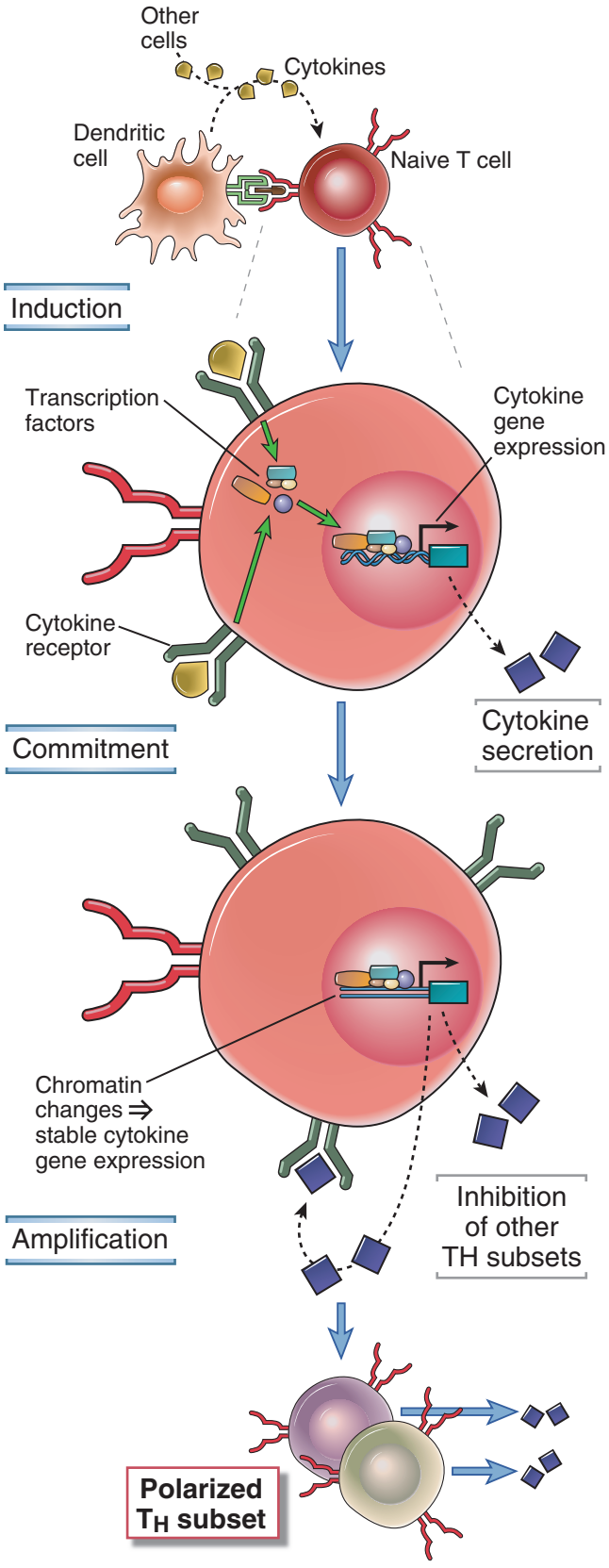


FIGURE 9–14 Development of T_H1, T_H2, and T_H17 subsets. Cytokines produced early in the innate or adaptive immune response to microbes promote the differentiation of naive $CD4^+$ T cells into T_H1, T_H2, or T_H17 cells by activating transcription factors that stimulate production of the cytokines of each subset (the early induction step). Progressive activation leads to stable changes in the expressed genes (commitment), and cytokines promote the development of each population and suppress the development of the other subsets (amplification). These principles apply to all three major subsets of $CD4^+$ effector T cells.

microbes and display microbial antigens are activated to produce cytokines (as well as costimulators, described earlier) as part of innate immune responses to the microbes (see Chapter 4). Different microbes may stimulate dendritic cells to produce distinct sets of cytokines, perhaps because the microbes are recognized by different microbial sensors in the cells. Other cells of innate immunity, such as NK cells and mast cells, also produce cytokines that influence the pattern of T cell subset development.

- ***Stimuli other than cytokines may also influence the pattern of helper T cell differentiation.*** Some studies indicate that different subsets of dendritic cells selectively promote either T_H1 or T_H2 differentiation; the same principle may be true for T_H17 cells. In addition, the genetic makeup of the host is an important determinant of the pattern of T cell differentiation. Inbred mice of some strains develop T_H2 responses to the same microbes that stimulate T_H1 differentiation in most other strains. Strains of mice that develop T_H2-dominant responses are susceptible to infections by intracellular microbes (see Chapter 15).
- ***The distinct cytokine profiles of differentiated cell populations are controlled by particular transcription factors that activate cytokine gene transcription and by chromatin modifications affecting cytokine gene loci.*** The transcription factors are themselves activated or induced by cytokines as well as by antigen receptor stimuli. Each subset expresses its own characteristic set of transcription factors. As the subsets become increasingly polarized, the gene loci encoding that subset's signature cytokines undergo histone modifications (changes in methylation and acetylation) and consequent chromatin remodeling events, so that these loci are "accessible" and in an "open" chromatin configuration, whereas the loci for other cytokines (those not produced by that subset) are in an inaccessible chromatin state. These epigenetic changes ensure that each subset can produce only its characteristic collection of cytokines. It is likely that epigenetic changes in cytokine gene loci correlate with stable phenotypes, and before these changes are established, the subsets may be plastic and convertible.
- ***Each subset of differentiated effector cells produces cytokines that promote its own development and may suppress the development of the other subsets.*** This feature of T cell subset development provides a powerful amplification mechanism. For instance, IFN-γ secreted by T_H1 cells promotes further T_H1 differentiation and inhibits the generation of T_H2 and T_H17 cells. Similarly, IL-4 produced by T_H2 cells promotes T_H2

differentiation, and IL-21 produced by T_H17 cells enhances T_H17 differentiation. Thus, each subset amplifies itself and may inhibit the other subsets. For this reason, once an immune response develops along one effector pathway, it becomes increasingly polarized in that direction, and the most extreme polarization is seen in chronic infections or in chronic exposure to environmental antigens, when the immune stimulation is prolonged.

- ***Differentiation of each subset is induced by the types of microbes which that subset is best able to combat.*** For instance, the development of T_H1 cells from antigen-stimulated T cells is driven by intracellular microbes, against which the principal defense is T_H1 mediated. Conversely, the immune system responds to helminthic parasites by the development of T_H2 cells, and the cytokines produced by these cells are critical for combating helminths. Similarly, T_H17 responses are induced by some bacteria and fungi and are most effective at defending against these microbes. The generation and effector functions of these differentiated T cells are an excellent illustration of the concept of *specialization of adaptive immunity,* which refers to the ability of the immune system to respond to different microbes in ways that are optimal for combating those microbes.

With this background, we proceed to a description of the signals for and mechanisms of development of each subset.

T_H1 Differentiation

T_H1 differentiation is driven mainly by the cytokines IL-12 and IFN-γ and occurs in response to microbes that activate dendritic cells, macrophages, and NK cells (Fig. 9-15). The differentiation of antigen-activated $CD4^+$ T cells to T_H1 effectors is stimulated by many intracellular bacteria, such as *Listeria* and mycobacteria, and by some parasites, such as *Leishmania,* all of which infect dendritic cells and macrophages. It is also stimulated by viruses and by protein antigens administered with strong adjuvants. A common feature of these infections and immunization conditions is that they elicit innate immune reactions that are associated with the production of certain cytokines, including IL-12, IL-18, and type I interferons. All these cytokines promote T_H1 development; of these, IL-12 is probably the most potent. Knockout mice lacking IL-12 are extremely susceptible to infections with intracellular microbes. IL-18 synergizes with IL-12, and type I interferons may be important for T_H1 differentiation in response to viral infections, especially in humans. Other microbes stimulate NK cells to produce IFN-γ, which is itself a strong T_H1-inducing cytokine and also acts on dendritic cells and macrophages to induce more IL-12 secretion. Once T_H1 cells have developed, they secrete IFN-γ, which promotes more T_H1 differentiation and thus strongly amplifies the reaction. In addition, IFN-γ inhibits the differentiation of naive $CD4^+$ T cells to the T_H2 and T_H17 subsets, thus promoting the polarization of the immune response in one direction. T cells may further enhance cytokine production by dendritic cells and macrophages, by virtue of CD40 ligand (CD40L) on activated

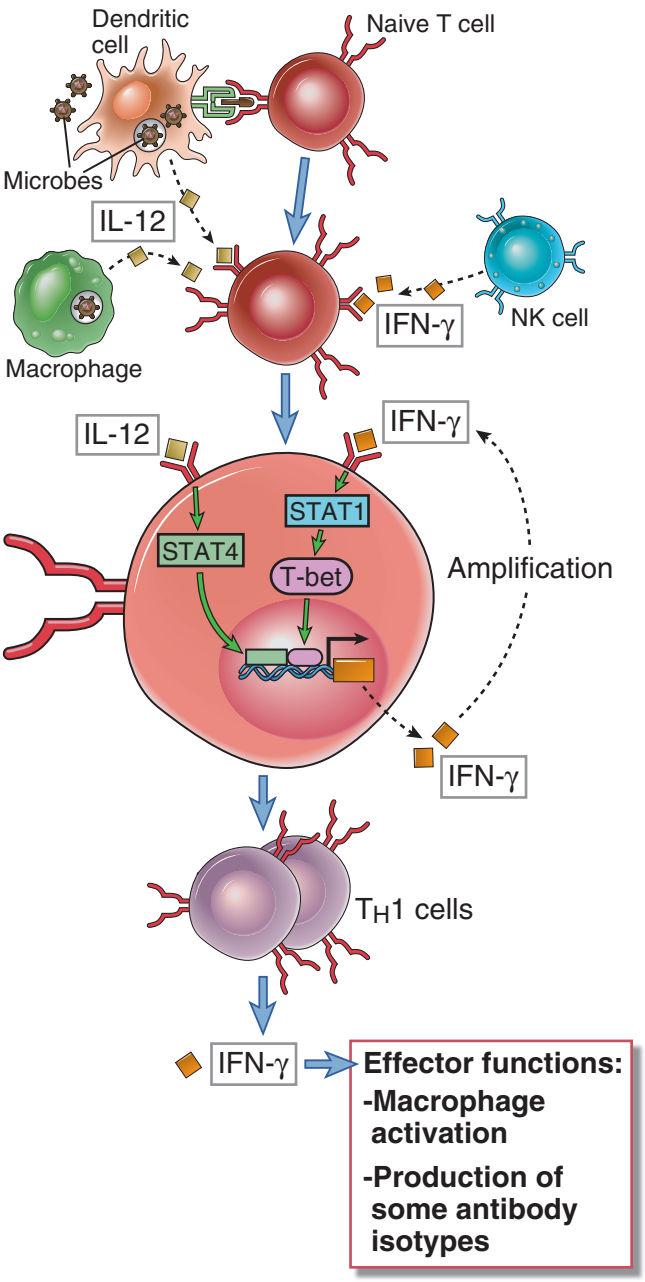


FIGURE 9–15 Development of T_H1 cells. IL-12 produced by dendritic cells and macrophages in response to microbes, including intracellular microbes, and IFN-γ produced by NK cells (all part of the early innate immune response to the microbes) activate the transcription factors T-bet, STAT1, and STAT4, which stimulate the differentiation of naive $CD4^+$ T cells to the T_H1 subset. IFN-γ produced by the T_H1 cells amplifies this response and inhibits the development of T_H2 and T_H17 cells.

T cells engaging CD40 on the APCs and stimulating IL-12 secretion.

IFN-γ and IL-12 stimulate T_H1 differentiation by activating the transcription factors T-bet, STAT1, and STAT4 (see Fig. 9-15). T-bet, a member of the T-box family of transcription factors, is considered to be the master regulator of T_H1 differentiation. T-bet expression is induced in naive $CD4^+$ T cells in response to antigen and IFN-γ. IFN-γ activates the transcription factor STAT1, which in turn stimulates expression of T-bet. T-bet then promotes

IFN-γ production through a combination of direct transcriptional activation of the IFN-γ gene and by inducing chromatin remodeling of the IFN-γ locus. The ability of IFN-γ to stimulate T-bet expression and the ability of T-bet to enhance IFN-γ transcription set up a positive amplification loop that drives differentiation of T cells toward the T_H1 phenotype. IL-12 contributes to T_H1 commitment by binding to receptors on antigen-stimulated $CD4^+$ T cells and activating the transcription factor STAT4, which further enhances IFN-γ production. Mice deficient in IL-12, IL-12 receptor, T-bet, or STAT4 cannot mount effective T_H1 responses to infections, and humans with genetic deficiencies in the IL-12R signaling pathway have impaired responses to infections with several kinds of intracellular bacteria.

T_H2 Differentiation

T_H2 differentiation is stimulated by the cytokine IL-4 and occurs in response to helminths and allergens (Fig. 9-16). Helminths and allergens cause chronic T cell stimulation, often without the strong innate immune responses that are required for T_H1 differentiation. Thus, T_H2 cells may develop in response to microbes and antigens that cause persistent or repeated T cell stimulation without much inflammation or the production of pro-inflammatory cytokines that drive T_H1 and T_H17 responses. The differentiation of antigen-stimulated T cells to the T_H2 subset is dependent on IL-4, which raises an interesting question: Because differentiated T_H2 cells are the major source of IL-4 during immune responses to protein antigens, where does the IL-4 come from before T_H2 cells develop? In some situations, such as helminthic infections, IL-4 produced by mast cells and, possibly, other cell populations, such as basophils recruited into lymphoid organs and eosinophils, may contribute to T_H2 development. Another possibility is that antigen-stimulated $CD4^+$ T cells secrete small amounts of IL-4 from their initial activation. If the antigen is persistent and present at high concentrations, the local concentration of IL-4 gradually increases. If the antigen also does not trigger inflammation with attendant IL-12 production, the result is increasing differentiation of T cells to the T_H2 subset. Once T_H2 cells have developed, the IL-4 they produce serves to amplify the reaction and inhibits the development of T_H1 and T_H17 cells.

IL-4 stimulates T_H2 development by activating the transcription factor STAT6, and STAT6, together with TCR signals, induces expression of GATA-3 (see Fig. 9-16). GATA-3 is a transcription factor that acts as a master regulator of T_H2 differentiation, enhancing expression of the T_H2 cytokine genes IL-4, IL-5, and IL-13, which are located in the same genetic locus. GATA-3 works by directly interacting with the promoters of these genes and also by causing chromatin remodeling, which opens up the locus for accessibility to other transcription factors. This is similar to the way in which T-bet influences IFN-γ expression. GATA-3 functions to stably commit differentiating cells toward the T_H2 phenotype, enhancing its own expression through a positive feedback loop. Furthermore, GATA-3 blocks T_H1 differentiation by inhibiting expression of the signaling chain of the IL-12 receptor. Knockout mice lacking IL-4, STAT6, or GATA-3 are deficient in T_H2 responses.

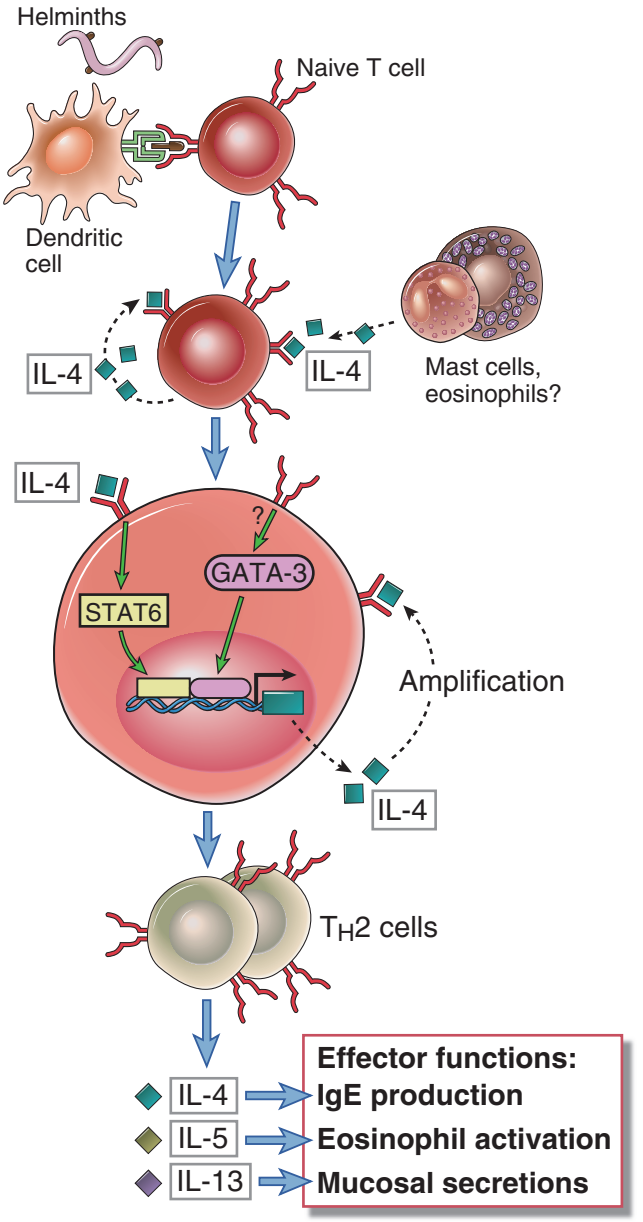


FIGURE 9–16 Development of T_H2 cells. IL-4 produced by activated T cells themselves or by mast cells and eosinophils, especially in response to helminths, activates the transcription factors GATA-3 and STAT6, which stimulate the differentiation of naive $CD4^+$ T cells to the T_H2 subset. IL-4 produced by the T_H2 cells amplifies this response and inhibits the development of T_H1 and T_H17 cells.

T_H17 Differentiation

The development of T_H17 cells is stimulated by pro-inflammatory cytokines produced in response to bacteria and fungi (Fig. 9-17). Various bacteria and fungi act on dendritic cells and stimulate the production of cytokines including IL-6, IL-1, and IL-23. Engagement of the lectin-like receptor Dectin-1 on dendritic cells by fungal products is a signal for the production of these cytokines. The combination of cytokines that drive T_H17 cell development may be produced not only in response to particular

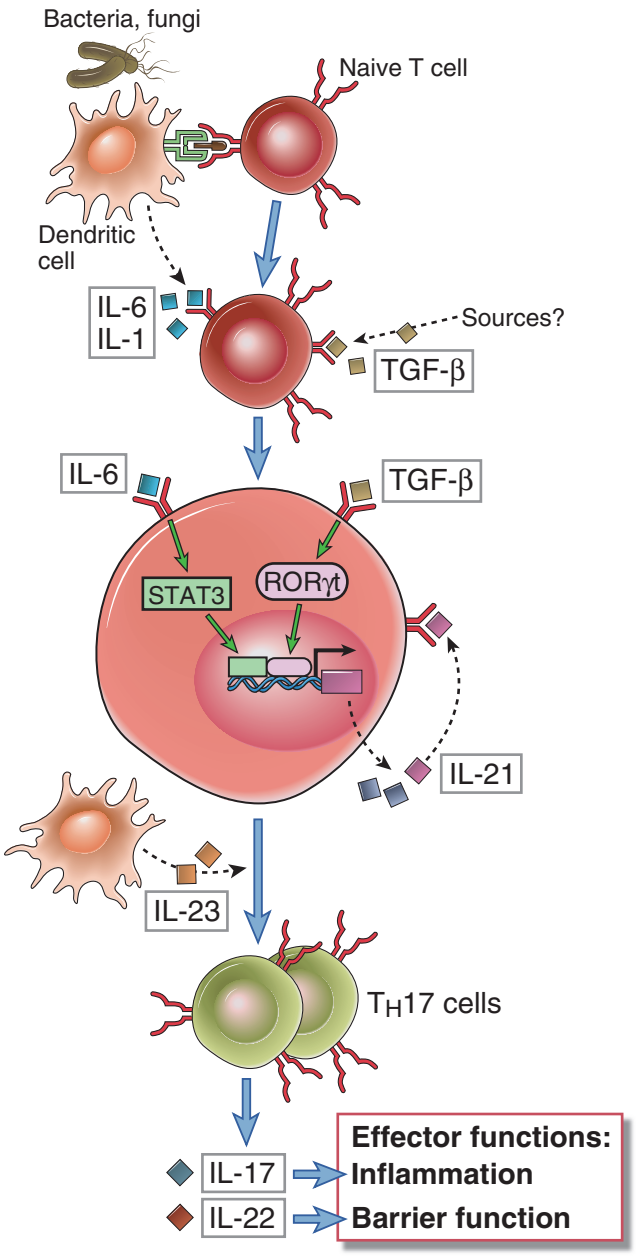


FIGURE 9–17 Development of T_H17 cells. IL-1 and IL-6 produced by APCs and transforming growth factor-β (TGF-β) produced by various cells activate the transcription factors RORγt and STAT3, which stimulate the differentiation of naive $CD4^+$ T cells to the T_H17 subset. IL-23, which is also produced by APCs, especially in response to fungi, stabilizes the T_H17 cells. TGF-β may promote T_H17 responses indirectly by suppressing T_H1 and T_H2 cells, both of which inhibit T_H17 differentiation (not shown in the figure). IL-21 produced by the T_H17 cells amplifies this response.

microbes, such as fungi, but also when cells infected with various bacteria and fungi undergo apoptosis and are ingested by dendritic cells. IL-23 may be more important for the proliferation and maintenance of T_H17 cells than for their induction. T_H17 differentiation is inhibited by IFN-γ and IL-4; therefore, strong T_H1 and T_H2 responses tend to suppress T_H17 development. A surprising aspect of T_H17 differentiation is that TGF-β, which is produced by many cell types and is an anti-inflammatory cytokine (see Chapter 14), promotes the development of proinflammatory T_H17 cells when other mediators of inflammation, such as IL-6 or IL-1, are present. Some experimental results indicate that TGF-β does not directly stimulate T_H17 development but is a potent suppressor of T_H1 and T_H2 differentiation and thus removes the inhibitory effect of these two subsets and allows the T_H17 response to develop under the influence of IL-6 or IL-1. According to this idea, the action of TGF-β in promoting T_H17 responses is indirect. T_H17 cells produce IL-21, which may further enhance their development, providing an amplification mechanism.

The development of T_H17 cells is dependent on the transcription factors RORγt and STAT3 (see Fig. 9-17). TGF-β and the inflammatory cytokines, mainly IL-6 and IL-1, work cooperatively to induce the production of RORγt, a transcription factor that is a member of the retinoic acid receptor family. RORγt is a T cell–restricted protein encoded by the *RORC* gene, so sometimes the protein may be referred to as RORc. The inflammatory cytokines, notably IL-6, activate the transcription factor STAT3, which functions with RORγt to drive the T_H17 response. Mutations in the gene encoding STAT3 are the cause of a rare human immune deficiency disease called Job's syndrome because patients present with multiple bacterial and fungal abscesses of the skin, resembling the biblical punishments visited on Job. These patients have defective T_H17 responses.

T_H17 cells appear to be especially abundant in mucosal tissues, particularly of the gastrointestinal tract, suggesting that the tissue environment influences the generation of this subset, perhaps by providing high local concentrations of TGF-β and other cytokines. This observation also suggests that T_H17 cells may be especially important in combating intestinal infections and in the development of intestinal inflammation. The development of T_H17 cells in the gastrointestinal tract is also dependent on the local microbial population.

The functions of differentiated effector cells of the $CD4^+$ lineage are mediated by surface molecules, primarily CD40 ligand, and by secreted cytokines. We will describe the cytokines produced by differentiated $CD4^+$ effector cells and their functions in Chapter 10.

Differentiation of $CD8^+$ T Cells into Cytotoxic T Lymphocytes

The activation of naive $CD8^+$ T cells requires antigen recognition and second signals, but the nature of the second signals may be different from those for $CD4^+$ cells. We have previously described the role of dendritic cells in presenting antigens to and costimulating naive $CD8^+$ cells.

The full activation of naive $CD8^+$ T cells and their differentiation into functional CTLs and memory cells may require the participation of $CD4^+$ helper cells. In other words, helper T cells can provide second signals for $CD8^+$ T cells. The requirement for helper cells may vary according to the type of antigen exposure. In the setting of a strong innate immune response to a microbe, if APCs are directly infected by the microbe, or if cross-presentation

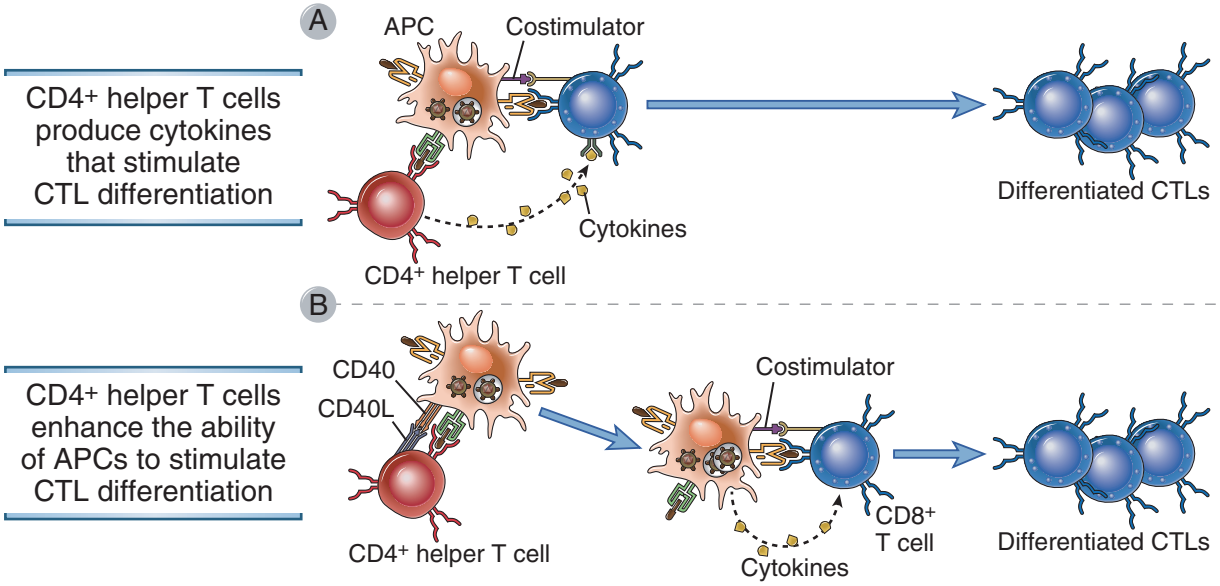


FIGURE 9–18 Role of helper T cells in the differentiation of CD8$^+$ T lymphocytes. CD4$^+$ helper T cells promote the development of CD8$^+$ CTLs by secreting cytokines that act directly on the CD8$^+$ cells **(A)** or by activating APCs to become more effective at stimulating the differentiation of the CD8$^+$ T cells **(B)**.

of microbial antigens is efficient, CD4$^+$ T cell help may not be required. CD4$^+$ helper T cells may be required for CD8$^+$ T cell responses to latent viral infections, organ transplants, and tumors, all of which tend to elicit relatively weak innate immune reactions. The varying importance of CD4$^+$ T cells in the development of CTL responses is illustrated by studies with mice that lack helper T cells. In these mice, some viral infections fail to generate effective CTLs or CD8$^+$ memory cells and are not eradicated, whereas other viruses do stimulate effective CTL responses. A lack of CD4$^+$ T cell helper function is the accepted explanation for the defects in CTL generation seen in individuals infected with HIV, which infects and eliminates only CD4$^+$ T cells.

Helper T cells may promote CD8$^+$ T cell activation by several mechanisms (Fig. 9-18). Helper T cells may secrete cytokines that stimulate the differentiation of CD8$^+$ T cells. Antigen-stimulated helper T cells express CD40 ligand (CD40L), which binds to CD40 on APCs and activates ("licenses") these APCs to make them more efficient at stimulating the differentiation of CD8$^+$ T cells.

Differentiation of CD8$^+$ T cells into effector CTLs involves acquisition of the machinery to perform target cell killing. The most specific feature of CTL differentiation is the development of membrane-bound cytoplasmic granules that contain proteins, including perforin and granzymes, whose function is to kill other cells (described in Chapter 10). In addition, differentiated CTLs are capable of secreting cytokines, mostly IFN-γ, that function to activate phagocytes. The molecular events in CTL differentiation involve transcription of genes encoding these effector molecules. Two transcription factors that are required for this program of new gene expression are T-bet (which we discussed in relationship to T_H1 differentiation earlier) and eomesodermin, which is structurally related to T-bet.

Development of Memory T Cells

T cell–mediated immune responses to an antigen usually result in the generation of memory T cells specific for that antigen, which may persist for years, even a lifetime. Thus, memory cells provide optimal defense against pathogens that are prevalent in the environment and may be repeatedly encountered. The success of vaccination is attributed in large part to the ability to generate memory cells on initial antigen exposure. Edward Jenner's classic experiment of successful vaccination of a child against smallpox is a demonstration of a memory response. Despite the importance of this historic observation, many fundamental questions about the generation of memory cells have still not been answered.

As expected, the size of the memory pool is proportional to the size of the naive antigen-specific population. Memory cells may develop from effector cells along a linear pathway, or effector and memory populations follow divergent differentiation and are two alternative fates of lymphocytes activated by antigen and other stimuli (Fig. 9-19). The mechanisms that determine whether an individual antigen-stimulated T cell will become a short-lived effector cell or enter the long-lived memory cell pool are not established. The signals that drive the development of memory cells are also not established. One possibility is that memory cells contain transcription factors that are different from those present in effector cells, and the nature of the transcription factors influences the choice of differentiation pathway.

Properties of Memory T Cells

Memory T cells have several characteristics that are responsible for their survival and rapid activation.

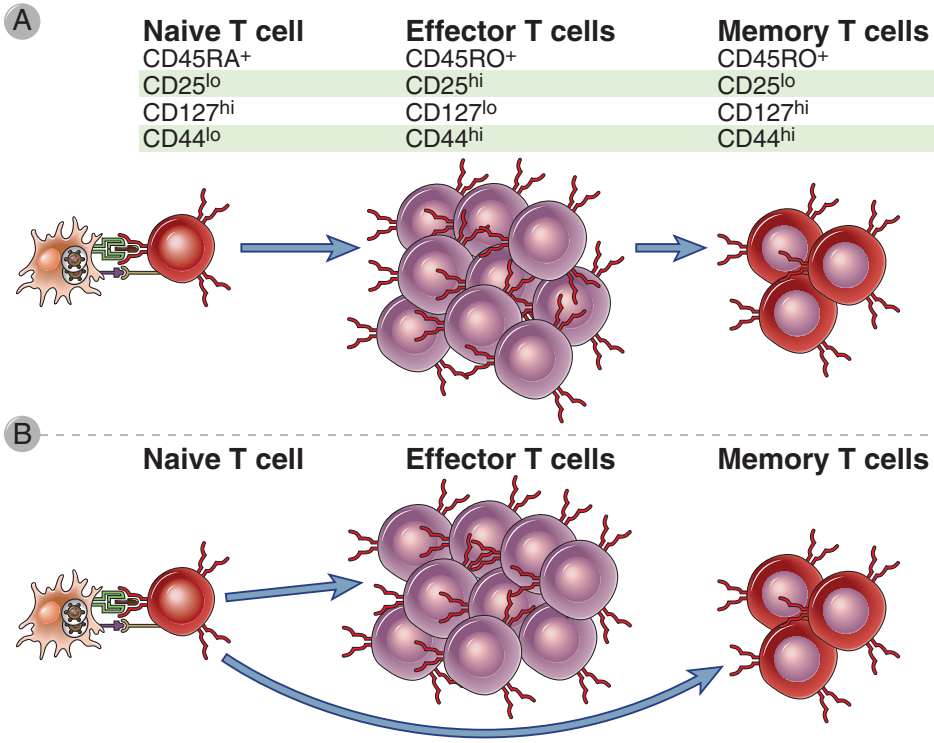


FIGURE 9–19 Development of memory T cells. In response to antigen and costimulation, naive T cells differentiate into effector and memory cells. Some of the phenotypic markers of these cell populations are shown in A. **A,** According to the linear model of memory T cell differentiation, most effector cells die and some survivors develop into the memory population. **B,** According to the branched differentiation model, effector and memory cells are alternative fates of activated T cells.

- ***The defining properties of memory cells are their ability to survive in a quiescent state after antigen is eliminated and to mount larger and more rapid responses to antigens than do naive cells.*** Whereas naive T cells live for weeks or months and are replaced by mature cells that develop in the thymus, memory T cells may survive for months or years. Thus, as humans age in an environment in which they are constantly exposed to and responding to infectious agents, the proportion of memory cells induced by these microbes compared with naive cells progressively increases. In individuals older than 50 years or so, half or more of circulating T cells may be memory cells. The rapid response of memory cells to antigen challenge has been documented in many studies done in humans and experimental animals. For example, in studies in mice, naive T cells respond to antigen in vivo in 5 to 7 days, and memory cells respond within 1 to 3 days (see Fig. 1-4, Chapter 1).
- ***The number of memory T cells specific for any antigen is greater than the number of naive cells specific for the same antigen.*** As we discussed earlier, proliferation leads to a large clonal expansion in all immune responses and differentiation into effector cells, most of which die after the antigen is eliminated. The surviving cells of the expanded clone are memory cells, and they are typically 10- to 100-fold more numerous than the pool of naive cells before antigen encounter. The increased clone size is a major reason that antigen challenge in a previously immunized individual induces a more robust response than the first immunization in a naive individual.
- ***Memory cells express increased levels of antiapoptotic proteins, which may be responsible for their prolonged survival.*** These proteins include Bcl-2 and Bcl-X_L, which promote mitochondrial stability and block apoptosis induced by a deficiency of survival signals (see Fig. 14-7, Chapter 14). The presence of these proteins allows memory cells to survive even after antigen is eliminated and innate immune responses have subsided, and the normal signals for T cell survival and proliferation are no longer present.
- ***Memory cells undergo slow proliferation, and this ability to self-renew may contribute to the long life span of the memory pool.*** The cycling of these cells may be driven by cytokines. Because of the capacity for self-renewal, memory cells have been likened to stem cells.
- ***The maintenance of memory cells is dependent on cytokines but does not require antigen recognition.*** The most important cytokine for the maintenance of memory $CD4^+$ and $CD8^+$ T cells is IL-7, which also plays a key role in early lymphocyte development (see Chapter 8) and in the survival of naive T cells (see Chapter 2). Predictably, high expression of the IL-7 receptor (CD127) is characteristic of memory T cells. Memory $CD8^+$ T cells also depend on the related cytokine IL-15 for their survival. IL-7 and IL-15 induce the expression of antiapoptotic proteins and stimulate low-level proliferation, both of which maintain populations of memory T cells during long periods. The independence of memory cells from antigen recognition has been best demonstrated by experiments in mice in which antigen receptors are genetically deleted after mature lymphocytes have developed. In these mice, the number of naive lymphocytes drops rapidly but memory cells are maintained.
- ***The gene loci for cytokines and other effector molecules may be fixed in an accessible configuration in memory***

cells. There is some evidence that the chromatin surrounding cytokine genes in $CD4^+$ memory T cells and genes encoding molecules such as perforin in memory $CD8^+$ T cells are in an accessible configuration, perhaps because of changes in methylation and acetylation of histones. As a result, these genes may be poised to respond rapidly to antigen challenge, accounting for the rapid effector responses of memory cells. Memory T cells are also less dependent on costimulation than are naive cells, allowing memory cells to respond to antigens presented by a wide range of APCs in peripheral tissues; in contrast, as we have discussed earlier in this chapter and in Chapter 6, naive T cells are dependent on antigen presentation by mature dendritic cells in lymphoid organs. The relative costimulation independence of memory T cells may also be related to the accessible state of the gene loci that encode molecules involved in T cell responses.

The most reliable phenotypic markers for memory T cells appear to be the surface expression of the IL-7 receptor and a protein of unknown function called CD27 and the absence of markers of naive and recently activated T cells (see Table 2-3, Chapter 2; and Fig. 9-19). In humans, most naive T cells express the 200-kD isoform of a surface molecule called CD45 that contains a segment encoded by an exon designated A. This CD45 isoform can be recognized by antibodies specific for the A-encoded segment and is therefore called CD45RA (for "restricted A"). In contrast, most memory T cells express a 180-kD isoform of CD45 in which the A exon RNA has been spliced out; this isoform is called CD45RO. However, this way of distinguishing naive from memory T cells is not perfect, and interconversion between $CD45RA^+$ and $CD45RO^+$ populations has been documented.

Both $CD4^+$ and $CD8^+$ memory T cells are heterogeneous and can be subdivided into subsets based on their homing properties and functions. **Central memory** T cells express the chemokine receptor CCR7 and L-selectin and home mainly to lymph nodes. They have a limited capacity to perform effector functions when they encounter antigen, but they undergo brisk proliferative responses and generate many effector cells on antigen challenge. **Effector memory** T cells, on the other hand, do not express CCR7 or L-selectin and home to peripheral sites, especially mucosal tissues. On antigenic stimulation, effector memory T cells produce effector cytokines such as IFN-γ or rapidly become cytotoxic, but they do not proliferate much. This effector subset, therefore, is poised for a rapid response to a repeated exposure to a microbe, but complete eradication of the infection may also require large numbers of effectors generated from the central memory T cells. It is unclear if all memory T cells can be classified into central and effector memory cells.

Memory T cells are also heterogeneous in terms of cytokine profiles. For example, some $CD4^+$ memory T cells may be derived from precursors before commitment to the T_H1, T_H2, or T_H17 phenotype, and when activated by re-exposure to antigen and cytokines, they can differentiate into any of these subsets. Other memory T cells may be derived from fully differentiated T_H1, T_H2, or T_H17 effectors and retain their respective cytokine profiles on reactivation. Memory $CD8^+$ T cells may also exist that maintain some of the phenotypic characteristics of differentiated CTLs.

DECLINE OF T CELL RESPONSES

Elimination of antigen leads to contraction of the T cell response, and this decline is responsible for maintaining homeostasis in the immune system. There are several reasons that the response declines. As the antigen is eliminated and the innate immune response associated with antigen exposure abates, the signals that normally keep activated lymphocytes alive and proliferating are no longer active. As mentioned earlier, costimulation and growth factors like IL-2 stimulate expression of the antiapoptotic proteins Bcl-2 and Bcl-X_L in the activated lymphocytes, and these proteins keep cells viable. As the level of costimulation and the amount of available IL-2 decrease, the levels of antiapoptotic proteins in the cells drop. At the same time, growth factor deprivation activates sensors of cellular stress (such as the BH3-only protein Bim), which trigger the mitochondrial pathway of apoptosis and are no longer opposed by the antiapoptotic proteins (see Fig. 14-7, Chapter 14). The net result of these changes is that most of the cells that were produced by activation die and the generation of newly activated cells declines, so the pool of antigen-activated lymphocytes contracts.

There has been much interest in the possibility that various regulatory mechanisms play a role in the normal contraction of immune responses. Such mechanisms might include the inhibitory receptors CTLA-4 and PD-1, apoptosis induced by death receptors of the TNF receptor superfamily (such as TNFRI and Fas), and regulatory T cells. However, it is still not established that these control mechanisms are essential for the normal decline of most immune responses.

SUMMARY

- T cell responses are initiated by signals that are generated by TCR recognition of peptide-MHC complexes on the surface of an APC and through signals provided at the same time by costimulators expressed on APCs.
- The best-defined costimulators are members of the B7 family, which are recognized by receptors of the CD28 family expressed on T cells. The expression of B7 costimulators on APCs is increased by encounter with microbes, providing a mechanism for generating optimal responses against infectious pathogens. Some members of the CD28 family inhibit T cell responses, and the outcome of T cell antigen recognition is determined by the balance between engagement of activating and inhibitory receptors of this family.
- T cell responses to antigen and costimulators include changes in the expression of surface molecules, synthesis of cytokines and cytokine

receptors, cellular proliferation, and differentiation into effector and memory cells.

- The surface molecules whose expression is induced on T cell activation include proteins that are involved in retention of T cells in lymphoid organs, growth factors for cytokines, effector and regulatory molecules, and molecules that influence the migration of the T cells.
- Shortly after activation, T cells produce the cytokine IL-2 and express high levels of the functional IL-2 receptor. IL-2 drives the proliferation of the cells, which can result in marked expansion of antigen-specific clones.
- $CD4^+$ helper T lymphocytes may differentiate into specialized effector T_H1 cells that secrete IFN-γ, which mediate defense against intracellular microbes, or into T_H2 cells that secrete IL-4 and IL-5, which favor IgE- and eosinophil/mast cell–mediated immune reactions against helminths, or into T_H17 cells, which promote inflammation and mediate defense against extracellular fungi and bacteria.
- The differentiation of naive $CD4^+$ T cells into subsets is induced by cytokines produced by APCs and by the T cells themselves. The differentiation program is governed by transcription factors that promote cytokine gene expression in the T cells and epigenetic changes in cytokine gene loci, which may be associated with stable commitment to a particular subset. Each subset produces cytokines that increase its own development and inhibit the development of the other subsets, thus leading to increasing polarization of the response.
- T cells of the $CD8^+$ subset proliferate and differentiate into cytotoxic T lymphocytes (CTLs), which express cytotoxic granules and can kill infected cells.
- Some activated T cells may differentiate into memory cells, which survive for long periods and respond rapidly to antigen challenge. The maintenance of memory cells is dependent on cytokines such as IL-7, which may promote the expression of antiapoptotic proteins and stimulate low-level cycling. Memory T cells are heterogeneous and consist of populations that differ in migration properties and functional responses.

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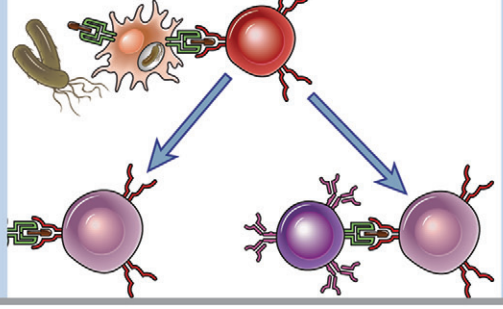
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CHAPTER

10

Effector Mechanisms of Cell-Mediated Immunity

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Cell-mediated immunity is the type of host defense that is mediated by T lymphocytes, and it serves as the defense mechanism against microbes that survive and replicate inside phagocytes and nonphagocytic cells. Historically, immunologists have divided adaptive immunity into humoral immunity, which can be adoptively transferred from an immunized donor to a naive host by antibodies in the absence of cells, and cell-mediated immunity, which can be adoptively transferred only by viable T lymphocytes. The effector phase of humoral immunity is triggered by the recognition of antigen by secreted antibodies; therefore, humoral immunity neutralizes and eliminates extracellular microbes and toxins that are accessible to antibodies, but it is not effective against microbes inside cells. In contrast, in cell-mediated immunity, the effector phase is initiated by the recognition of antigens by T cells. T lymphocytes recognize protein antigens of microbes that are displayed on the surfaces of infected cells as peptides bound to self major histocompatibility complex (MHC) molecules. Therefore, cell-mediated immunity is specific for cell-associated microbes. Defects in cell-mediated immunity result in increased susceptibility to infection by viruses and intracellular bacteria as well as some extracellular bacteria and fungi that are ingested by phagocytes. T cell–mediated reactions are also important in allograft rejection (see Chapter 16), anti-tumor immunity (see Chapter 17), and immune-mediated inflammatory diseases (see Chapter 18). In Chapter 9, we described the activation of T lymphocytes and the differentiation of naive T cells into effector and memory cells. In this chapter, we discuss the reactions of effector T cells in cell-mediated immunity.

TYPES OF CELL-MEDIATED IMMUNE REACTIONS

Different populations of T cells have evolved to combat different types of infectious pathogens. Thus, cell-mediated immunity provides excellent examples of the specialization of adaptive immunity. There are several important general principles of cell-mediated immune reactions.

- ***Effector T cells of the $CD4^+$ lineage link specific recognition of microbes with the recruitment and activation of other leukocytes that destroy the microbes*** (Fig. 10-1A). The nature of the leukocytes that are recruited and activated is determined by the subset of $CD4^+$ effector T cells that are induced in the immune response. In general, T_H1 cells activate macrophages, T_H17 reactions are dominated by neutrophils (and variable numbers of macrophages), and T_H2 cells recruit and activate eosinophils. Each type of leukocyte is specially adapted to destroy particular types of microbes. This cooperation of T lymphocytes and other leukocytes illustrates an important link between adaptive and innate immunity: by means of cytokine

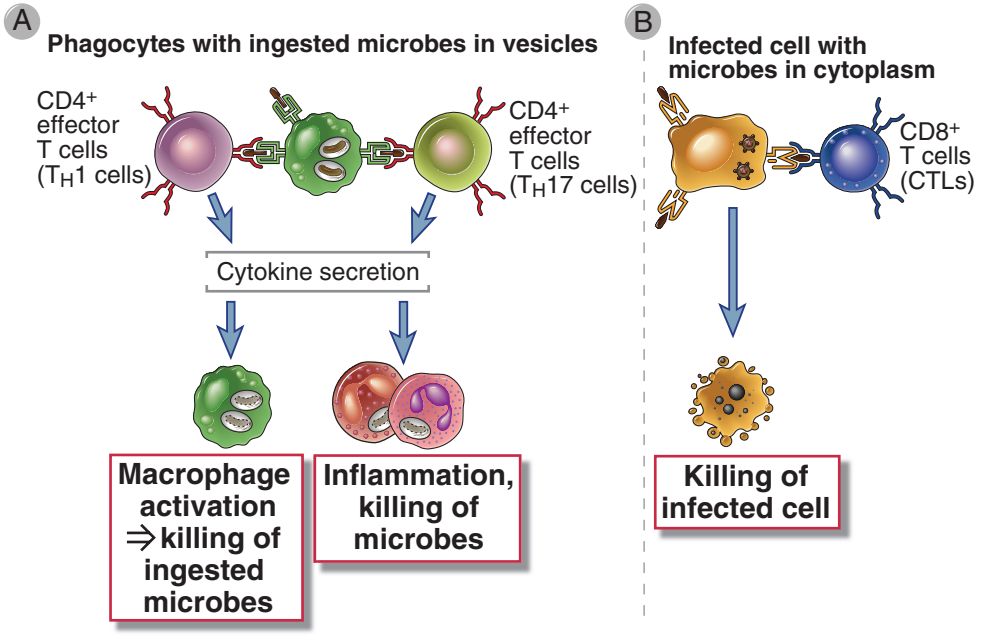


FIGURE 10–1 Types of T cell–mediated immune reactions. A, $CD4^+$ T cells recognize antigens of phagocytosed and extracellular microbes and produce cytokines that activate the phagocytes to kill the microbes and stimulate inflammation. $CD8^+$ T cells can also secrete cytokines and participate in similar reactions. **B,** $CD8^+$ cytotoxic T lymphocytes (CTLs) recognize antigens of microbes residing in the cytoplasm of infected cells and kill the cells.

secretion, T cells stimulate the function and focus the activity of nonspecific effector cells of innate immunity (such as phagocytes and eosinophils), thereby converting these cells into agents of adaptive immunity.

- ***The adaptive immune response to microbes that are phagocytosed and live within the phagosomes of macrophages is mediated by T_H1 cells, which recognize microbial antigens and activate the phagocytes to destroy the ingested microbes.*** Macrophages are major cells of host defense early after infection, that is, during innate immune responses (see Chapter 4). Their function is to ingest and kill microbes. Many microbes have developed mechanisms that enable them to survive and even to replicate within phagocytes, so innate immunity is unable to eradicate infections by such microbes. In these situations, T_H1 cells function to enhance the microbicidal actions of macrophages and thus to eliminate the infection.
- ***The response to extracellular microbes, including many fungi and bacteria, is mediated by T_H17 cells.*** These cells recruit neutrophils (and some monocytes), which ingest and destroy the microbes.
- ***The response to helminthic parasites is mediated by T_H2 cells,*** which stimulate the production of immunoglobulin E (IgE) antibodies and activate eosinophils and mast cells to eliminate the helminths.
- ***The adaptive immune response to microbes that infect and replicate in the cytoplasm of various cell types, including nonphagocytic cells, is mediated by $CD8^+$ cytotoxic T lymphocytes (CTLs), which kill infected cells and eliminate the reservoirs of infection*** (Fig. 10-1B). If the infected cells lack intrinsic microbicidal ability, the infection can be eradicated only by destroying these cells. CTL-mediated killing is also a mechanism for elimination of microbes that are taken up by phagocytes but escape from phagosomes into the cytosol, where they are not susceptible to the microbicidal activities of phagocytes.
- ***T cell–dependent inflammation may damage normal tissues.*** Inflammation, consisting of leukocyte recruitment and activation, accompanies many of the reactions of $CD4^+$ T lymphocytes and may be injurious under various conditions. This T cell–dependent injurious reaction is called **delayed-type hypersensitivity (DTH)**, the term *hypersensitivity* referring to tissue damage caused by an immune response. DTH frequently occurs together with protective cell-mediated immunity against microbes and may be the cause of much of the pathology associated with certain types of infection (see Chapters 15 and 18).

Cell-mediated immune responses consist of the development of effector T cells from naive cells in peripheral lymphoid organs, migration of these effector T cells and other leukocytes to sites of infection, and either cytokine-mediated activation of leukocytes to destroy microbes or direct killing of infected cells (Fig. 10-2). The development of effector T cells involves the sequence of antigen recognition, clonal expansion, and differentiation that is characteristic of all adaptive immune responses; these processes were described in Chapter 9. In this chapter, we describe the reactions of effector T cells and their roles in host defense and tissue injury. We start with the process that brings effector T cells to the site of infection or tissue damage.

MIGRATION OF EFFECTOR T LYMPHOCYTES TO SITES OF INFECTION

Some effector T cells exit the lymphoid organs where they were generated and preferentially home to sites of infection in peripheral tissues, where they are needed to eliminate microbes during the effector phase of adaptive immune responses (Fig. 10-3). In Chapter 3, we described naive T cell recirculation through lymphoid tissues and

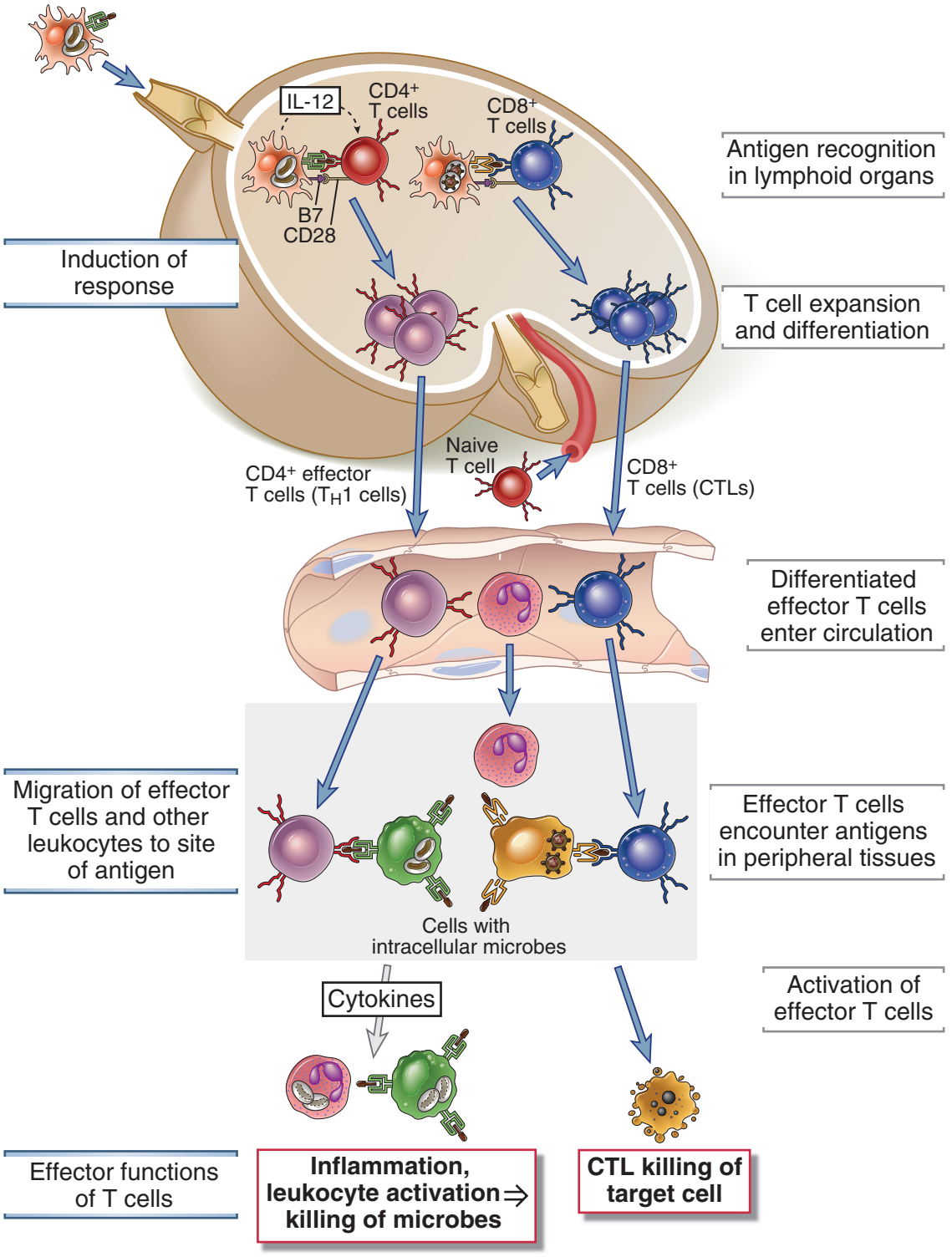


FIGURE 10–2 The induction and effector phases of cell-mediated immunity.
Induction of response: $CD4^+$ T cells and $CD8^+$ T cells recognize peptides that are derived from protein antigens and presented by dendritic cells in peripheral lymphoid organs. The T lymphocytes are stimulated to proliferate and differentiate into effector (and memory) cells, which enter the circulation.

Migration of effector T cells and other leukocytes to the site of antigen: Effector T cells and other leukocytes migrate through blood vessels in peripheral tissues by binding to endothelial cells that have been activated by cytokines produced in response to infection in these tissues.

Effector functions of T cells: Effector T cells recognize the antigen in the tissues and respond by secreting cytokines that recruit more leukocytes and activate phagocytes to eradicate the infection. CTLs also migrate to tissues and kill infected cells.

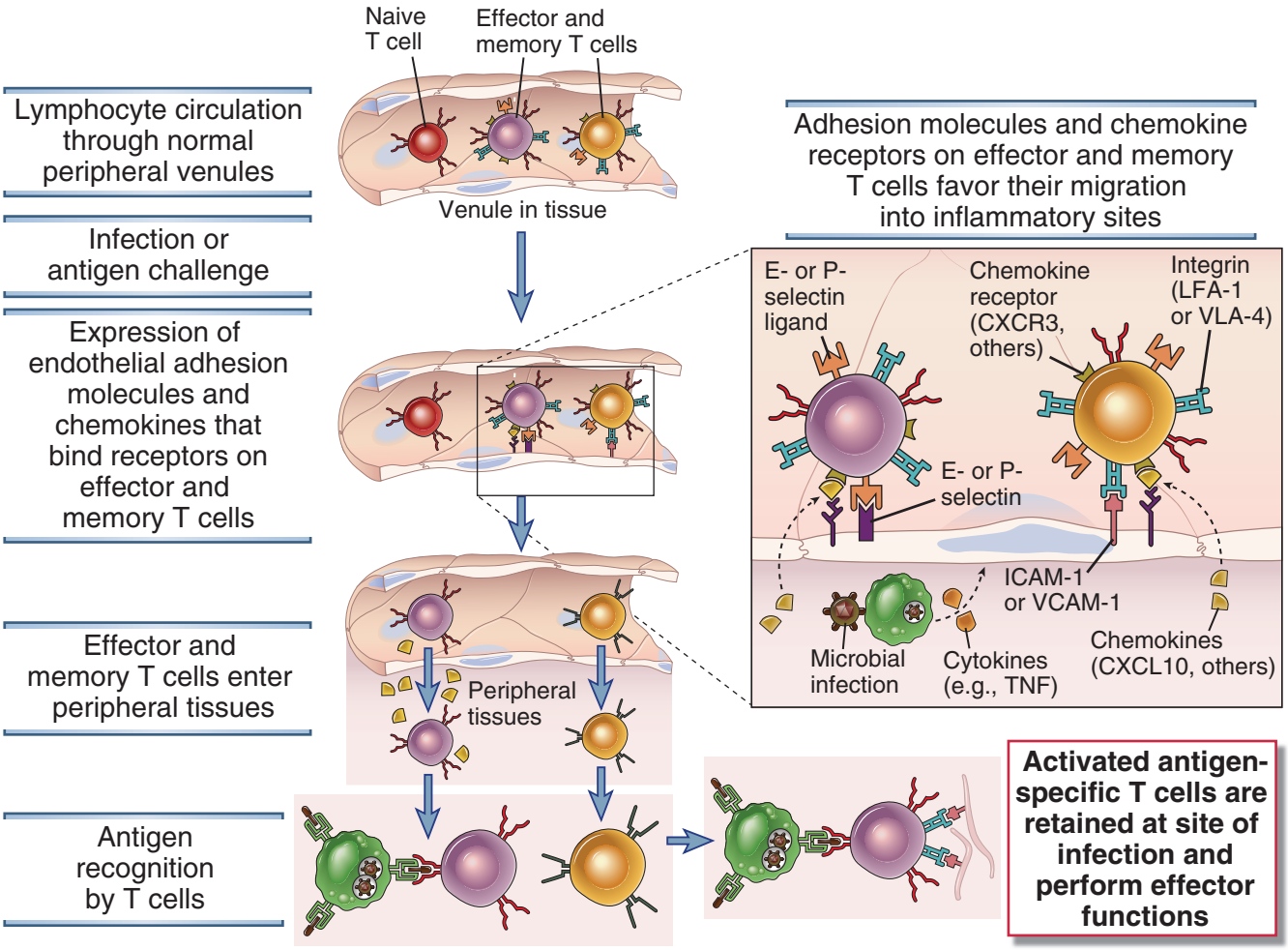


FIGURE 10–3 Migration and retention of effector and memory T cells at sites of infection. Previously activated effector and memory T cells, but not naive cells, migrate through endothelium that is activated by cytokines (e.g., TNF) produced at a site of infection. Activated endothelial cells express E- and P-selectins and ligands for integrins, and effector T cells express selectin ligands and integrins. These adhesion molecules mediate the interaction between the T cells and endothelium. Chemokines play additional critical roles in the migration of the T cells through the vessel wall. In extravascular tissue, the T cells that specifically recognize antigen are activated and retained, whereas T cells that do not encounter the antigen for which they are specific either die or return to the circulation, largely through lymphatic vessels (not shown).

effector T cell migration into nonlymphoid tissues and the roles of adhesion molecules and chemokines in these processes. The differentiation of naive T cells into effector cells, which occurs in the peripheral lymphoid organs, is associated with a change in expression of the chemokine receptors and adhesion molecules that determine the migratory behavior of these cells. The expression of molecules involved in naive T cell homing into lymph nodes, including L-selectin and CCR7, decreases shortly after antigen-induced activation of the naive T cells, and the cell surface expression of the sphingosine 1-phosphate receptor S1PR1 increases. As a result, the effector cells that develop are no longer constrained to stay in the node and are attracted to enter the blood or efferent lymphatics, which ultimately drain into the blood through the thoracic duct. Furthermore, unlike naive T cells, effector T cells express chemokine receptors that bind chemokines produced at sites of infection and adhesion molecules that bind to endothelial adhesion molecules that are induced on postcapillary venules by cytokines, including IL-1 and tumor necrosis factor (TNF), that are produced at infection sites. Therefore, after they enter the circulation, effector T cells home preferentially to sites of infection.

T_H1, T_H2, and T_H17 subsets of $CD4^+$ T cells each have distinct homing phenotypes that direct them to migrate into different sites of infections. For example, during differentiation from naive precursors, T_H1 cells gain the capacity to produce functional ligands that bind E-selectin and P-selectin, whereas T_H2 cells express lower levels of selectin ligands. This aspect of T_H1 differentiation involves T-bet–dependent expression of glycosyltransferases that are required for synthesis of the carbohydrate moieties to which the selectins bind. Furthermore, the chemokine receptors CXCR3 and CCR5, which bind to chemokines elaborated in tissues during innate immune responses, are expressed at high levels by T_H1 cells but not by T_H2 cells. Therefore, T_H1 cells tend to be abundant at sites of infection where the infectious agents trigger strong innate immune reactions; these agents include many bacteria

and viruses. In general, CTLs migrate in similar ways as T_H1 cells. In contrast, T_H2 cells express the chemokine receptors CCR3, CCR4, and CCR8, which recognize chemokines that are highly expressed at sites of helminth infection or allergic reactions, particularly in mucosal tissues, and so T_H2 cells tend to migrate to these tissues. T_H17 cells express CCR6, which binds the chemokine CCL20, and migration of T_H17 cells into inflammatory sites is dependent on CCR6. CCL20 is produced by various tissue cells and macrophages in many bacterial and fungal infections.

After effector T cells enter the site of infection and are activated once again by antigen, they produce more cytokines and chemokines and stimulate much greater leukocyte migration. This later, enhanced inflammation is sometimes called **immune inflammation** to indicate the role of T lymphocytes (immune cells) in the process.

The migration of effector T cells from the circulation to peripheral sites of infection is largely independent of antigen, but cells that recognize antigen in extravascular tissues may be preferentially retained there (see Fig. 10-3). T cell migration through blood and lymphatic vessels is controlled mainly by adhesion molecules and chemokines, which will engage T cells of any antigen specificity. Therefore, this process of migration results in the recruitment of circulating effector T cells to inflammatory sites, regardless of antigen specificity, ensuring that the maximum possible number of previously activated T cells have the opportunity to locate infectious microbes and to eradicate the infection. Some memory T cells also migrate into peripheral nonlymphoid tissues regardless of antigen specificity. Once in the tissues, the T cells encounter microbial antigens presented by macrophages and other antigen-presenting cells (APCs). T cells that specifically recognize antigens receive signals through their antigen receptors that increase the affinity of integrins for their ligands. Two of these integrins, VLA-4 and VLA-5, bind to fibronectin in extracellular matrices, and a third adhesion molecule, CD44, which is also highly expressed on effector and memory T cells, binds to hyaluronan. As a result, antigen-specific effector and memory T cells that encounter the antigen are preferentially retained at the extravascular site where the antigen is present. T cells not specific for the antigen that migrate into a site of inflammation may die in the tissue or return through lymphatic vessels to the circulation.

EFFECTOR FUNCTIONS OF CD4+ HELPER T CELLS

Effector T cells of the $CD4^+$ lineage function by secreted cytokines and cell surface molecules to activate other cells to eliminate microbes. $CD4^+$ T cells also participate indirectly in host defense by helping B lymphocytes to produce high-affinity antibodies against extracellular microbes and by promoting the development of fully functional CTLs that combat intracellular microbes such as viruses. The roles of helper T cells in antibody responses are described in Chapter 11 and in CTL responses in Chapter 9. Here our focus is on the roles of $CD4^+$ T cells as the effector cells of cell-mediated immunity.

The functions of $CD4^+$ effector cells in cell-mediated immunity can be divided into several steps (Fig. 10-4):

- ***Recruitment of other leukocytes.*** The recruitment of neutrophils, monocytes, and eosinophils to the site of the reaction is mediated by chemokines produced by the T cells themselves and by other cells in response to cytokines secreted by the T cells. As we have mentioned previously and will discuss in more detail later, different subsets of $CD4^+$ effector cells recruit different types of leukocytes into the reaction.
- ***Activation of the recruited leukocytes.*** The mechanisms by which $CD4^+$ T cells activate other leukocytes involve T cell expression of the surface protein CD40 ligand (CD40L) and secretion of cytokines. The CD40L-mediated pathway is best defined for T_H1-mediated activation of macrophages and is described in this context later. The roles of cytokines in activating different leukocyte populations are also described later for each subset of effector T cells.
- ***Amplification of the response.*** As in all adaptive immune responses, there are several positive feedback loops that serve to amplify the response. For instance, cytokines produced by T cells activate macrophages to produce other cytokines that in turn act on the T cells and increase their responses.
- ***Downregulation of the response.*** Because effector T cells are typically short-lived, they die after performing their function. As the antigen is eliminated, the stimuli for propagating the response are lost, and the response declines over time. Special control mechanisms may also operate to limit effector responses. For instance, both T_H1 cells and activated macrophages produce the cytokine IL-10, which functions mainly to inhibit further T_H1 differentiation and macrophage activation. Additional inhibitory mechanisms, such as other anti-inflammatory cytokines and receptors that turn off T cell activation, may also be involved in controlling T cell–mediated responses.

With this overview, we proceed to a discussion of the functions of the three major subsets of effector $CD4^+$ T cells in cell-mediated immunity.

Functions of T_H1 Cells

The principal function of T_H1 cells is to activate macrophages to ingest and destroy microbes (Fig. 10-5). Recall that phagocytosed intracellular microbes are powerful stimuli for the generation of T_H1 cells (see Chapter 9). Thus, T_H1 effector cells develop in response to the pathogens that these cells are designed to eradicate, an excellent example of the specialization of adaptive immunity. The same reaction of T_H1-mediated macrophage activation is involved in injurious delayed-type hypersensitivity, which is a component of many inflammatory diseases, and in granulomatous inflammation, which is typical of tuberculosis and is also seen in some other infectious and inflammatory disorders. These pathologic reactions are described in Chapter 18.

Before discussing the activation of macrophages and how they destroy microbes, we describe the properties of

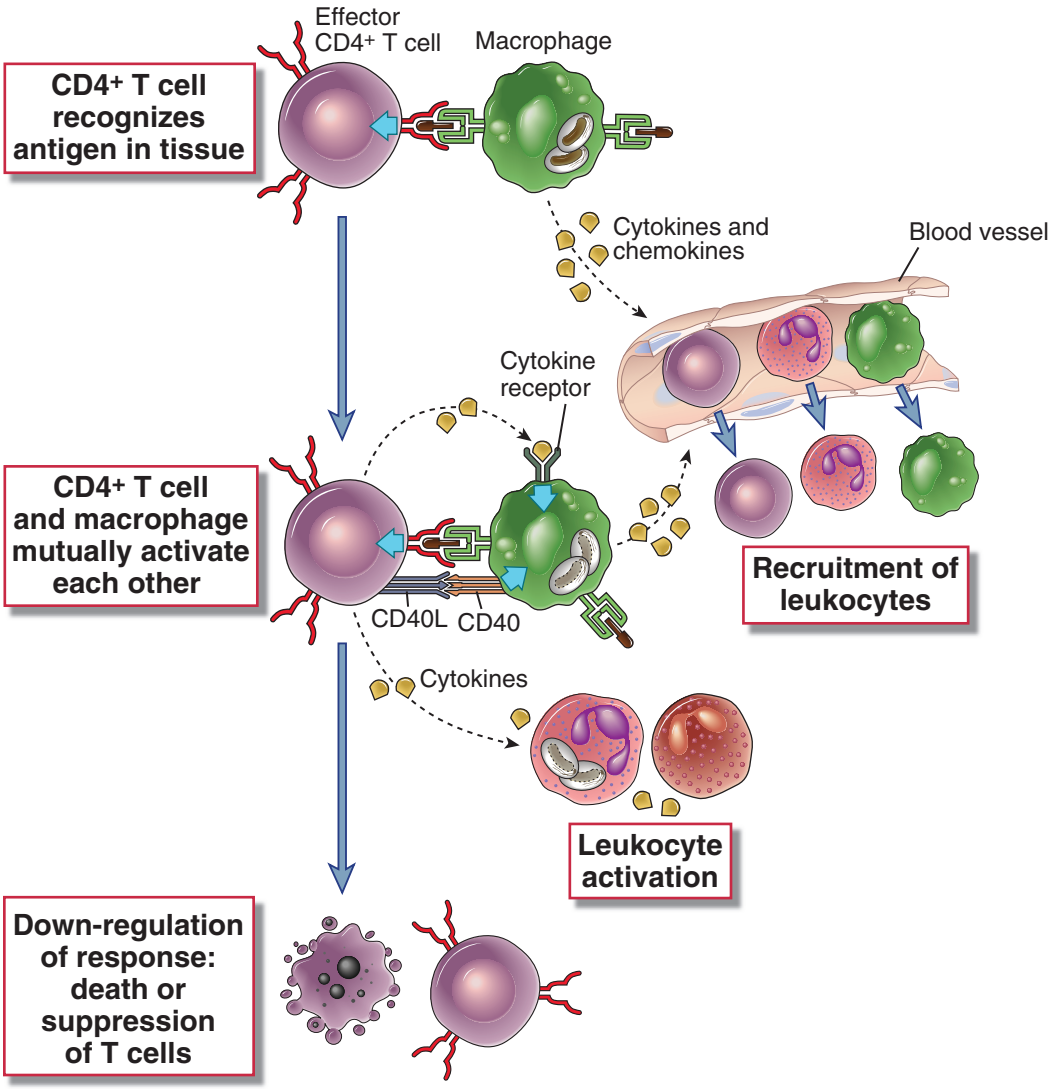


FIGURE 10–4 Sequence of events in the reactions of effector CD4$^+$ T cells. The sequence of events in the functional responses of effector CD4$^+$ T cells is shown. Effector cells are recruited from the circulation, activated by antigens displayed by macrophages that have phagocytosed microbes, and secrete cytokines that amplify and control the reaction. The T cells use CD40L and cytokines to activate phagocytes and cytokines to recruit more leukocytes.

interferon-γ (IFN-γ), the cytokine responsible for most of the specialized functions of T_H1 cells.

Cytokines Produced by T_H1 Cells

The signature cytokine of T_H1 cells is IFN-γ. T_H1 cells also produce TNF, some chemokines, and other cytokines.

Interferon-γ

IFN-γ is the principal macrophage-activating cytokine and serves critical functions in immunity against intracellular microbes. IFN-γ is also called immune or type II interferon. Although it has some antiviral activity, it is not a potent antiviral cytokine, and it functions mainly as an activator of effector cells of the immune system.

IFN-γ is a homodimeric protein belonging to the type II cytokine family (see Chapter 7). In addition to CD4$^+$ T_H1 cells, IFN-γ is also produced by NK cells and CD8$^+$ T cells. NK cells secrete IFN-γ in response to activating ligands on the surface of infected or stressed host cells (see Chapter 2) or in response to IL-12; in this setting, IFN-γ functions as a mediator of innate immunity. In adaptive immunity, T cells produce IFN-γ in response to antigen recognition, and production is enhanced by IL-12 and IL-18.

The receptor for IFN-γ is composed of two structurally homologous polypeptides belonging to the type II cytokine receptor family, called IFNγR1 and IFNγR2. IFN-γ binds to and induces the dimerization of the two receptor chains. This leads to activation of the associated JAK1 and JAK2 kinases and ultimately to phosphorylation and dimerization of STAT1, which stimulates transcription of several genes (see Chapter 7). IFN-γ–induced genes encode many different molecules that mediate the biologic activities of this cytokine, described next.

The functions of IFN-γ are important in cell-mediated immunity against intracellular microbes (see Fig. 10-5).

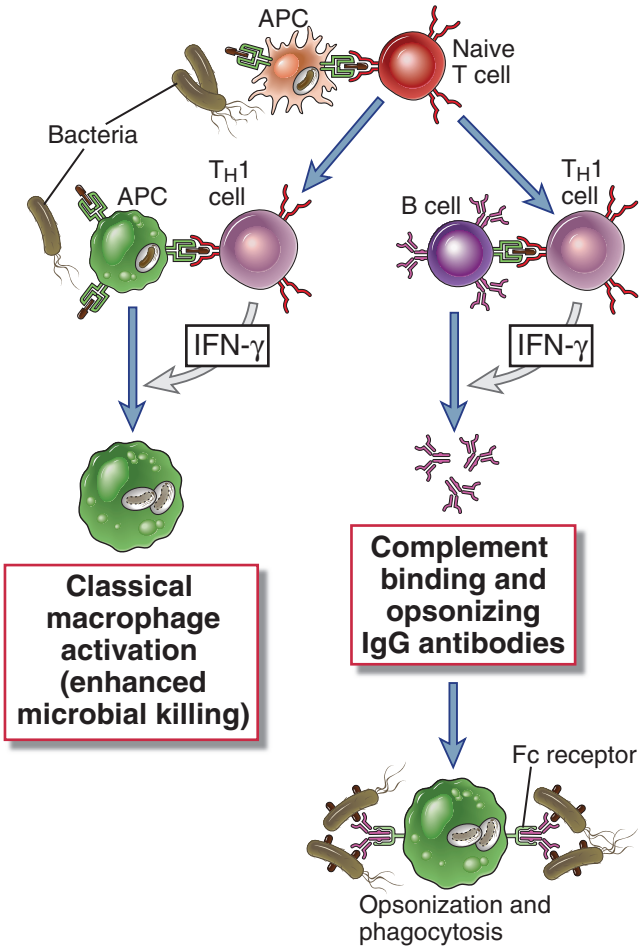


FIGURE 10–5 Functions of T_H1 cells. $CD4^+$ T cells that differentiate into T_H1 cells secrete IFN-γ, which acts on macrophages to increase phagocytosis and killing of microbes in phagolysosomes and on B lymphocytes to stimulate production of IgG antibodies that opsonize microbes for phagocytosis. The cells also produce TNF, which activates neutrophils and promotes inflammation (not shown).

- ***IFN-γ activates macrophages to kill phagocytosed microbes, the hallmark of "classically activated" macrophages.*** In innate immune reactions, IFN-γ is produced by NK cells and acts on macrophages together with Toll-like receptor (TLR) signals delivered by microbes (see Chapter 4) to trigger macrophage activation. In adaptive cell-mediated immunity, IFN-γ produced by T_H1 cells works together with CD40 ligand, also expressed by the T cells, to activate macrophages. IFN-γ activates numerous signaling pathways and transcription factors, most importantly STAT1, and TLR and CD40 signals activate the transcription factors nuclear factor κB (NF-κB) and activation protein 1 (AP-1). These transcription factors stimulate the expression of several enzymes in the phagolysosomes of macrophages, including phagocyte oxidase, which induces the production of reactive oxygen species (ROS); inducible nitric oxide synthase (iNOS), which stimulates the production of nitric oxide (NO); and lysosomal enzymes. These substances destroy ingested microbes in the vesicles and are responsible for the microbicidal function of activated macrophages.
- ***IFN-γ acts on B cells to promote switching to certain IgG subclasses, notably IgG2a or IgG2c) (in mice), and to inhibit switching to IL-4–dependent isotypes, such as IgE.*** The IgG subclasses induced by IFN-γ bind to Fcγ receptors on phagocytes and activate complement, and both these mechanisms promote the phagocytosis of opsonized microbes (see Chapter 12). Thus, IFN-γ induces antibody responses that also participate in phagocyte-mediated elimination of microbes, in concert with the direct macrophage-activating effects of this cytokine. The mechanism of isotype switching and the role of cytokines in this process are described in Chapter 11. This action of IFN-γ on B cells is better established in mice than in humans.
- ***IFN-γ promotes the differentiation of $CD4^+$ T cells to the T_H1 subset and inhibits the differentiation of T_H2 and T_H17 cells.*** These actions of IFN-γ serve to amplify the T_H1 response and were described in Chapter 9.
- ***IFN-γ stimulates expression of several different proteins that contribute to enhanced MHC-associated antigen presentation and the initiation and amplification of T cell–dependent immune responses*** (see Fig. 6-9, Chapter 6). These proteins include MHC molecules; many proteins involved in antigen processing, including the transporter associated with antigen processing (TAP); components of the proteasome; HLA-DM; and B7 costimulators on APCs.

The actions of IFN-γ together result in increased ingestion of microbes and the destruction of the ingested pathogens. Individuals with rare inherited inactivating mutations in the IFN-γ receptor and knockout mice lacking IFN-γ or the IFN-γ receptor or molecules required for T_H1 differentiation or IFN-γ signaling (IL-12, T-bet, STAT1) are susceptible to infections with intracellular microbes, such as mycobacteria, because of defective macrophage-mediated killing of the microbes.

Other T_H1 Cytokines

In addition to IFN-γ, T_H1 cells produce TNF and various chemokines, which contribute to the recruitment of leukocytes and enhanced inflammation. Somewhat surprisingly, T_H1 cells are also important sources of IL-10, which functions mainly to inhibit dendritic cells and macrophages and thus to suppress T_H1 activation. This is an example of a negative feedback loop in T cell responses.

T_H1-Mediated Classical Macrophage Activation and Killing of Phagocytosed Microbes

In cell-mediated immune responses against phagocytosed microbes, T cells specifically recognize microbial antigens but phagocytes actually destroy the pathogens. This fundamental concept was first appreciated from studies of cell-mediated immunity to the intracellular bacterium *Listeria monocytogenes* (Fig. 10-6). It was shown in the 1950s that mice infected with a low dose of *Listeria* were protected from challenge with higher, lethal doses. Protection could be transferred to naive animals with lymphocytes (later shown to be T lymphocytes) from the infected mice but not with serum. In vitro, the bacteria were killed not by T cells from immune animals but by

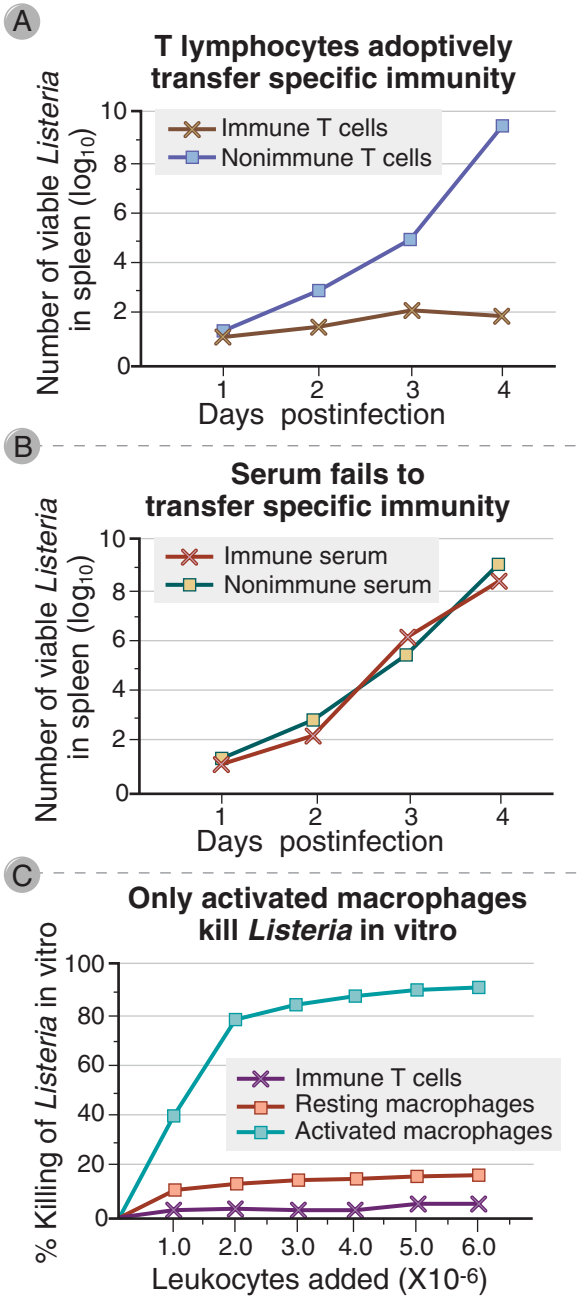


FIGURE 10–6 Cell-mediated immunity to *Listeria monocytogenes*. Immunity to *L. monocytogenes* is measured by inhibition of bacterial growth in the spleens of animals inoculated with a known dose of viable bacteria. Such immunity can be transferred to normal mice by T lymphocytes **(A)** but not by serum **(B)** from syngeneic mice previously immunized with killed or low doses of *L. monocytogenes*. In an in vitro assay of cell-mediated immunity, the bacteria are actually killed by activated macrophages and not by T cells **(C).**

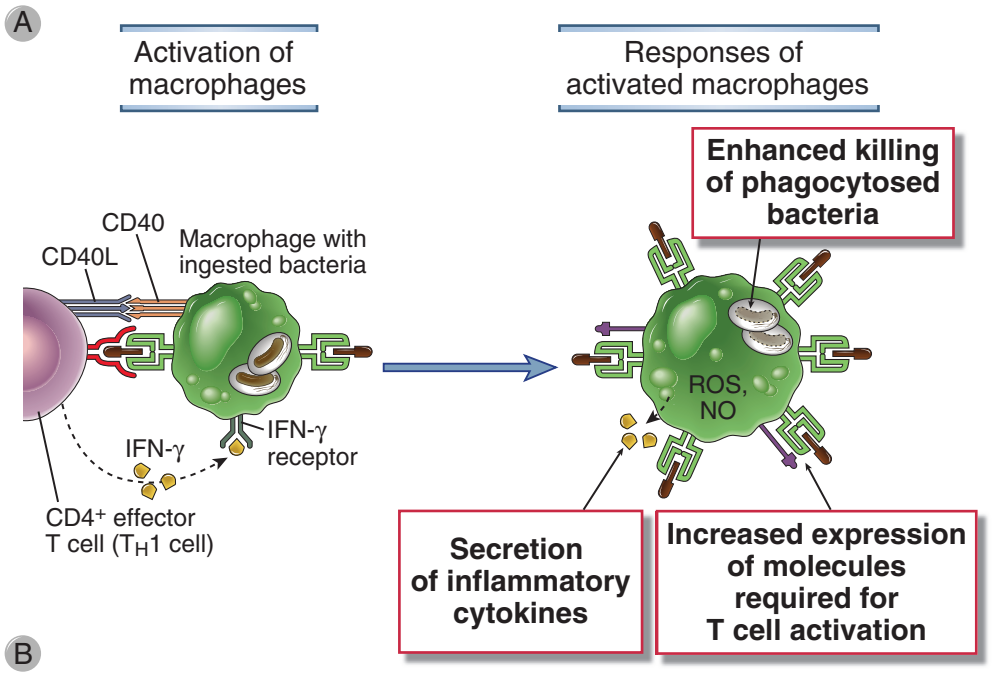
activated macrophages, emphasizing the central role of macrophages in the execution of effector function.

At any site of infection, as part of the innate immune response, monocytes are recruited from blood into tissues by chemokines produced by macrophages and other cells resident at the site (see Chapter 4). These monocytes mature into tissue macrophages and first attempt to phagocytose and destroy the pathogen. If the microbe has evolved to resist elimination by the macrophages, it survives within the phagosomes. In these infected cells, microbial peptides are processed and presented as peptides associated with class II MHC molecules. At the same time, T_H1 effector cells are generated in an adaptive immune response in secondary lymphoid tissues, by processes described in Chapter 9. These T cells are recruited to the site of infection, where they recognize antigenic peptides (the same as those that initiated the response) displayed by the microbe-bearing macrophages. The macrophages are exposed to signals from the T_H1 effector cells, which activate the macrophages to kill the ingested microbes. Activation consists of increased expression of various proteins that endow activated macrophages with the capacity to perform specialized functions, such as microbial killing. In the following sections, we describe the T cell signals that activate macrophages in cell-mediated immune reactions and the functions of these macrophages.

$CD4^+$ T_H1 cells activate macrophages by contact-mediated signals delivered by CD40L-CD40 interactions and by IFN-γ (Fig. 10-7). When the T_H1 cells are stimulated by antigen, the cells express CD40L on their surface and secrete IFN-γ. The actions of IFN-γ on macrophages, described earlier, synergize with the actions of CD40 ligand, and together they are potent stimuli for macrophage activation. The importance of the CD40 pathway in cell-mediated immunity is illustrated by the immunologic defects in humans with inherited mutations in CD40L (X-linked hyper-IgM syndrome) and in mice in which the gene for CD40 or CD40L is knocked out (see Chapter 20). All these disorders are characterized by severe deficiencies in cell-mediated immunity to intracellular microbes, and children with the X-linked hyper-IgM syndrome often succumb to infection by the intracellular pathogen *Pneumocystis jiroveci*. As expected, these patients and knockout mice also have defects in helper T cell–dependent antibody production. The requirement for interactions between the surface molecules CD40 on the macrophages and CD40L on the T cells ensures that macrophages that are presenting antigens to the T cells (i.e., the macrophages that are harboring intracellular microbes) are also the macrophages that are most efficiently activated by the T cells. The role of IFN-γ as the major macrophage-activating cytokine was discussed before. The same principles are applicable to the T cell–dependent activation of B lymphocytes—helper T cells stimulate B lymphocyte proliferation and differentiation by CD40-mediated signals and cytokines (see Chapter 11).

Activated macrophages kill phagocytosed microbes mainly by the actions of reactive oxygen species, nitric oxide, and lysosomal enzymes. All these potent microbicidal agents are produced within the lysosomes of macrophages and kill ingested microbes after phagosomes fuse with lysosomes (see Chapter 4, Figure 4-12). These toxic substances may also be released into adjacent tissues, where they kill extracellular microbes and may cause damage to normal tissues. This pathway of macrophage activation is called classical (Fig. 10-8) to distinguish it from alternative activation, described later.

Activated macrophages are involved in several other reactions of host defense (see Fig. 10-7). They stimulate



B

Macrophage response	Role in cell-mediated immunity
Production of reactive oxygen species, nitric oxide, increased lysosomal enzymes	Killing of microbes in phagolysosomes (effector function of macrophages)
Secretion of cytokines (TNF, IL-1, IL-12) and chemokines	TNF, IL-1, chemokines: leukocyte recruitment (inflammation) IL-12: T_H1 differentiation, IFN-γ production
Increased expression of B7 costimulators, MHC molecules	Increased T cell activation (amplification of T cell response)

FIGURE 10–7 Macrophage activation by T_H1 cells. A, Macrophages are activated by CD40L-CD40 interactions and by IFN-γ expressed by T_H1 cells and perform several functions that kill microbes, stimulate inflammation, and enhance the antigen-presenting capacity of the cells. **B,** The principal molecules that mediate the functions of macrophages are listed. Macrophages are also activated during innate immune reactions and perform the same functions (see Chapter 4).

inflammation through the secretion of cytokines, mainly TNF, IL-1, and chemokines, and short-lived lipid mediators such as prostaglandins, leukotrienes, and platelet-activating factor. The collective action of these macrophage-derived cytokines and lipid mediators is to recruit more leukocytes, which improves the ability to destroy infectious organisms. Activated macrophages *amplify cell-mediated immune responses* by becoming more efficient APCs because of increased levels of molecules involved in antigen processing and increased surface expression of class II MHC molecules and costimulators and by producing cytokines (such as IL-12) that stimulate T lymphocyte differentiation into effector cells.

Some tissue injury may normally accompany T_H1 cell–mediated immune reactions to microbes because the microbicidal products released by activated macrophages and neutrophils are capable of injuring normal tissue and do not discriminate between microbes and host tissue. However, this tissue injury is usually limited in extent and duration, and it resolves as the infection is cleared. As mentioned earlier, delayed hypersensitivity is an example of a T_H1-mediation reaction that can cause significant tissue injury (see Chapter 18).

Functions of T_H2 Cells

T_H2 cells stimulate IgE- and eosinophil-mediated reactions that serve to eradicate helminthic infections (Fig. 10-9). Helminths are too large to be phagocytosed by neutrophils and macrophages and may be more resistant to the microbicidal activities of these phagocytes than are most bacteria and viruses. Therefore, special mechanisms are needed for defense against helminthic infections. T_H2 cells secrete IL-4, IL-5, and IL-13, which work cooperatively to eradicate these infections. We first describe the properties of these cytokines and then their roles in host defense.

Cytokines Produced by T_H2 Cells

The functions of T_H2 cells are mediated by IL-4, which induces IgE antibody responses; IL-5, which activates eosinophils; and IL-13, which has diverse actions.

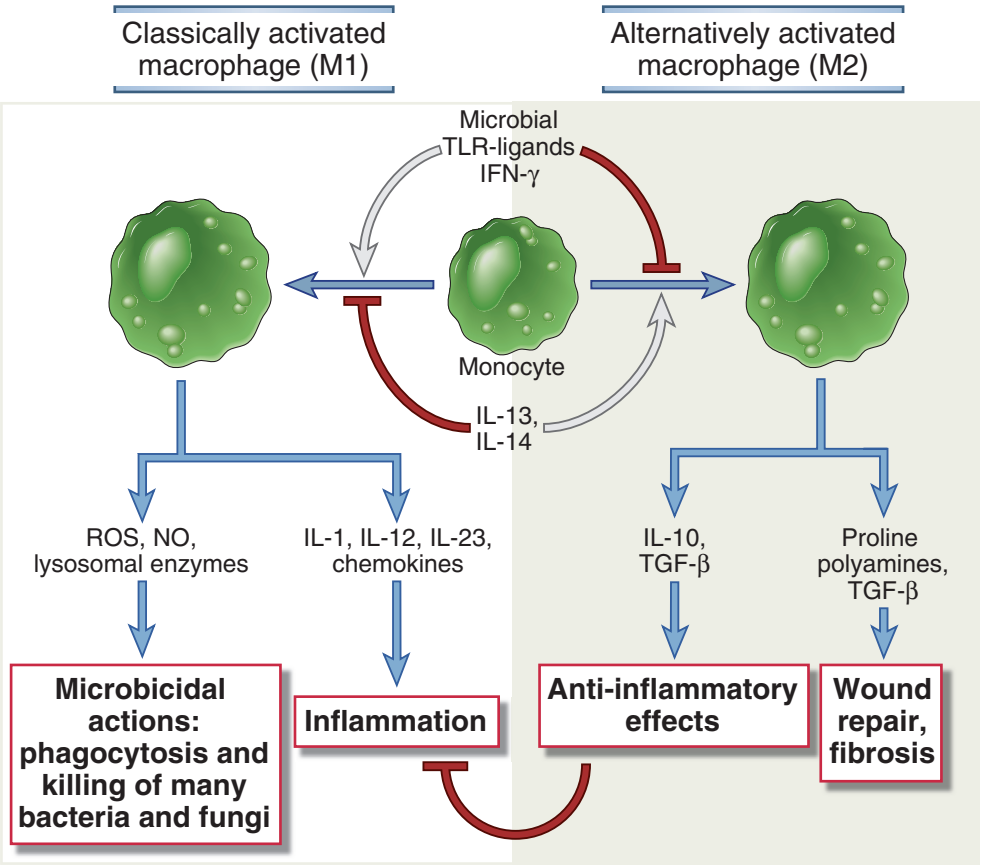


FIGURE 10–8 Classical and alternative macrophage activation. Subsets of activated macrophages. Different stimuli activate monocytes-macrophages to develop into functionally distinct populations. Classically activated macrophages are induced by microbial products and cytokines, particularly IFN-γ, and are microbicidal and involved in potentially harmful inflammation. Alternatively activated macrophages are induced by IL-4 and IL-13 produced by T_H2 cells and other leukocytes and are important in tissue repair and fibrosis.

Interleukin-4

IL-4 is the major stimulus for the production of IgE antibodies and for the development of T_H2 cells from naive $CD4^+$ helper T cells. IL-4 is the signature cytokine of the T_H2 subset and functions as both an inducer and an effector cytokine of these cells.

IL-4 is a member of the four–α-helical cytokine family. The principal cellular sources of IL-4 are $CD4^+$ T lymphocytes of the T_H2 subset and activated mast cells. The IL-4 receptor of lymphoid cells consists of a cytokine-binding α chain that is a member of the type I cytokine receptor family, associated with the γ_c chain shared by other cytokine receptors. This IL-4Rαγ_c receptor signals by the JAK-STAT pathway (JAK3 or JAK4 and STAT6) and by a pathway that involves the insulin response substrate (IRS) called IRS-2. IL-4 and IL-13 activate the STAT6 protein, which induces transcription of genes that account for many of the actions of these cytokines. IL-4 also binds to the IL-13 receptor (described below).

IL-4 has important actions on several cell types.

- ***IL-4 stimulates B cell Ig heavy chain class switching to the IgE isotype.*** The mechanisms of class switching are described in Chapter 11. Knockout mice lacking IL-4 have less than 10% of normal IgE levels. IgE antibodies play a role in eosinophil-mediated defense against helminthic (and some arthropod) infections. IgE is also the principal mediator of immediate hypersensitivity (allergic) reactions, and production of IL-4 is important for the development of allergies (see Chapter 19). IL-4 also enhances switching to IgG4 (in humans, or the homologous IgG1 in mice) and inhibits switching to the IgG2a and IgG3 isotypes in mice, both of which are stimulated by IFN-γ. This is one of several reciprocal antagonistic actions of IL-4 and IFN-γ. IL-13 can also contribute to switching to the IgE isotype.
- ***IL-4 stimulates the development of T_H2 cells and functions as an autocrine growth factor for differentiated T_H2 cells.*** This function of IL-4 was described in Chapter 9.
- ***IL-4, together with IL-13, contributes to an alternative form of macrophage activation*** that is distinct from the macrophage response to IFN-γ and is described later. In fact, IL-4 and IL-13 suppress IFN-γ–mediated classical macrophage activation and thus inhibit defense against intracellular microbes.
- ***IL-4 (and IL-13) stimulate peristalsis in the gastrointestinal tract, and IL-13 increases mucus secretion from airway and gut epithelial cells.*** Both these actions contribute to elimination of microbes at epithelial surfaces.
- ***IL-4 and IL-13 stimulate the recruitment of leukocytes,*** notably eosinophils, by promoting expression of

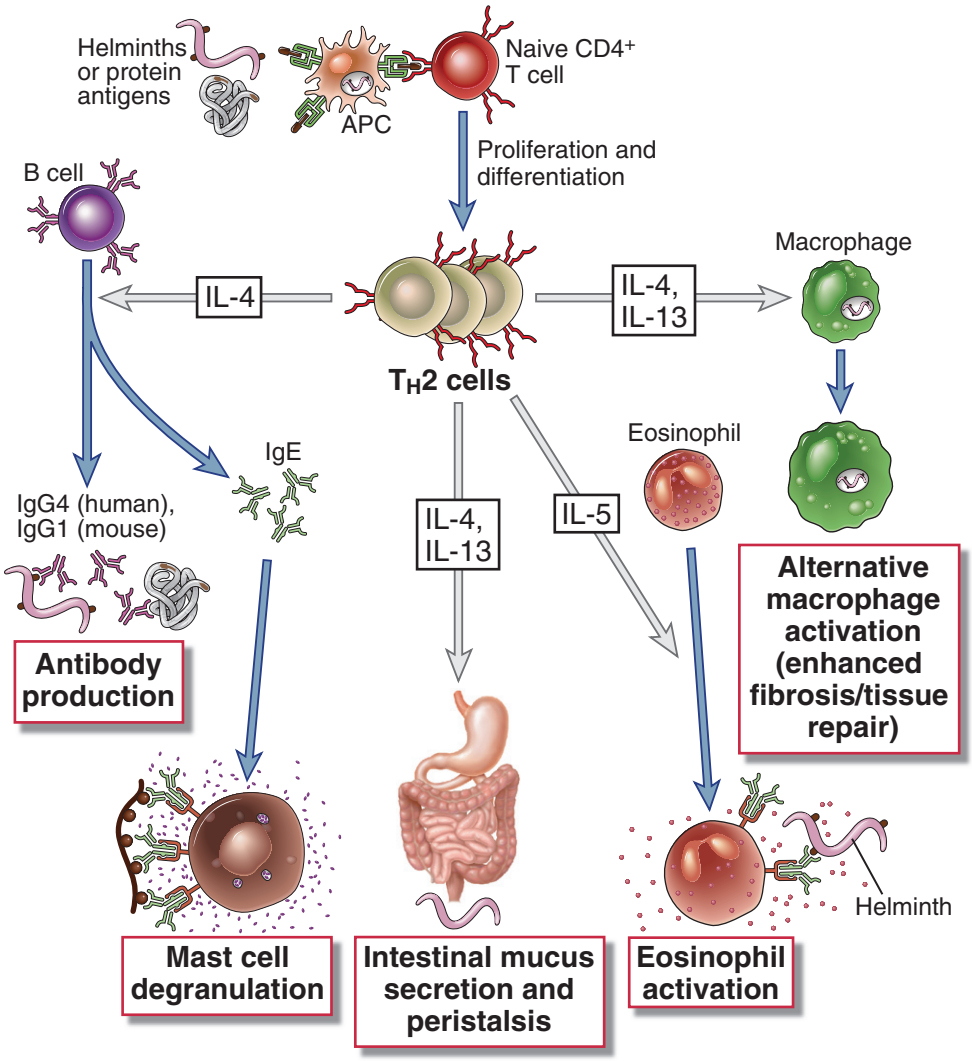


FIGURE 10–9 Functions of T_H2 cells. $CD4^+$ T cells that differentiate into T_H2 cells secrete IL-4, IL-5, and IL-13. IL-4 (and IL-13) act on B cells to stimulate production of antibodies that bind to mast cells, such as IgE. IL-4 is also an autocrine growth and differentiation cytokine for T_H2 cells. IL-5 activates eosinophils, a response that is important for defense against helminthic infections. IL-4 and IL-13 are involved in barrier immunity, induce an alternative pathway of macrophage activation, and inhibit classical, T_H1-mediated macrophage activation.

adhesion molecules on endothelium and the secretion of chemokines that bind chemokine receptors expressed on eosinophils.

Interleukin-13

IL-13 is structurally and functionally similar to IL-4 and also plays a key role in defense against helminths (see Chapter 15) and in allergic diseases (see Chapter 19). IL-13 is a member of the four–α-helical cytokine family, with limited sequence homology but significant structural similarity to IL-4. IL-13 is produced mainly by the T_H2 subset, but basophils, eosinophils, and NKT cells may also produce the cytokine. The functional IL-13 receptor is a heterodimer of the IL-4Rα chain and the IL-13Rα1 chain. This complex can bind both IL-4 and IL-13 with high affinity and accounts for the fact that most of the biologic effects of IL-13 are shared with IL-4. The receptor is expressed on a wide variety of cells, including B cells, mononuclear phagocytes, dendritic cells, eosinophils, basophils, fibroblasts, endothelial cells, and bronchial epithelial cells. T cells do not express the IL-13 receptor. IL-13R signaling is similar to IL-4R signaling.

IL-13 works together with IL-4 in producing biologic effects associated with allergic inflammation, discussed in detail in Chapter 19, and in defense against helminths. Some of the actions of IL-13 overlap those of IL-4, and others are distinct. IL-13 functions with IL-4 to induce alternative macrophage activation, which contributes to tissue repair and fibrosis. IL-13 stimulates mucus production by airway epithelial cells, an important component of allergic reactions such as asthma. As mentioned before, both IL-13 and IL-4 can activate B cells to switch to IgE and some IgG isotypes and recruit leukocytes. Unlike IL-4, IL-13 is not involved in T_H2 differentiation.

Interleukin-5

IL-5 is an activator of eosinophils and serves as the principal link between T cell activation and eosinophilic inflammation. It is a homodimer of a polypeptide containing a four–α-helical domain and is a member of the type I cytokine family. It is produced by T_H2 cells and by activated mast cells. The IL-5 receptor is a heterodimer composed of a unique α chain and a common β chain (β_c), which is also part of the IL-3 and granulocyte-macrophage colony-stimulating factor (GM-CSF)

receptors (see Fig. 7-23, Chapter 7). The major IL-5–induced signaling pathway involves JAK2 and STAT3.

The principal actions of IL-5 are to activate mature eosinophils and to stimulate the growth and differentiation of eosinophils. Activated eosinophils are able to kill helminths. Eosinophils express Fc receptors specific for IgE and some IgG antibodies and are thereby able to bind to microbes, such as helminths, that are opsonized by these antibodies. IL-5 also stimulates the production of IgA antibodies.

Roles of T_H2 Cells in Host Defense

T_H2 cells function in defense against helminth infections by several mechanisms (see Fig. 10-9).

- ***IgE- and eosinophil-mediated reactions.*** IL-4 (and IL-13) stimulates the production of helminth-specific IgE antibodies, which opsonize the helminths and promote the binding of eosinophils. IL-5 activates the eosinophils and these cells release their granule contents, including major basic protein and major cationic protein, which are capable of destroying even the tough integuments of helminths (see Chapters 15 and 19).
- ***Activation of mast cells.*** Mast cells express high-affinity Fcε receptors and may be activated by IgE-coated helminths and other antigens that bind IgE, resulting in degranulation. The granule contents of mast cells include vasoactive amines, and mast cells secrete cytokines such as TNF and chemokines, and lipid mediators, all of which induce local inflammation that helps to destroy the parasites. Mast cell mediators are also responsible for the vascular abnormalities and inflammation in allergic reactions (see Chapter 19).
- ***Barrier immunity.*** Cytokines produced by T_H2 cells are involved in blocking entry and promoting expulsion of microbes from mucosal organs. For instance, IL-13 stimulates mucus production, and IL-4 and IL-13 may stimulate peristalsis in the gastrointestinal system. Thus, T_H2 cells play an important role in host defense at the barriers with the external environment, sometimes called *barrier immunity*.
- ***Alternative macrophage activation.*** IL-4 and IL-13 activate macrophages to express enzymes that promote collagen synthesis and fibrosis. The macrophage response to T_H2 cytokines has been called *alternative macrophage activation* (see Fig. 9-8) to distinguish it from the activation induced by IFN-γ, which was characterized first (and hence the designation "classical") and which results in potent microbicidal functions. Macrophages that are activated by T_H2 cytokines contribute to tissue remodeling and fibrosis in the setting of chronic parasitic infections and allergic disease. Alternatively activated macrophages may also serve to initiate repair after diverse types of tissue injury that may not involve infectious agents or immune responses; in these situations, the activating cytokines, such as IL-4, may be produced by eosinophils and other cell types in tissues. Alternatively activated macrophages induce the formation of fibrous tissue by secreting growth factors that stimulate fibroblast proliferation (platelet-derived growth factor), collagen synthesis (transforming growth factor-β [TGF-β]), and new blood vessel formation or angiogenesis (fibroblast growth factor). T_H2 cytokines suppress classical macrophage activation and interfere with protective T_H1-mediated immune responses to intracellular infections (see Chapter 15). Suppression of classical macrophage activation is, in part, because IL-4 stimulates production of cytokines such as IL-10 and TGF-β that inhibit T_H1 development and function.

Functions of T_H17 Cells

T_H17 cells secrete cytokines that recruit leukocytes, mainly neutrophils, to sites of infection (Fig. 10-10). Because neutrophils are a major defense mechanism against extracellular bacteria and fungi, T_H17 cells play an especially important role in defense against these infections.

Cytokines Produced by T_H17 Cells

T_H17 cells produce several cytokines. Most of the inflammatory actions of these cells are mediated by IL-17, but other cytokines produced by this subset may also contribute.

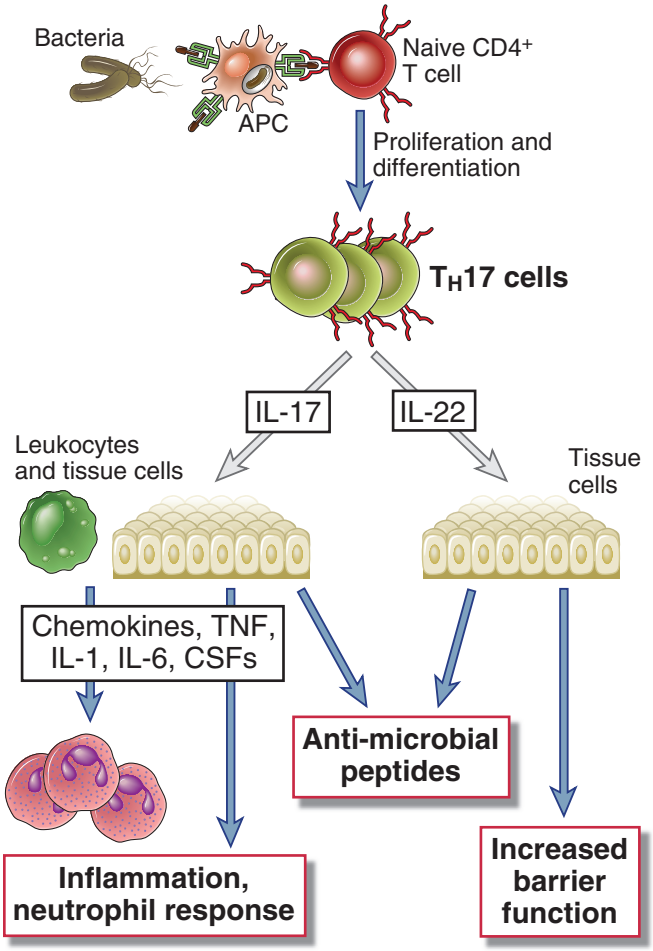


FIGURE 10–10 Functions of T_H17 cells. Cytokines produced by T_H17 cells stimulate local production of chemokines and inflammation and the production of antimicrobial peptides (defensins) and promote epithelial barrier functions.

Interleukin-17

IL-17 is an unusual cytokine because neither it nor its receptor is homologous to any other known cytokine-receptor pair. The IL-17 family includes six structurally related proteins, of which IL-17A and IL-17F are the most similar, and the immunologic activities seem to be mediated primarily by IL-17A. IL-17A and IL-17F are produced mainly by T_H17 cells, whereas the other members of the family are produced by diverse cell types. IL-17 receptors are multimeric and expressed on a wide range of cells. Their structure and signaling mechanisms are not well defined.

IL-17 is an important link between T cell–mediated adaptive immunity and the innate immune system, especially the inflammatory component of innate responses.

- ***IL-17 induces neutrophil-rich inflammatory reactions.*** It stimulates the production of chemokines and other cytokines (such as TNF) that recruit neutrophils and, to a lesser extent, monocytes to the site of T cell activation. It also enhances neutrophil generation by increasing the production of G-CSF and the expression of its receptors.
- ***IL-17 stimulates the production of antimicrobial substances,*** including defensins, from numerous cell types.

Other T_H17 Cytokines

IL-22 is a member of the IL-10 cytokine family. It is produced by activated T cells, particularly T_H17 cells, and by NK cells. The actions of IL-22 appear contradictory. Some studies indicate that it contributes to inflammation and tissue injury, but the bulk of the available data suggests that it is produced in epithelial tissues, especially of the skin and gastrointestinal tract, and serves to maintain epithelial integrity, mainly by promoting the barrier function of epithelia and by stimulating repair reactions.

IL-21 is produced by activated $CD4^+$ T cells, including T_H17 cells, which has a wide variety of effects on B and T cells and NK cells. The IL-21 receptor belongs to the type I cytokine receptor family, consists of a ligand-binding chain and the γ_c subunit, and activates a JAK-STAT signaling pathway in which STAT3 is especially prominent. An important function of IL-21 is in antibody responses, especially the reactions that occur in germinal centers (see Chapter 11). IL-21 is required for the *generation of follicular helper T cells* and is also produced by follicular helper cells and stimulates B cells in germinal centers. IL-21 has also been shown to promote the differentiation of T_H17 cells, especially in humans, providing an autocrine pathway for amplifying T_H17 responses. Some of the other reported actions of IL-21 include increasing the proliferation and effector function of $CD8^+$ T cells and NK cells.

Roles of T_H17 Cells in Host Defense

The principal effector function of T_H17 cells is to induce neutrophilic inflammation, which serves to destroy extracellular bacteria and fungi (see Fig. 10-10). The ability of IL-17 to recruit neutrophils accounts for the central role of T_H17 cells in adaptive immune reactions in which neutrophilic inflammation is prominent. The recruited neutrophils ingest and kill extracellular microbes, including fungi and bacteria. The importance of this role of T_H17 cells is illustrated by the inherited disease called the hyper-IgE syndrome (or Job's syndrome), which is characterized by increased susceptibility to cutaneous fungal and bacterial infections, and is caused by mutations in the transcription factor STAT3, which is essential for the development of T_H17 cells (see Chapter 9).

T_H17 cells are also important in the pathogenesis of to many inflammatory diseases, such as psoriasis, inflammatory bowel disease, rheumatoid arthritis, and multiple sclerosis. Antibodies that block the development or functions of T_H17 cells are in clinical trials for several of these diseases. T_H1 and T_H17 cells may both be present in the lesions in these diseases, and their relative contribution to the development and propagation of the disorders is an area of active research. T_H17 cells may also serve to maintain normal epithelial function in the gut and skin, mainly by virtue of the actions of IL-22.

EFFECTOR FUNCTIONS OF $CD8^+$ CYTOTOXIC T LYMPHOCYTES

$CD8^+$ CTLs eliminate intracellular microbes mainly by killing infected cells (see Fig. 10-1B). The development of a $CD8^+$ CTL response to infection proceeds through similar steps as those described for $CD4^+$ T cell responses, including antigen-mediated stimulation of naive $CD8^+$ T cells in lymphoid organs, clonal expansion, differentiation, and migration of differentiated CTLs into tissues. These events were described in Chapter 9. In addition to direct cell killing, $CD8^+$ T cells secrete IFN-γ and thus contribute to macrophage activation in host defense and in hypersensitivity reactions. Here we discuss the mechanisms by which differentiated CTLs kill cells harboring microbes.

Mechanisms of CTL-Mediated Cytotoxicity

CTL-mediated killing involves specific recognition of target cells and delivery of proteins that induce cell death. CTLs kill targets that express the same class I–associated antigen that triggered the proliferation and differentiation of naive $CD8^+$ T cells from which they are derived and do not kill adjacent uninfected cells that do not express this antigen. In fact, even the CTLs themselves are not injured during the killing of antigen-expressing targets. This specificity of CTL effector function ensures that normal cells are not killed by CTLs reacting against infected cells. The killing is highly specific because an "immunologic synapse" (see Chapter 6) is formed at the site of contact of the CTL and the antigen-expressing target, and the molecules that actually perform the killing are secreted into the synapse and cannot diffuse to other nearby cells.

The process of CTL-mediated killing of targets consists of antigen recognition, activation of the CTLs, delivery of the "lethal hit" that kills the target cells, and release of the CTLs (Fig. 10-11). Each of these steps is controlled by specific molecular interactions.

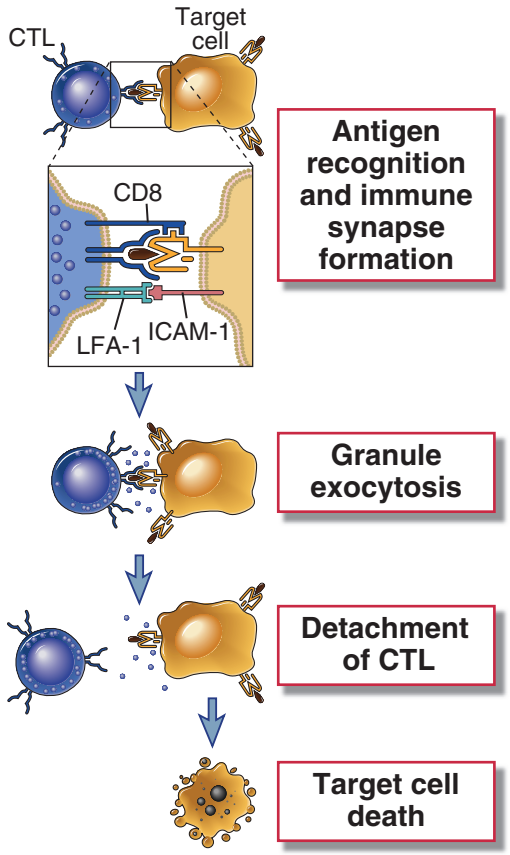


FIGURE 10–11 Steps in CTL-mediated lysis of target cells. A CTL recognizes the antigen-expressing target cell and is activated. Activation results in the release of granule contents from the CTL into the target cell through the area of contact (the immunologic synapse). Granule contents deliver a lethal hit to the target. The CTL may detach and kill other target cells. The formation of conjugates between a CTL and its target and activation of the CTL also require interactions between accessory molecules (LFA-1, CD8) on the CTL and their specific ligands on the target cell; these are not shown.

Recognition of Antigen and Activation of CTLs

The CTL binds and reacts to the target cell by using its antigen receptor, coreceptor (CD8), and adhesion molecules. To be efficiently recognized by CTLs, target cells must express class I MHC molecules complexed to a peptide (the complex serving as the ligand for the T cell receptor (TCR) and the CD8 coreceptor) and intercellular adhesion molecule 1 (ICAM-1, the principal ligand for the LFA-1 integrin). The CTLs and their target cells form tight conjugates (Fig. 10-12). This immunologic synapse (see Chapter 7) formed between the two cells is characterized by a ring of close apposition between the CTL and target cell membranes, mediated by LFA-1–ICAM-1 binding, and an enclosed gap or space inside the ring. Distinct regions of the CTL membrane can be observed by immunofluorescence microscopy within the ring, including a signaling patch, which includes the TCR, protein kinase C-θ, and Lck, and a secretory domain, which appears as a gap to one side of the signaling patch. This interaction results in the initiation of biochemical signals that activate the CTL, which are essentially the same as the signals involved in the activation of helper T cells (see Chapter 7). Cytokines and costimulators provided by dendritic cells, which are required for the differentiation of naive $CD8^+$ T cells into CTLs, are not necessary for triggering the effector function of CTLs (i.e., target cell killing). Therefore, once $CD8^+$ T cells specific for an antigen have differentiated into fully functional CTLs, they can kill any nucleated cell that displays that antigen.

In addition to the T cell receptor, $CD8^+$ CTLs express receptors that are also expressed by NK cells, which contribute to both regulation and activation of CTLs. Some of these receptors belong to the killer immunoglobulin receptor (KIR) family, discussed in Chapter 4, and recognize class I MHC molecules on target cells but are not specific for a particular peptide-MHC complex. These KIRs transduce inhibitory signals that may serve to prevent CTLs from killing normal cells. In addition, CTLs express the NKG2D receptor, described in Chapter 4, that recognizes class I MHC–like molecules MIC-A, MIC-B, and ULBP, expressed on infected or neoplastic cells. NKG2D may serve to deliver signals that act together with TCR recognition of antigen to enhance killing activity.

Killing of Target Cells by CTLs

Within a few minutes of a CTL's antigen receptor recognizing its antigen on a target cell, the target cell undergoes changes that induce it to die by apoptosis. Target cell death occurs during the next 2 to 6 hours and proceeds even if the CTL detaches. Thus, the CTL is said to deliver a lethal hit to the target cell. The principal mechanism of CTL-mediated target cell killing is the delivery of cytotoxic proteins stored within cytoplasmic granules (also called secretory lysosomes) to the target cell, thereby triggering apoptosis of the target cell (Fig. 10-13). As discussed earlier, CTL recognition of the target leads to activation of the CTL, one consequence of which is cytoskeleton reorganization such that the microtubule organizing center of the CTL moves to the area of the cytoplasm near the contact with the target cell. The cytoplasmic granules of the CTL are transported along microtubules and become concentrated in the region of the synapse, and the granule membrane fuses with the plasma membrane at the secretory domain. Membrane fusion results in exocytosis of the CTL's granule contents into the confined space within the synaptic ring, between the plasma membranes of the CTL and target cell.

The cytotoxic proteins in the granules of CTLs (and NK cells) include granzymes and perforin. **Granzymes** A, B, and C are serine proteases that cleave proteins after aspartate residues. **Perforin** is a membrane-perturbing molecule homologous to the C9 complement protein. The granules also contain and a sulfated proteoglycan, **serglycin**, which serves to assemble a complex containing granzymes and perforin. The main function of perforin is to facilitate delivery of the granzymes into the cytosol of the target cell. How this is accomplished is still not well understood. Perforin may polymerize and form aqueous pores in the target cell membrane through which granzymes enter, but there is no proof that this is

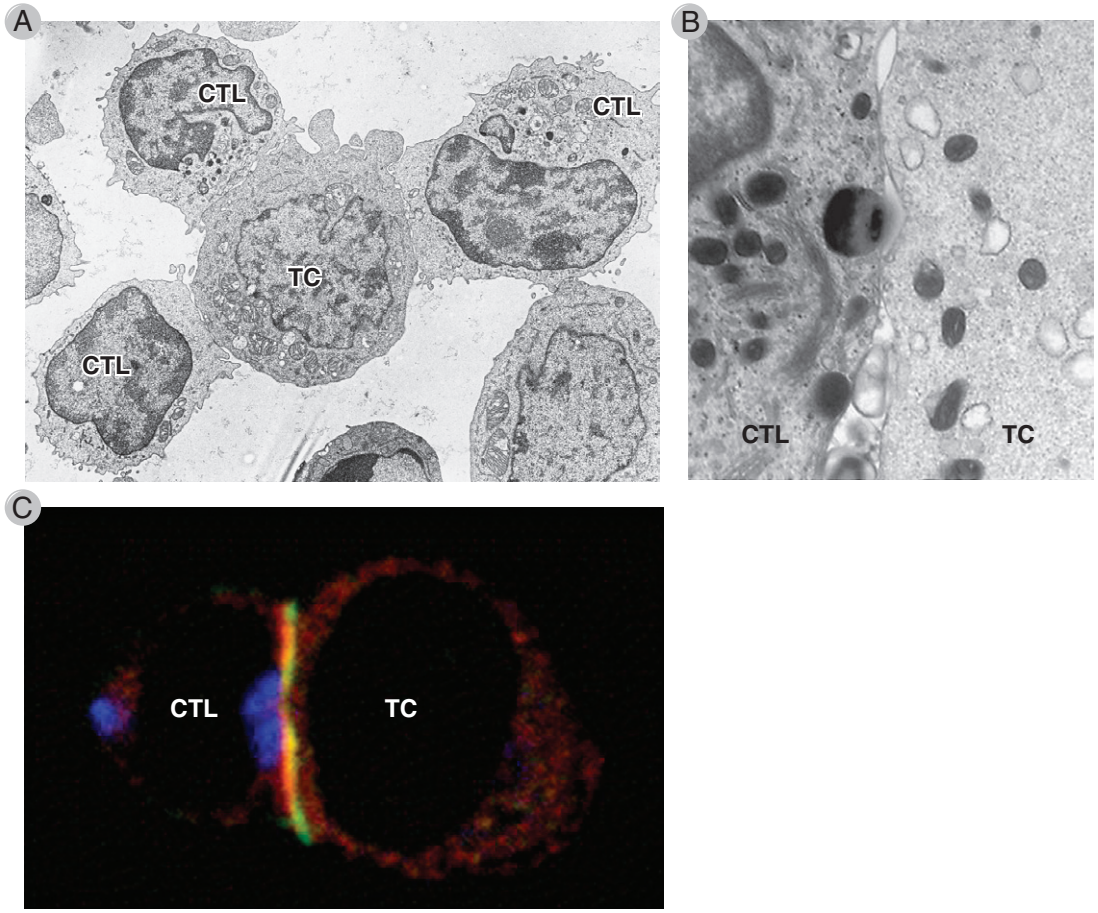


FIGURE 10–12 Formation of conjugates between CTLs and a target cell. A, Electron micrograph of three CTLs from a cloned cell line specific for the human MHC molecule HLA-A2 binding to an HLA-A2–expressing target cell (TC) within 1 minute after the CTLs and targets are mixed. Note that in the CTL on the upper left, the granules have been redistributed toward the target cell. *(Courtesy of Dr. P. Peters, Netherlands Cancer Institute, Amsterdam.)* **B,** Electron micrograph of the point of membrane contact between a CTL *(left)* and target cell *(right)*. Two CTL granules are near the synapse. Several mitochondria are also visible. *(Reprinted from Stinchcombe JC, G Bossi, S Booth, and GM Griffiths. The immunological synapse of CTL contains a secretory domain and membrane bridges. Immunity 8:751-761, 2001, © Cell Press, with permission from Elsevier).* **C,** Confocal fluorescence micrograph of an immune synapse between a CTL *(left)* and target cell *(right)* stained with antibodies against cathepsins in a secretory granule (blue), LFA-1 (green), and the cytoskeletal protein talin (red). The image demonstrates the central location of the secretory granule and the peripheral location of the adhesion molecule LFA-1 and associated cytoskeletal protein talin. *(Reprinted from Stinchcombe JC, and GM Griffiths. The role of the secretory immunological synapse in killing by CD8+ CTL. Seminars in Immunology 15:301-305, © 2003 Elsevier Science Ltd., with permission from Elsevier.)*

critical for CTL-mediated cell killing. According to another current model, complexes of granzyme B, perforin, and serglycin are discharged from the CTL onto the target cell and are internalized into endosomes by receptor-mediated endocytosis. Perforin may act on the endosomal membrane to facilitate the release of the granzymes into the target cell cytoplasm. Once in the cytoplasm, the granzymes cleave various substrates, including caspases, and initiate apoptotic death of the cell. For example, granzyme B activates caspase-3 as well as the Bcl-2 family member Bid, which triggers the mitochondrial pathway of apoptosis (see Fig. 14-7, Chapter 14). Another protein found in human CTL (and NK cell) granules, called granulysin, can alter the permeability of target cell and microbial membranes, but its importance in cell killing by CTLs is not established.

CTLs also use a granule-independent mechanism of killing that is mediated by interactions of membrane molecules on the CTLs and target cells. On activation, CTLs express a membrane protein, called **Fas ligand (FasL),** that binds to the death receptor Fas, which is expressed on many cell types. This interaction also results in activation of caspases and apoptosis of Fas-expressing targets (see Fig. 14-7, Chapter 14). Studies with knockout mice lacking perforin, granzyme B, or FasL indicate that granule proteins are the principal mediators of killing by $CD8^+$ CTLs. Some $CD4^+$ T cells are also capable of killing target cells (which, of course, must express class II MHC–associated peptides to be recognized by the $CD4^+$ cells). $CD4^+$ T cells are deficient in perforin and granzymes, and FasL may be more important for their killing activity.

After delivering the lethal hit, the CTL is released from its target cell, which usually occurs even before the target cell goes on to die. CTLs themselves are not injured during target cell killing, probably because the directed granule exocytosis process during CTL-mediated killing preferentially delivers granule contents into the target cell and away from the CTL. In addition, CTL granules

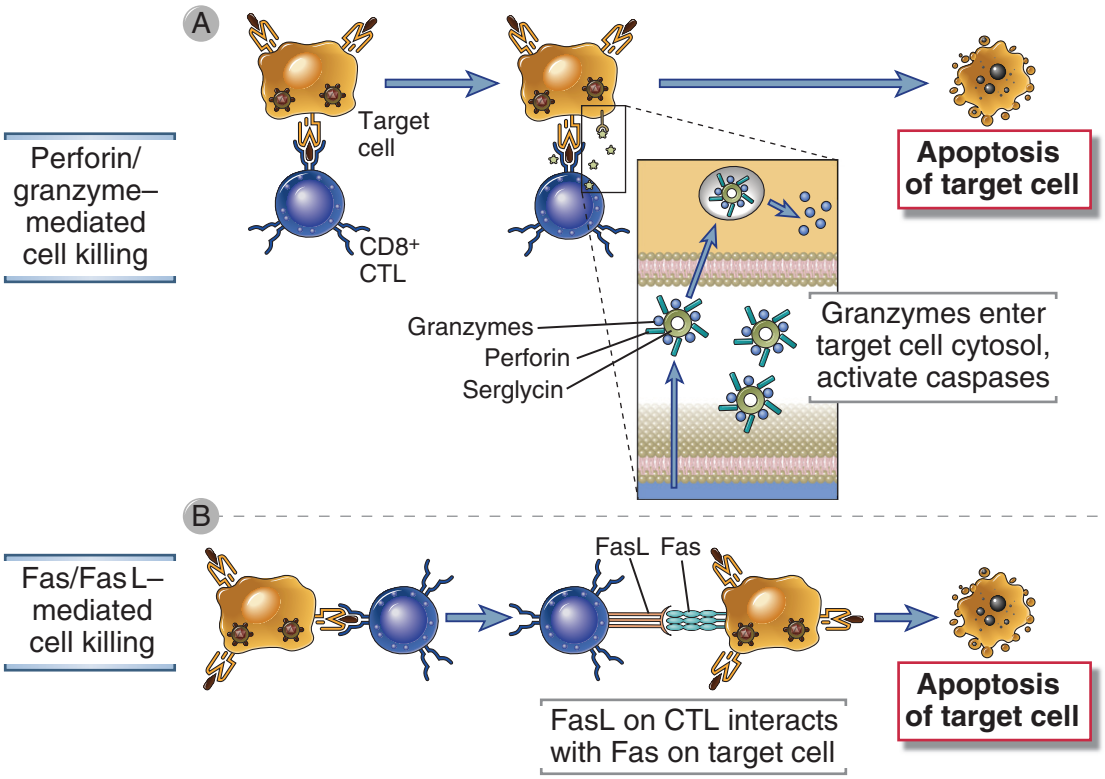


FIGURE 10–13 Mechanisms of CTL-mediated killing of target cells. CTLs kill target cells by two main mechanisms. **A,** Complexes of perforin and granzymes are released from the CTL by granule exocytosis and enter target cells. The granzymes are delivered into the cytoplasm of the target cells by a perforin-dependent mechanism, and they induce apoptosis. **B,** FasL is expressed on activated CTLs, engages Fas on the surface of target cells, and induces apoptosis.

contain a proteolytic enzyme called cathepsin B, which is delivered to the CTL surface on granule exocytosis, where it degrades errant perforin molecules that come into the vicinity of the CTL membrane.

Roles of $CD8^+$ CTLs in Host Defense

In infections by intracellular microbes, the killing activity of CTLs is important for eradication of the reservoir of infection (see Fig. 10-1B). There are two types of situations in which cells cannot destroy microbes that infect them. First, some viruses live and replicate in cells that are incapable of destroying microbes (such as hepatitis viruses in liver cells). Second, even in phagocytes, some microbes escape from vesicles and live in the cytoplasm, where the microbicidal mechanisms of phagocytes are ineffective because these mechanisms are largely restricted to vesicles (to protect the cells from damage). Such infections can be eliminated only by destroying the infected cells, and in adaptive immune responses, $CD8^+$ CTLs are the principal mechanism for killing infected cells. In addition, the caspases that are activated in target cells by granzymes and FasL cleave many substrates and activate enzymes that degrade DNA, but they do not distinguish between host and microbial proteins. Therefore, by activating nucleases in target cells, CTLs can initiate the destruction of microbial DNA as well as the target cell genome, thereby eliminating potentially infectious DNA. The massive expansion of $CD8^+$ T cells that follows infections (see Fig. 9-12, Chapter 9) provides a large pool of CTLs to combat these infections. Defects in the development and activity of CTLs result in increased susceptibility to viral and some bacterial infections and reactivation of latent virus infections (such as infection by the Epstein-Barr virus), which are normally kept in check by virus-specific CTLs.

Destruction of infected cells by CTLs is a cause of tissue injury in some diseases. For instance, in infection by hepatitis B and C viruses, the infected liver cells are killed by the host CTL (and NK cell) response and not by the viruses. These viruses are not cytopathic, but the host senses and reacts against the infectious microbe and is not able to distinguish microbes that are intrinsically harmful or relatively harmless (see Chapter 18).

FUNCTIONS OF OTHER T CELL SUBSETS

The majority of T cells are $CD4^+$ helper cells and $CD8^+$ CTLs. In addition to these, there are smaller populations of T cells that have distinct features and probably serve specialized functions in host defense. The best defined of these subsets are γδ T cells and NKT cells. Both of these subsets have common characteristics that distinguish them from $CD4^+$ and $CD8^+$ T cells.

- γδ T cells and NKT cells recognize a wide variety of antigens, many of which are not peptides, and these

are not displayed by class I and class II MHC molecules on APCs.
- The antigen receptors of many γδ T cells and NKT cells have limited diversity, suggesting that both cell types may have evolved to recognize a small group of microbes. Because of this feature, these T cells are often said to be at the crossroads of innate and adaptive immunity.
- Both cell types are abundant in epithelial tissues, such as the gastrointestinal tract.

γδ T Cells

The antigen receptor of MHC-restricted $CD4^+$ and $CD8^+$ T lymphocytes is a heterodimer composed of α and β chains (see Chapter 7). There is a second type of clonally distributed receptor composed of heterodimers of γ and δ chains, which are homologous to the α and β chains of the TCRs found on $CD4^+$ and $CD8^+$ T lymphocytes. T cells expressing the γδ TCR represent a lineage distinct from the more numerous αβ-expressing T cells. The percentages of γδ T cells vary widely in different tissues and species, but overall, less than 5% of all T cells express this form of TCR. The γδ heterodimer associates with the CD3 and ζ proteins in the same way as TCR αβ heterodimers do, and TCR-induced signaling events typical of αβ-expressing T cells are also observed in γδ T cells. Although the theoretical potential diversity of the γδ TCR is even greater than the diversity of the αβ TCR, in reality, only a limited number of γ and δ V regions are expressed in some subsets of these cells, and there is little or no junctional diversity.

Different populations of γδ T cells may develop at distinct times during ontogeny, contain different V regions, reside in different tissues, and have a limited capacity to recirculate among these tissues. In mice, many skin γδ T cells develop in neonatal life and express one particular TCR with essentially no variability in the V region, whereas many of the γδ T cells in the vagina, uterus, and tongue appear later and express another TCR with a different V region. The limited diversity of the γδ TCRs in many tissues suggests that the ligands for these receptors may be invariant and conserved. One intriguing feature of γδ T cells is their abundance in epithelial tissues of certain species. For example, more than 50% of lymphocytes in the small bowel mucosa of mice and chickens, called **intraepithelial lymphocytes**, are γδ T cells. In mouse skin, most of the intraepidermal T cells express the γδ receptor. Equivalent cell populations are not as abundant in humans; only about 10% of human intestinal intraepithelial T cells express the γδ TCR. γδ T cells in lymphoid organs express more diverse TCRs than the epithelial γδ cells.

γδ T cells do not recognize MHC-associated peptide antigens and are not MHC restricted. Some γδ T cell clones recognize small phosphorylated molecules, alkyl amines, or lipids that are commonly found in mycobacteria and other microbes and that may be presented by "non-classical" class I MHC–like molecules. Other γδ T cells recognize protein or nonprotein antigens that do not require processing or any particular type of APCs for their presentation. Many γδ T cells are triggered by microbial heat shock proteins. A working hypothesis for the specificity of γδ T cells is that they may recognize antigens that are frequently encountered at epithelial boundaries between the host and the external environment.

A number of biologic activities have been ascribed to γδ T cells, including secretion of cytokines and killing of infected cells, but the function of these cells remains poorly understood. It has been postulated that this subset of T cells may initiate immune responses to microbes at epithelia, before the recruitment and activation of antigen-specific αβ T cells. However, mice lacking γδ T cells, created by targeted disruption of the γ or δ TCR gene, have little or no immunodeficiency and only a modest increase in susceptibility to infections by some intracellular bacteria.

NKT Cells

A small population of T cells also expresses markers that are found on NK cells, such as CD56; these are called NKT cells. The TCR α chains expressed by a subset of NKT cells have limited diversity, and in humans, these cells are characterized by a V region encoded by a rearranged Vα24-Jα18 gene segment, with little or no junctional diversity, associated with one of three β chains. Because of this limited diversity, these cells are also called invariant NKT (iNKT) cells. Other NKT cells exist that have quite diverse antigen receptors. All NKT cell TCRs recognize lipids that are bound to class I MHC–like molecules called CD1 molecules. αβ T cells that do not express NKT markers but recognize CD1-associated lipid antigens have also been described, and these cells may be $CD4^+$, $CD8^+$, or $CD4^-CD8^-$ αβ T cells. NKT cells and other lipid antigen–specific T cells are capable of rapidly producing cytokines such as IL-4 and IFN-γ after activation, and they may help marginal zone B cells to produce antibodies against lipid antigens. NKT cells may mediate protective innate immune responses against some pathogens, such as mycobacteria (which have lipid-rich cell walls), and invariant NKT cells may even regulate adaptive immune responses primarily by secreting cytokines. However, the roles of these cells in protective immunity or disease in humans are unclear.

SUMMARY

- Cell-mediated immunity is the adaptive immune response stimulated by microbes inside host cells. It is mediated by T lymphocytes and can be transferred from immunized to naive individuals by T cells and not by antibodies.
- Differentiated effector T cells are recruited preferentially to peripheral tissue sites of infection and tissue injury, and cells that recognize antigen in the tissues are retained. These events are mediated mainly by adhesion molecules and chemokines.
- Both $CD4^+$ and $CD8^+$ T cells contribute to cell-mediated immunity, but each subset has unique effector functions for the eradication of infections.

- $CD4^+$ T_H1 cells recognize antigens of microbes that have been ingested by phagocytes and activate the phagocytes to kill the microbes. The activation of macrophages by T_H1 cells is mediated by IFN-γ and CD40L-CD40 interactions. Activated macrophages kill phagocytosed microbes ingested into phagolysosomes by the actions of reactive oxygen and nitrogen species and enzymes (called classical macrophage activation). Activated macrophages also stimulate inflammation and can damage tissues.
- $CD4^+$ T_H2 cells recognize antigens produced by helminths and other microbes as well as environmental antigens associated with allergies. IL-4, secreted by activated T_H2 cells, promotes B cell isotype switching and production of IgE, which may coat helminths and mediate mast cell deregulation and inflammation. IL-5 secreted by activated T_H2 cells activates eosinophils to release granule contents that destroy helminths but may also damage host tissues. IL-4 and IL-13 together provide protection at epithelial barriers (barrier immunity) and induce an alternative form of macrophage activation that generates macrophages that mediate tissue repair and fibrosis.
- $CD4^+$ T_H17 cells stimulate neutrophil-rich inflammatory responses that eradicate extracellular bacteria and fungi. T_H17 cells may also be important in mediating tissue damage in autoimmune diseases.
- $CD8^+$ CTLs kill cells that express peptides derived from cytosolic antigens (e.g., viral antigens) that are presented in association with class I MHC molecules. CTL-mediated killing is mediated mainly by granule exocytosis, which releases granzymes and perforin. Perforin facilitates granzyme entry into the cytoplasm of target cells, and granzymes initiate several pathways of apoptosis. $CD8^+$ T cells also secrete IFN-γ and thus may participate in defense against phagocytosed microbes and in DTH reactions.
- γδ T cells and NKT cells are small populations of lymphocytes that express antigen receptors of limited diversity and recognize a wide variety of antigens, including peptides and small molecules (γδ cells) and lipids (NKT cells). Some of these cells are located in epithelia and are believed to respond against conserved microbial antigens. The functions of these cells are not well defined.

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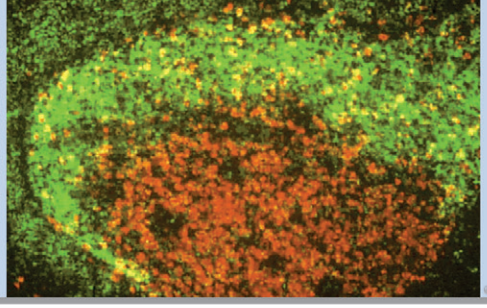
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CHAPTER

11

B Cell Activation and Antibody Production

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Humoral immunity is mediated by secreted antibodies, which are produced by cells of the B lymphocyte lineage. Two types of microbial antigens can induce robust antibody responses. First, multivalent antigens of microbial origin can activate B cells through the B cell receptor (BCR), often accompanied by signals provided by engagement of pattern recognition receptors on B cells by microbial products, but without T cell help. Second, microbial protein antigens can be presented by B cells to helper T cells, resulting in T-dependent responses in which helper T cells drive B cell activation. In both cases, antibodies are secreted and bind to the antigens of extracellular bacteria, viruses, and other microbes and function to neutralize and eliminate these pathogens. The elimination of different types of microbes requires several effector mechanisms that are mediated by distinct antibody isotypes. In general, antibodies produced with help from T cells bind more tightly to antigens and serve more diverse functions than antibodies produced without T cell help, and this is why antibodies against protein antigens (the stimulators of T cells) are the most effective mediators of humoral immunity. This chapter describes the molecular and cellular events of the humoral immune response, in particular the stimuli that induce B cell proliferation and differentiation and how these stimuli influence the type of antibody that is produced. The mechanisms by which antibodies eliminate microbes are described in Chapter 12.

GENERAL FEATURES OF HUMORAL IMMUNE RESPONSES

The earliest studies of adaptive immunity were devoted to analyses of serum antibodies produced in response to microbes, toxins, and model antigens. Much of our current understanding of adaptive immune responses and the cellular interactions that take place during such responses has evolved from studies of antibody production. We begin with a summary of some of the key features of B cell activation and antibody production.

- ***The activation of B cells results in their proliferation, leading to clonal expansion, followed by***

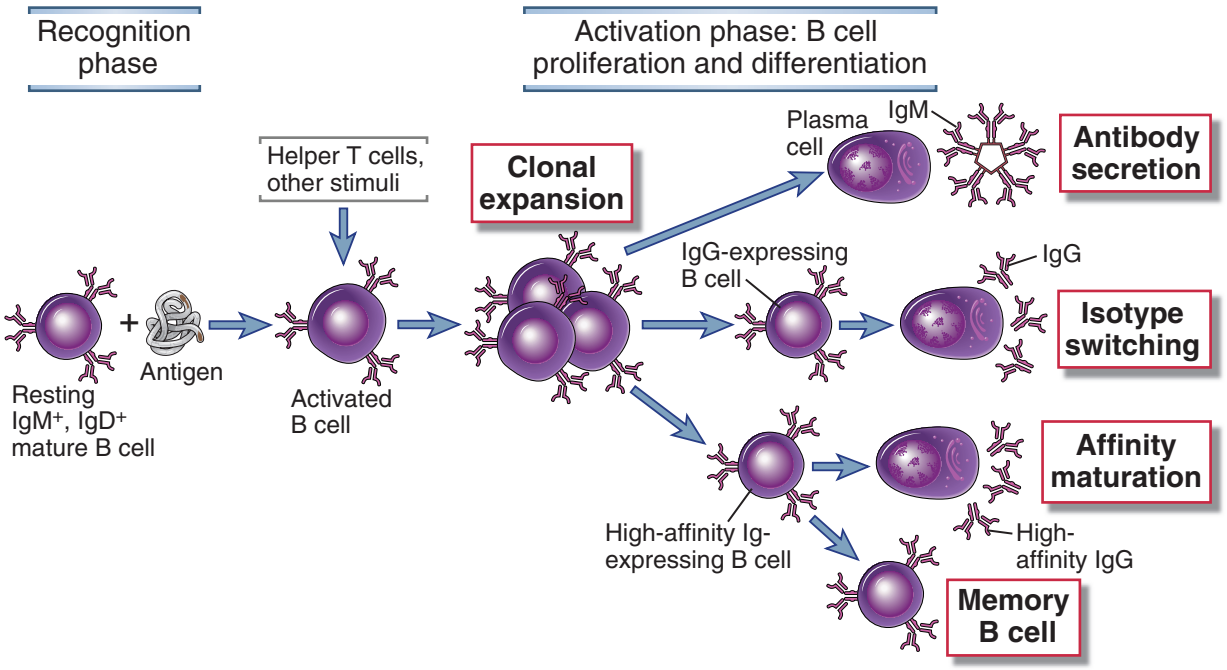


FIGURE 11–1 Phases of the humoral immune response. The activation of B cells is initiated by specific recognition of antigens by the surface Ig receptors of the cells. Antigen and other stimuli, including helper T cells, stimulate the proliferation and differentiation of the specific B cell clone. Progeny of the clone may produce IgM or other Ig isotypes (e.g., IgG), may undergo affinity maturation, or may persist as memory cells.

differentiation, culminating in the generation of memory B cells and antibody-secreting plasma cells (Fig. 11-1). As we discussed in Chapter 8, mature antigen-responsive B lymphocytes develop from bone marrow precursors before antigenic stimulation and populate peripheral lymphoid tissues, which are the sites where lymphocytes interact with foreign antigens. Humoral immune responses are initiated by the recognition of antigens by specific B lymphocytes. Antigen binds to membrane IgM and IgD on mature, naive B cells and activates these cells. Activation leads to proliferation of antigen-specific cells and their differentiation, generating memory B cells and antibody-secreting plasma cells. A single B cell may, within a week, give rise to as many as 5000 antibody-secreting cells, which produce more than 10^{12} antibody molecules per day. This tremendous expansion is needed to keep pace with rapidly dividing microbes. Some activated B cells begin to produce antibodies other than IgM and IgD; this process is called heavy chain isotype (class) switching. As a humoral immune response develops, activated B cells that produce antibodies that bind to antigens with increasing affinity progressively dominate the response; this process is called affinity maturation.

- ***The type and amount of antibodies produced vary according to the type of antigen driving the immune response, the involvement of T cells, a prior history of antigen exposure, and the anatomic site at which activation occurs.*** The influence of these factors on the humoral immune response is summarized later and discussed throughout the chapter.
- ***Antibody responses to protein antigens require that the antigen be specifically recognized and internalized by B cells and that a peptide fragment of the internalized protein be presented to $CD4^+$ helper T lymphocytes that then activate these B cells.*** For this reason, proteins are classified as thymus-dependent or T-dependent antigens. The term *helper T lymphocyte* arose from the realization that these cells stimulate, or help, B lymphocytes to produce antibodies. A specialized type of helper T cell, called a follicular helper T cell, facilitates the formation of germinal centers, which are specialized structures in lymphoid organs generated during T-dependent humoral immune responses.
- ***Antibody responses to multivalent nonprotein antigens with repeating determinants, such as polysaccharides, some lipids, and nucleic acids, do not require antigen-specific helper T lymphocytes.*** Multivalent antigens (so called because each antigen molecule contains multiple identical epitopes) are therefore called thymus-independent or T-independent antigens. These responses are elicited by engagement of the BCR and may be enhanced by the triggering of pattern recognition receptors on B cells and myeloid cells and by cytokines.
- ***Some of the progeny of activated B cells are long-lived antibody-secreting plasma cells, which continue to produce antibodies for months or years, and others are long-lived memory cells.*** Humoral immune responses are initiated in peripheral lymphoid organs, such as the spleen for blood-borne antigens, draining lymph nodes for antigens entering through the skin and other epithelia, and mucosal lymphoid tissues for some inhaled and ingested antigens. Antibodies produced at these sites enter the circulation or are transported into the lumens of mucosal organs and mediate their protective effects wherever antigens are present.

In T-dependent responses, plasma cells or their precursors migrate from germinal centers in the peripheral lymphoid organs, where they are produced, to the bone marrow, where they live for many years. These long-lived plasma cells secrete antibodies that provide immediate protection whenever a microbe recognized by those antibodies infects the individual. Some progeny of B cells activated in a T-dependent manner may differentiate into memory cells, which mount rapid responses on subsequent encounters with the antigen. The differentiation of activated B cells into plasma cells or memory cells depends on signals from receptors on B cells, including the antigen receptor and key cytokine receptors, that induce the expression of specific transcription factors that control cell fate decisions.

- ***Heavy chain isotype switching and affinity maturation are typically seen in helper T cell–dependent humoral immune responses to protein antigens.*** Isotype switching primarily results from the stimulation of B cells by helper T cells. CD40 ligand (CD40L) on the surface of activated helper T cells and cytokines secreted by these T cells are the main molecular drivers that induce B cells to undergo the switching process. Affinity maturation is also dependent on the activation of B cells by CD40L on T cells; it involves the somatic mutation of rearranged Ig V genes in activated B cells and the subsequent selection of B cells with a high affinity for the original antigen. Whereas some isotype switching can occur outside lymphoid follicles, somatic mutation and isotype switching occur largely in germinal centers.
- ***Primary and secondary antibody responses to protein antigens differ qualitatively and quantitatively*** (Fig. 11-2). Primary responses result from the activation of previously unstimulated naive B cells, whereas secondary responses are due to the stimulation of expanded clones of memory B cells. Therefore, the secondary response develops more rapidly than does the primary response, and larger amounts of antibodies are produced in the secondary response. Heavy chain isotype switching and affinity maturation also increase with repeated exposure to protein antigens.
- ***Distinct subsets of B cells respond preferentially to different types of antigens*** (Fig. 11-3). Follicular B cells in peripheral lymphoid organs primarily make antibody responses to protein antigens that require collaboration with helper T cells. Marginal zone B cells in the spleen and other lymphoid tissues recognize multivalent antigens, such as blood-borne polysaccharides, and mount primarily T cell–independent antibody responses. B-1 B cells also mediate largely T cell–independent responses, but in mucosal tissues and the peritoneum.

In the following sections, we will initially discuss the interaction of antigen with B cells and then the role of helper T cells in B cell responses to protein antigens and the mechanisms of isotype switching and affinity maturation. We will conclude with a discussion of T-independent antibody responses.

ANTIGEN RECOGNITION AND ANTIGEN-INDUCED B CELL ACTIVATION

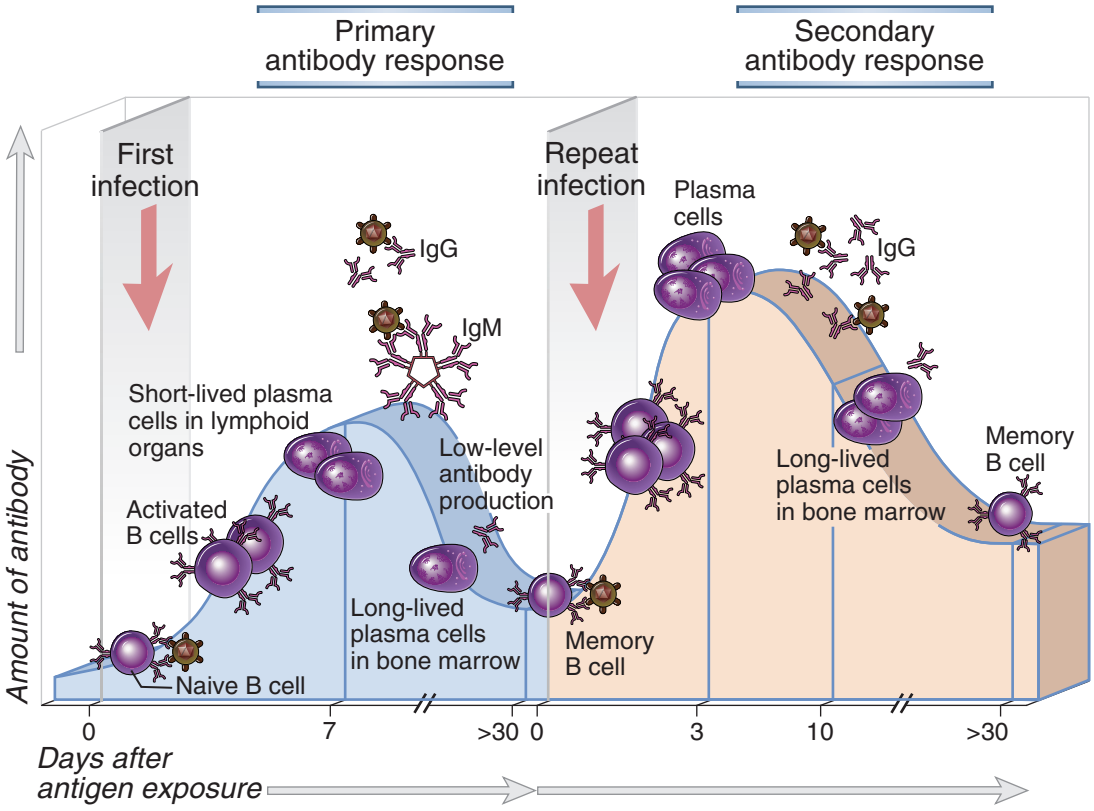
To initiate antibody responses, antigens have to be captured and transported to the B cell areas of lymphoid organs. The antigens then initiate the process of B cell activation, often working in concert with other signals that are generated during innate immune responses triggered by microbes during infections or by adjuvants in vaccines. We next describe these early events in B cell activation.

Antigen Capture and Delivery to B Cells

Mature B lymphocytes migrate from one secondary lymphoid organ to the next in search of antigen. Naive B cells reside in and circulate through the follicles of peripheral lymphoid organs (the spleen, lymph nodes, and mucosal lymphoid tissues) in search of their cognate antigen (see Chapters 2 and 3). Most B cells enter follicles and are called follicular B cells or recirculating B cells. Entry into the follicles is guided by the chemokine CXCL13 secreted by follicular dendritic cells and other stromal cells in the follicle. CXCL13 binds to the CXCR5 chemokine receptor on recirculating naive B cells and attracts these cells into the follicles. As we discuss later, the same chemokine-receptor pair is also important during immune responses because it can attract a subset of activated T cells to the follicle. Naive follicular B cells survive for limited periods until they encounter antigen (see Chapter 2). Follicular B cell survival depends on signals from the BCR as well as on inputs received from a tumor necrosis factor (TNF) superfamily cytokine called BAFF (B cell–activating factor of the TNF family, also known as BLyS, for B lymphocyte stimulator), which provides maturation and survival signals through the BAFF receptor. BAFF and a related ligand, APRIL, can activate two other receptors, TACI and BCMA, which participate in later stages of B cell activation and differentiation (and will be discussed later). These cytokines are produced mainly by myeloid cells in lymphoid follicles and in the bone marrow.

Antigen may be delivered to naive B cells in lymphoid organs in different forms and by multiple routes. Antigens that enter by crossing an epithelial barrier as well as antigens in the circulation are capable of activating B cells and are brought to B cell zones by several mechanisms (Fig. 11-4).

- Most antigens from tissue sites are transported to lymph nodes by afferent lymphatic vessels that drain into the subcapsular sinus of the nodes. Soluble antigens, generally smaller than 70 kD, may reach the B cell zone through conduits that extend between the subcapsular sinus and the follicle and interact directly with specific B cells.
- Subcapsular sinus macrophages capture large microbes and antigen-antibody complexes and deliver these to follicles, which lie under the sinus.
- Many relatively large antigens that enter the node through afferent lymphatic vessels are not captured by subcapsular sinus macrophages but are too large to



Feature	Primary response	Secondary response
Peak response	Smaller	Larger
Antibody isotype	Usually IgM > IgG	Relative increase in IgG and, under certain situations, in IgA or IgE
Antibody affinity	Lower average affinity, more variable	Higher average affinity (affinity maturation)
Induced by	All immunogens	Only protein antigens

FIGURE 11–2 Primary and secondary humoral immune responses. In a primary immune response, naive B cells are stimulated by antigen, become activated, and differentiate into antibody-secreting cells that produce antibodies specific for the eliciting antigen. Some of the antibody-secreting plasma cells migrate to and survive in the bone marrow, where they continue to produce antibodies for long periods. Long-lived memory B cells are also generated during the primary response. A secondary immune response is elicited when the same antigen stimulates these memory B cells, leading to more rapid proliferation and differentiation and production of greater quantities of specific antibody than are produced in the primary response. The principal characteristics of primary and secondary antibody responses are summarized in the table. These features are typical of T cell–dependent antibody responses to protein antigens.

enter the conduits. These antigens may be captured in the medullary region by resident dendritic cells and transported into follicles, where they can activate B cells.

- Antigens in immune complexes may bind to complement receptors (in particular the complement receptor type 2 or CR2) on marginal zone B cells, and these cells can transfer the immune complex–containing antigens to follicular B cells.
- Antigen in immune complexes may also bind to CR2 on the surface of follicular dendritic cells and be presented to antigen-specific B cells.
- Blood-borne pathogens may be captured by plasmacytoid dendritic cells in the blood and transported to the spleen, where they may be delivered to marginal zone B cells.
- Polysaccharide antigens can be captured by macrophages in the marginal zone of splenic lymphoid follicles and displayed or transferred to B cells in this area.

In all these cases, the antigen that is presented to B cells is generally in its intact, native conformation and is not processed by antigen-presenting cells. This, of

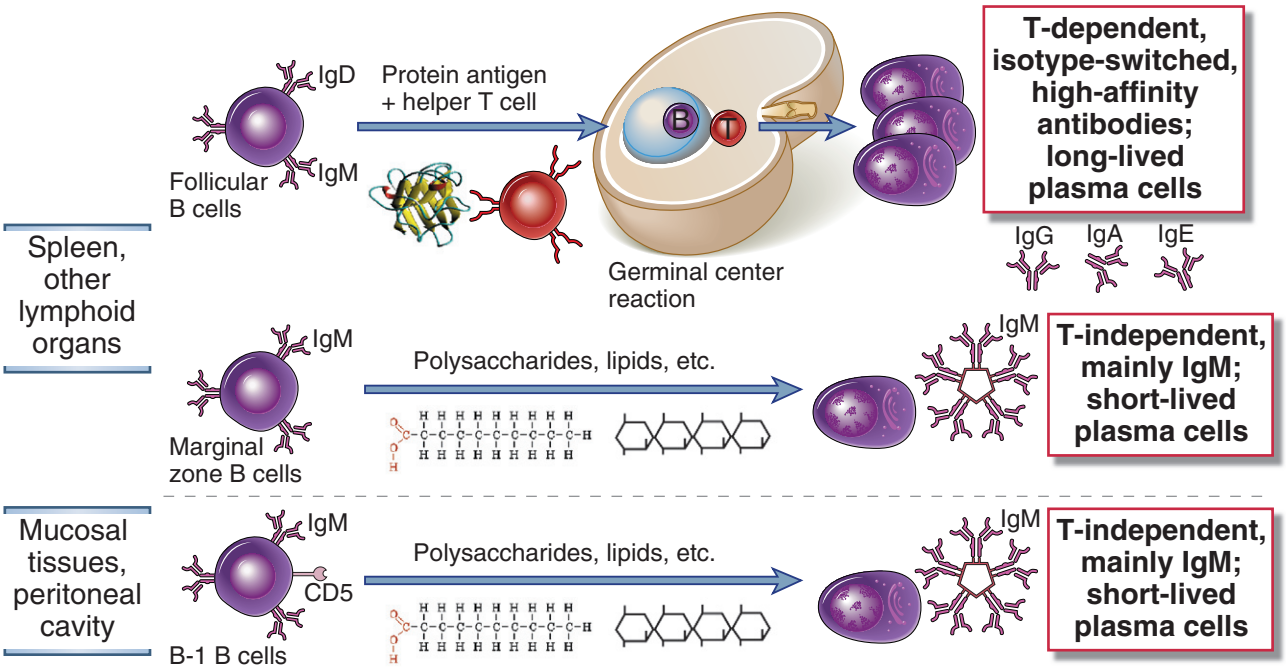


FIGURE 11–3 Distinct B cell subsets mediate different types of antibody responses. Follicular B cells are recirculating cells that receive T cell help when they respond to protein antigens and thus initiate T-dependent antibody responses. These responses can lead to the formation of germinal centers, where class switching and somatic mutation of antibody gene occur, resulting in specialized high-affinity antibody responses. T-independent responses to multivalent antigens such as lipids, polysaccharides, and nucleic acids are mediated mainly by marginal zone B cells in the spleen and B-1 cells in mucosal sites. These functional distinctions between subsets are not absolute.

course, is one of the important distinctions between the forms of antigens recognized by B and T lymphocytes (see Chapter 6).

Activation of B Cells by Antigens and Other Signals

The activation of antigen-specific B lymphocytes is initiated by the binding of antigen to membrane Ig molecules, which, in conjunction with the associated Igα and Igβ proteins, make up the antigen receptor complex of mature B cells. The B lymphocyte antigen receptor, described in Chapter 7, serves two key roles in B cell activation. First, binding of antigen to the receptor delivers biochemical signals to the B cells that initiate the process of activation (see Chapter 7). Second, the receptor internalizes the bound antigen into endosomal vesicles, and if the antigen is a protein, it is processed into peptides that may be presented on the B cell surface for recognition by helper T cells. This antigen-presenting function of B cells will be considered later in the context of T-dependent B cell activation.

Although antigen recognition can initiate B cell responses, by itself it is usually inadequate to stimulate significant B cell proliferation and differentiation. For full responses to be induced, other stimuli cooperate with BCR engagement, including complement proteins, pattern recognition receptors, and, in the case of protein antigens, helper T cells (discussed later).

B cell activation is facilitated by the CR2/CD21 coreceptor on B cells, which recognizes complement fragments covalently attached to the antigen or that are part of immune complexes containing the antigen (Fig. 11-5). Complement activation is typically seen with microbes, which activate this system in the absence of antibodies by the alternative and lectin pathways, and in the presence of antibodies by the classical pathway (see Chapters 4 and 12). In all these situations, complement fragments are generated that bind to the microbes. One of these fragments, called C3d, is recognized by the complement receptor CR2 (also called CD21), which enhances the strength of BCR signaling and thus functions as a coreceptor for B cells (see Chapter 7). Some nonmicrobial polysaccharides also activate complement by the alternative or lectin pathway, and this is one reason that such antigens are able to induce antibody responses without T cell help.

Microbial products engage Toll-like receptors on B cells, which also enhances B cell activation (see Fig. 11-5). Human B cells express several Toll-like receptors (TLRs), including TLR5, which recognizes bacterial flagellin; endosomal TLR7, which recognizes single-stranded RNA; and TLR9, which is specific for unmethylated CpG-rich DNA in endosomes (see Chapter 4). Murine B cells also express TLR4 on the cell surface. These pattern recognition receptors directly activate B cells. In addition, the activation of myeloid cells through pattern recognition receptors can promote B cell activation indirectly in two ways. Dendritic cells activated through TLRs contribute significantly to helper T cell activation (see Chapter 9). Myeloid cells activated by TLRs may secrete APRIL and BAFF, cytokines that can induce T-independent B cell responses.

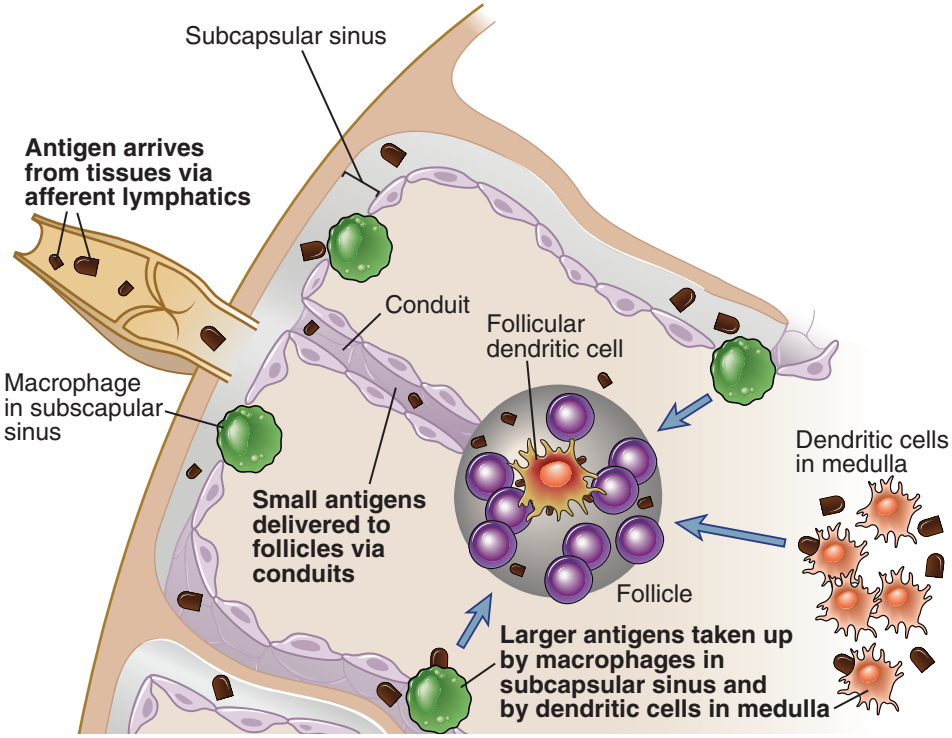


FIGURE 11–4 Pathways of antigen delivery to follicular B cells. Antigen is delivered to B cells in follicles largely through afferent lymphatics that drain into the subcapsular sinus of the lymph node. Small antigens may reach the follicle through conduits. Larger antigens may be captured by subcapsular sinus macrophages and delivered to the follicle, or they may directly access dendritic cells in the medulla that may be involved in delivering antigen not only to the T cell zone but also to B cell–containing follicles.

Functional Responses of B Cells to Antigens

Distinct cellular events are induced by antigen-mediated cross-linking of the BCR by different types of antigens: multivalent antigens initiate B cell proliferation and differentiation, and protein antigens prepare B cells for subsequent interactions with helper T cells. Antigen receptor cross-linking by some antigens can stimulate several important changes in B cells (Fig. 11-6). The previously resting cells enter into the G_1 stage of the cell cycle, and this is accompanied by increases in cell size, cytoplasmic RNA, and biosynthetic organelles such as ribosomes The survival of the stimulated B cells is enhanced as a result of the production of various anti-apoptotic proteins, notably Bcl-2 (see Fig. 14-7, Chapter 14), and the cells may proliferate and secrete some antibody. Activation of B cells by antigen results in increased expression of class II major histocompatibility complex (MHC) molecules and B7 costimulators, because of which antigen-stimulated B cells are more efficient activators of helper T lymphocytes than are naive B cells. The expression of receptors for several T cell–derived cytokines is also increased, which enables antigen-stimulated B lymphocytes to respond to cytokines secreted by helperT cells. The expression of chemokine receptors may change, resulting in movement of the B cells out of the follicles.

The importance of signaling by the BCR complex for the subsequent responses of the cells may vary with the nature of the antigen. Most T-independent antigens, such as polysaccharides, display multiple identical epitopes on each molecule or on a cell surface. Therefore, such multivalent antigens effectively cross-link many B cell antigen receptors and initiate responses even though they are not recognized by helper T lymphocytes. In contrast, many naturally occurring globular protein antigens possess only one copy of each epitope per molecule. Therefore, such protein antigens cannot simultaneously bind to and cross-link multiple Ig molecules, and their ability to activate the BCR is limited, so they do not typically induce signals that can drive B cell proliferation and differentiation. However, some protein antigens may be displayed as multivalent arrays on the surfaces of microbes or cells, or they may be multivalent because they are in aggregates. Protein antigens are also internalized by the BCR and processed and displayed to helper T cells, which are potent stimulators of B lymphocyte proliferation and differentiation. In fact, in T-dependent responses, a major function of membrane Ig may be not signaling but binding and internalization of the antigen for subsequent presentation to helper T cells. Therefore, protein antigens may only activate the BCR to enhance antigen presentation and the migration of antigen-specific B cells toward the T cell zone.

After specific B cells recognize antigens, the subsequent steps in humoral immune responses are very different in T-dependent and T-independent responses. We next describe the activation of B cells by protein antigens and helper T cells.

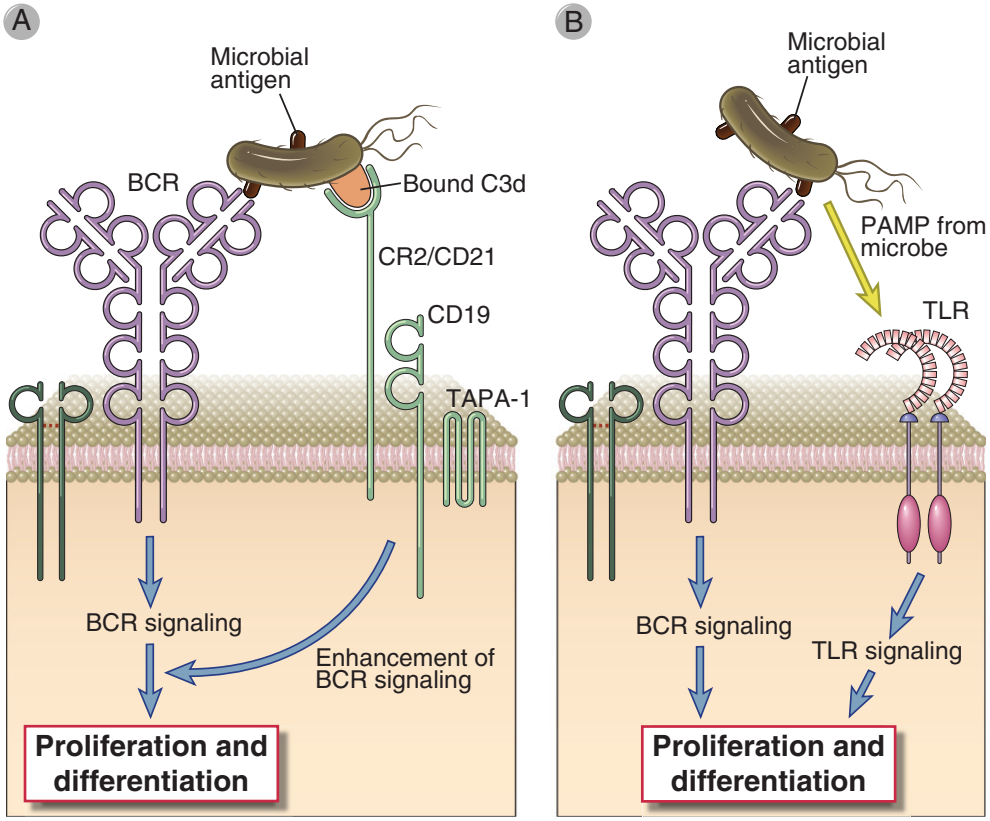


FIGURE 11–5 Role of CR2 and TLRs in B cell activation. In immune responses to microbes, activation of B cells through the BCR may be enhanced by complement-coated antigen that can simultaneously ligate the BCR and complement receptor 2 (CR2) **(A)**, and may also involve the contemporaneous activation of Toll-like receptors (TLRs) on B cells by molecules (so-called pathogen-associated molecular patterns [PAMPs]) derived from the microbe **(B)**.

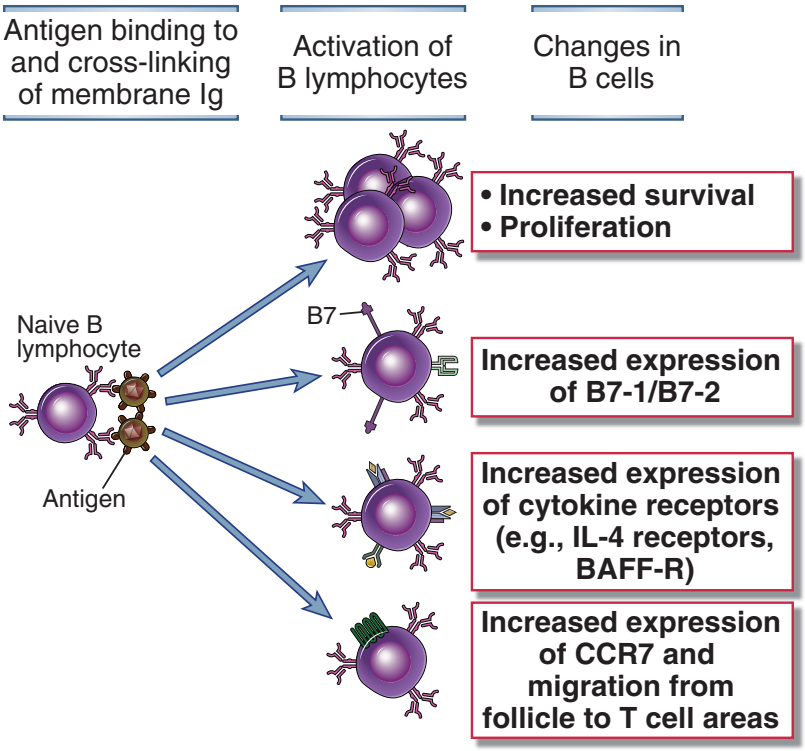


FIGURE 11–6 Functional responses induced by antigen-mediated cross-linking of the BCR complex. Antigen-induced cross-linking of the B cell antigen receptor induces several cellular responses including proliferation, expression of new cell surface molecules including costimulators and cytokine receptors, and altered migration within the lymph node of the cells as a result of the expression of CCR7.

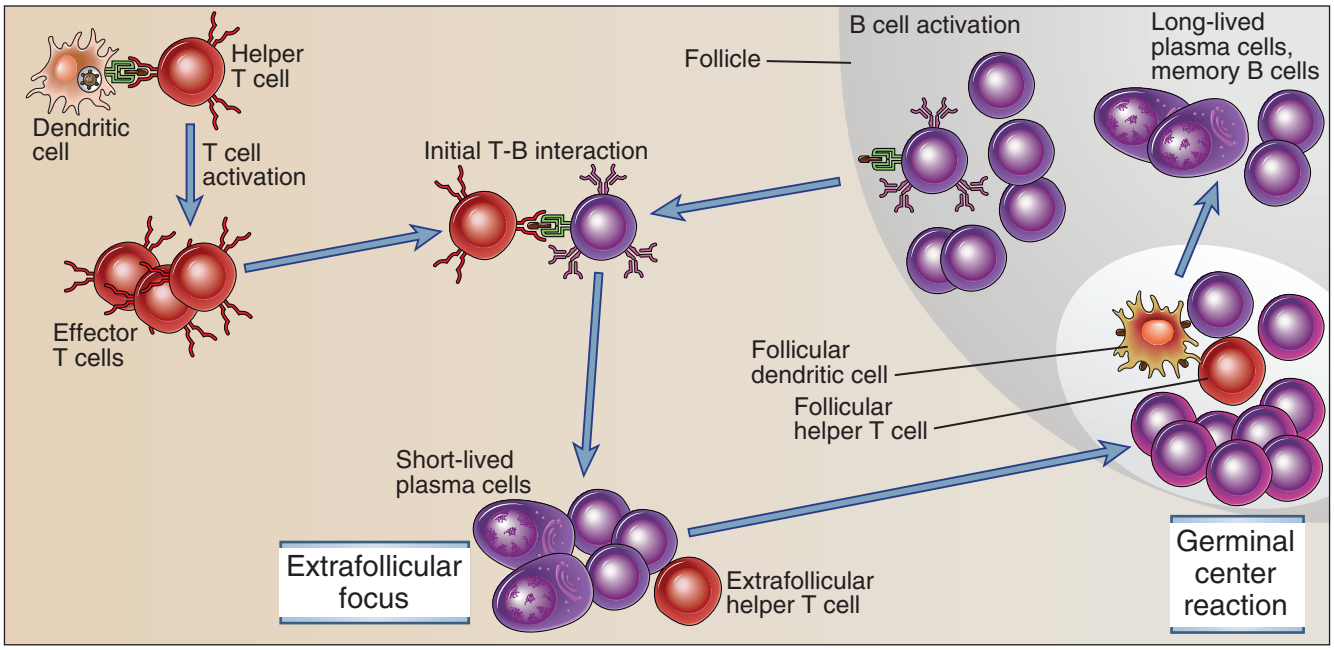


FIGURE 11–7 Sequence of events in humoral immune responses to T cell–dependent protein antigens. Immune responses are initiated by the recognition of antigens by B cells and helper T cells. The activated lymphocytes migrate toward one another and interact, resulting in B cell proliferation and differentiation. Restimulation of B cells by helper T cells in extrafollicular sites leads to early isotype switching and short-lived plasma cell generation. The late events occur in germinal centers and include somatic mutation and the selection of high-affinity cells (affinity maturation), additional isotype switching, memory B cell generation, and the generation of long-lived plasma cells.

HELPER T CELL–DEPENDENT ANTIBODY RESPONSES TO PROTEIN ANTIGENS

Antibody responses to protein antigens require recognition and processing of the antigen by B cells, followed by presentation of a peptide fragment of the antigen to helper T cells, leading to cooperation between the antigen-specific B and T lymphocytes. The helper function of T lymphocytes was discovered by experiments performed in the late 1960s, which showed that antibody responses required the presence of both bone marrow cells (now known to contain mature B lymphocytes) and thymus-derived cells (which were T lymphocytes). Subsequent experiments showed that only the bone marrow cells produced the antibody, but their activation required the thymic cells, which were called helper cells. These classic experimental studies were among the first formal proof of the importance of interactions between two completely different cell populations in the immune system. It took many years to establish that most helper T cells are $CD4^+CD8^-$ lymphocytes that recognize peptide antigens presented by class II MHC molecules. One of the important accomplishments of immunology has been the elucidation of the mechanisms of T-B cell interactions and the actions of helper T cells in antibody responses.

The Sequence of Events During T Cell–Dependent Antibody Responses

Protein antigens are recognized by specific B and T lymphocytes in peripheral lymphoid organs, and the activated cell populations come together in these organs to initiate humoral immune responses (Fig. 11-7). The interaction between helper T cells and B lymphocytes is initiated by the recognition of protein antigens, and the sequence of events that drive B cell proliferation and differentiation is as follows:

- Antigen is taken up by dendritic cells that have also been activated by microbial products and presented to naive helper T cells in the T cell zones of lymphoid organs.
- Helper T cells are initially activated by the dendritic cells presenting antigenic peptides on class II MHC molecules and also expressing costimulatory ligands such as the B7 molecules (see Chapters 6 and 9).
- Activated helper T cells express CD40L and also chemokine receptors that promote their migration toward the follicle following a chemokine gradient.
- B cells in the lymphoid follicles are activated by antigen, which may be in soluble form or displayed by other cells.
- B cells process and present the antigen, alter their cell surface chemokine receptor profile, and migrate toward the T cell zone.
- Activated helper T cells and B cells interact at the boundary of the T cell zone and follicle, where the B cells are activated by CD40L on the helper T cells and by cytokines that the T cells secrete.
- Small extrafollicular B cell foci form in the medulla of the lymph node or between the periarteriolar lymphoid sheath and the red pulp of the spleen. B cells in these foci undergo low levels of isotype switching and somatic mutation and generate short-lived plasma cells that secrete antibodies.

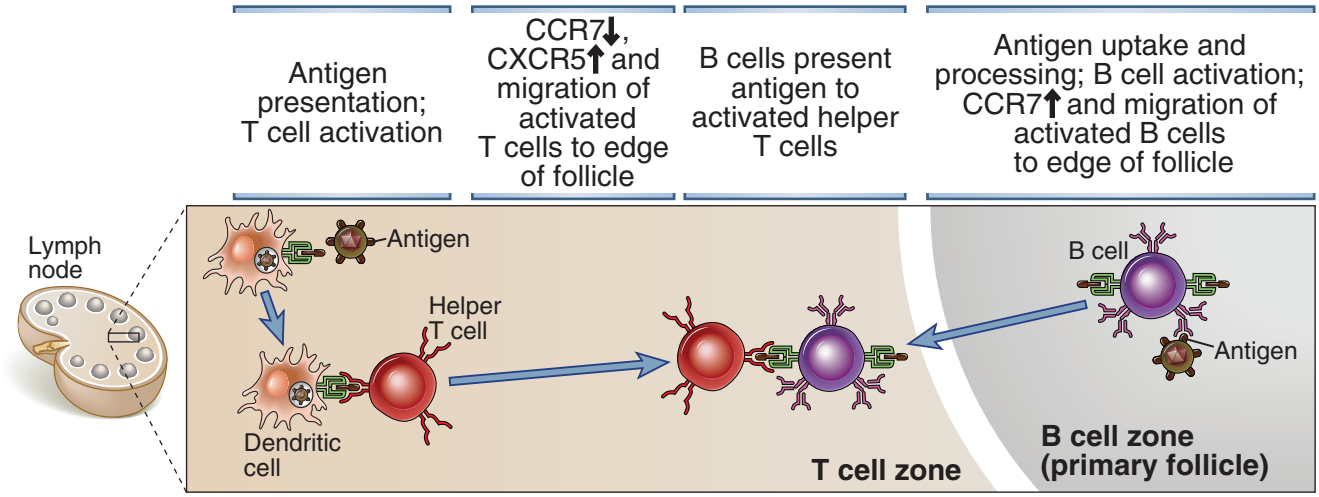


FIGURE 11–8 Migration of B cells and helper T cells and T-B interaction. Antigen-activated helper T cells and B cells move toward one another in response to chemokine signals and make contact adjacent to the edge of primary follicles. In this location, the B cell presents antigen to the T cell, and the B cell receives activating signals from the T cell.

- Some activated helper T cells are induced during B:T interactions to differentiate into T follicular helper cells (T_{FH} cells).
- Activated B cells and T_{FH} cells migrate into the follicle, where the B cells are activated by T_{FH} cells. Germinal centers form within the follicles and are the sites of extensive B cell proliferation, isotype switching, somatic mutation, selection events that lead to affinity maturation, memory B cell generation, and induction of long-lived plasma cells that migrate to the bone marrow.

In the following sections, we describe each of these steps in detail.

Initial Activation and Migration of Helper T Cells and B Cells

The activation of specific B and T cells by antigen is essential for their functional interaction and brings them into proximity to enhance the possibility that the antigen-specific B and T cells will locate one another (Fig. 11-8). The frequency of naive B cells or T cells specific for a given epitope of an antigen is as low as 1 in 10^5 to 1 in 10^6 lymphocytes, and both populations have to be activated and the specific B and T cells have to find each other and physically interact to generate strong antibody responses. Helper T cells that have been activated by antigen and costimulation are induced to proliferate, express CD40L, and secrete cytokines. They also down-regulate the chemokine receptor CCR7 and increase the expression of CXCR5 and as a result leave the T cell zone and migrate toward the follicle. As mentioned earlier, CXCL13, the ligand for CXCR5, is secreted by follicular dendritic cells and other follicular stromal cells, and it contributes to the migration of activated $CD4^+$ T cells toward the follicle.

Although protein antigens, being monovalent, typically do not provide strong enough signals to induce much B cell proliferation and differentiation, they can activate B cells and initiate a number of events. BCR engagement by these antigens results in reduced cell surface expression of the chemokine receptor CXCR5 and increased expression of CCR7, which is normally expressed on T cells. As a result, activated B cells migrate toward the T cell zone drawn by a gradient of CCL19 and CCL21, the ligands for CCR7. B cells activated by protein antigens can also express CD69, which blocks surface expression of sphingosine 1-phosphate receptors, causing retention of activated B cells in lymph nodes (see Chapter 3). Protein antigens are endocytosed by the B cell and presented in a form that can be recognized by helper T cells, and this represents the next step in the process of T-dependent B cell activation.

Antigen Presentation by B Cells and the Hapten-Carrier Effect

Protein antigens that are recognized by specific B cell antigen receptors are endocytosed and delivered to a vesicular compartment for processing, leading to the association of a linear peptide derived from the protein with a class II MHC molecule that can bind and display the peptide (Fig. 11-9). This peptide is presented on the B cell surface to a helper T cell that was previously activated in the T cell zone when its TCR recognized an identical peptide presented by a dendritic cell that had encountered the same antigen. Because the BCR recognizes an epitope of the native protein with high affinity, specific B cells bind and present the antigen much more efficiently (i.e., at much lower concentrations) than do other B cells not specific for the antigen. This is why B cells specific for an antigen respond preferentially to that antigen, compared with other "bystander" cells. A protein antigen that elicits a T-dependent B cell response therefore makes use of at least two epitopes when activating specific B cells. A surface epitope on the native protein is recognized with high specificity by a B cell, and an internal linear peptide epitope is subsequently released from the protein, binds class II MHC molecules, and is

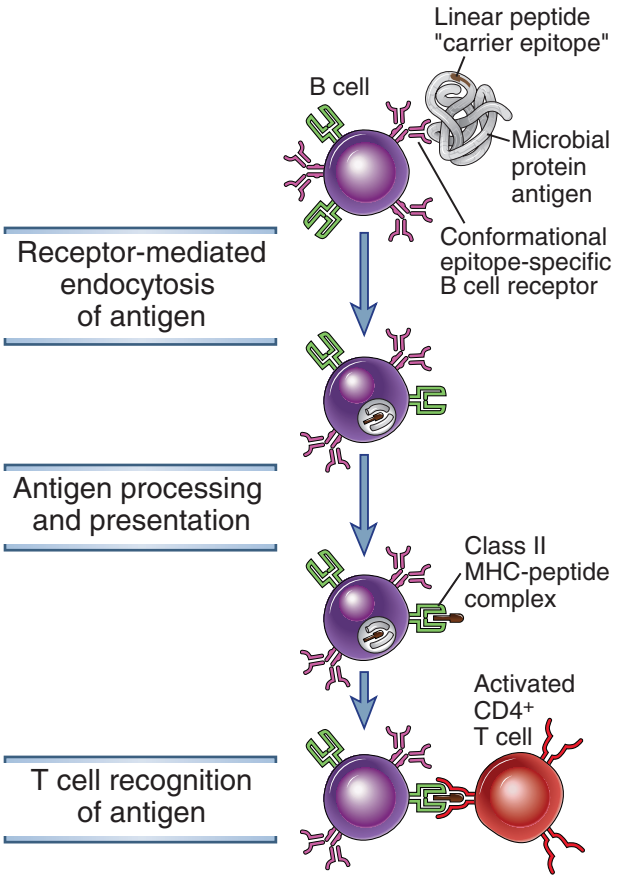


FIGURE 11–9 Antigen presentation on B cells to helper T cells. Protein antigens bound to membrane Ig are endocytosed and processed, and peptide fragments are presented in association with class II MHC molecules. Helper T cells that were previously activated by dendritic cells recognize the MHC-peptide complexes on the B cells and then stimulate B cell responses. Activated B cells also express costimulators (not shown) that enhance helper T cell responses. In hapten-carrier responses, the protein is conjugated to a hapten (the B cell epitope) and is internalized by a hapten-specific B cell, which processes the antigen and presents the linear peptide (the T cell epitope, sometimes known as the carrier determinant) on class II MHC molecules to an activated helper T cell.

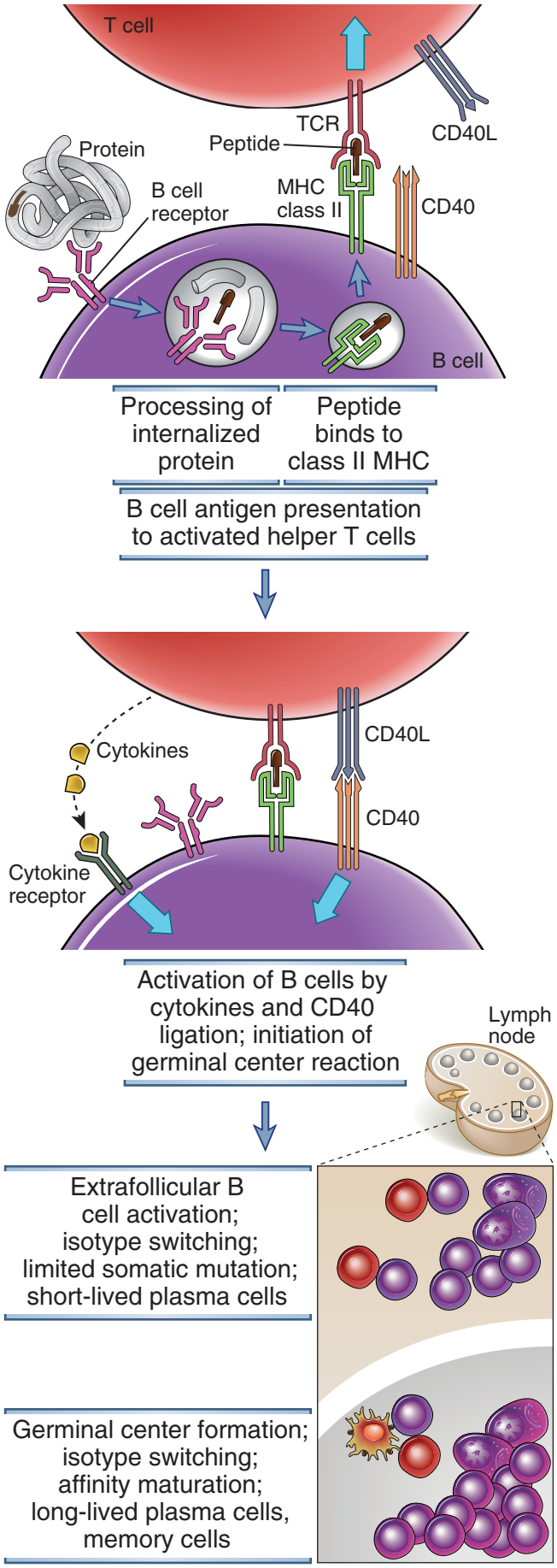
recognized by helper T cells. The antibodies that are subsequently secreted are usually specific for conformational determinants of the native antigen because membrane Ig on B cells is capable of binding conformational epitopes of proteins, and the same Ig is secreted by plasma cells derived from those B cells. This feature of B cell antigen recognition determines the fine specificity of the antibody response and is independent of the fact that helper T cells recognize only linear epitopes of processed peptides. In fact, a single B lymphocyte specific for a native epitope may bind and endocytose a protein and present multiple different peptides complexed with class II MHC molecules to different helper T cells, but the resultant antibody response remains specific for the native protein.

The principles outlined here for T-B cell collaboration help explain a phenomenon that is known as the hapten-carrier effect. Analysis of antibody responses to hapten-carrier conjugates was among the earliest approaches that demonstrated how antigen presentation by B lymphocytes contributes to the development of humoral immune responses. Haptens, such as dinitrophenol, are small chemicals that can be bound by specific antibodies but are not immunogenic by themselves. If, however, haptens are coupled to proteins, which serve as carriers, the conjugates are able to induce antibody responses against the haptens. There are three important characteristics of anti-hapten antibody responses to hapten-protein conjugates. First, such responses require both hapten-specific B cells and protein (carrier)–specific helper T cells. Second, to stimulate a response, the hapten and carrier portions have to be physically linked and cannot be administered separately. Third, the interaction is class II MHC restricted, that is, the helper T cells cooperate only with B lymphocytes that express class II MHC molecules that are identical to those that were involved in the initial activation of naive T cells by dendritic cells. All these features of antibody responses to hapten-protein conjugates can be explained by the antigen-presenting functions of B lymphocytes. Hapten-specific B cells bind the antigen through the hapten determinant, endocytose the hapten-carrier conjugate, and present peptides derived from the carrier protein to carrier-specific helper T lymphocytes (see Fig. 11-9). Thus, the two cooperating lymphocytes recognize different epitopes of the same complex antigen. The hapten is responsible for efficient carrier uptake, which explains why hapten and carrier must be physically linked. The requirement for MHC-associated antigen presentation for T cell activation accounts for the MHC restriction of T cell–B cell interactions.

The characteristics of humoral responses elucidated for hapten-carrier conjugates apply to all protein antigens in which one intrinsic determinant, usually a native conformational determinant, is recognized by B cells (and is therefore analogous to the hapten) and another determinant, in the form of a class II MHC–associated linear peptide, is recognized by helper T cells (and is analogous to the carrier that is the source of the peptide). The hapten-carrier effect is the basis for the development of conjugate vaccines, discussed later in the chapter.

Role of CD40L:CD40 Interaction in T-Dependent B Cell Activation

On activation, helper T cells express CD40 ligand (CD40L), which engages its receptor, CD40, on antigen-stimulated B cells at the T-B interface and induces subsequent proliferation and differentiation initially in extrafollicular foci and later in germinal centers (Fig. 11-10). CD40 is a member of the TNF receptor superfamily. Its ligand, CD40L (CD154), is a trimeric membrane protein that is homologous to TNF. CD40 is constitutively expressed on B cells, and CD40L is expressed on the surface of helper T cells after activation by antigen and costimulators. When these activated helper T cells interact physically with antigen-presenting B cells, CD40L recognizes CD40 on the B cell surface. CD40L binding to CD40 results in the conformational alteration of preformed CD40 trimers, and this induces the association of cytosolic proteins called TRAFs (TNF receptor–associated factors) with the cytoplasmic domain of CD40. The TRAFs recruited to



CD40 initiate enzyme cascades that lead to the activation and nuclear translocation of transcription factors, including NF-κB and AP-1, which collectively stimulate B cell proliferation and increased synthesis and secretion of Ig. Similar signaling pathways are activated by TNF receptors (see Chapter 7). CD40-induced transcription factor induction is also crucial for subsequent germinal center formation and for the synthesis of activation-induced deaminase (AID), an enzyme that is required for somatic mutation and isotype switching, as will be discussed later. T cell–mediated dendritic cell and macrophage activation also involves the interaction of CD40L on activated helper T cells with CD40 on dendritic cells and macrophages (see Chapter 10). Thus, this pathway of contact-dependent cellular responses is a general mechanism for the activation of target cells by helper T lymphocytes and is not unique to antibody production.

Mutations in the CD40L gene result in a disease called the X-linked hyper-IgM syndrome, which is characterized by defects in antibody production, isotype switching, affinity maturation, and memory B cell generation in response to protein antigens, as well as deficient cell-mediated immunity (see Chapter 20). Similar abnormalities are seen in CD40 or CD40L gene knockout mice. Interestingly, a DNA virus called the Epstein-Barr virus (EBV) infects human B cells and induces their proliferation. This may lead to immortalization of the cells and the development of lymphomas. The cytoplasmic tail of a transforming protein of EBV called LMP1 (latent membrane protein 1) associates with the same TRAF molecules as does the cytoplasmic domain of CD40, and this apparently triggers B cell proliferation. Thus, EBV LMP1 is functionally homologous to a physiologic B cell signaling molecule, and EBV has apparently co-opted a normal pathway of B lymphocyte activation for its own purpose, which is to promote survival and proliferation of cells that the virus has infected.

In addition to CD40L on helper T cells activating B cells, helper T cells also secrete cytokines that contribute to B cell responses. The best defined roles of T cell–derived cytokines in humoral immune responses are in isotype switching, described later. Several cytokines have also been implicated in the early steps of B cell proliferation and differentiation, but it is not clear if any are actually essential for these responses.

The initial interaction of activated helper T cells with antigen-specific B cells at the edge of the follicle induces some proliferation and differentiation of B cells and leads

FIGURE 11–10 Mechanisms of helper T cell–mediated B cell activation. Activated helper T cells that migrate toward the B cell zone express CD40L and their T cell receptors recognize peptide–class II MHC complexes on B cells that have been triggered by antigen and have in turn migrated to the interface between T and B cell zones. CD40L on the activated T helper cell then binds to CD40 on antigen-activated B cells and initiates B cell proliferation and differentiation. Cytokines bind to cytokine receptors on the B cells and also stimulate B cell responses. Two types of differentiation events may occur—the formation of extrafollicular foci and the induction of a germinal center B cell reaction.

to the formation of a collection of cells called an extrafollicular focus.

Extrafollicular B Cell Activation

After the initial interaction of B cells with helper T cells at the interface between the follicle and the T cell zone, subsequent activation of B cells by helper T cells can occur at two different locations, one outside the follicles and the other in the follicles, in germinal centers. The nature of the B cell response differs in these locations (Table 11-1). Extrafollicular foci of T-dependent B cell activation are generated relatively early in an immune response. Germinal centers, in which specialized follicular helper T (T_{FH}) cells trigger B cells to undergo numerous changes, appear a few days later.

B cells that are activated by helper T cells through CD40L in the extrafollicular foci that may undergo some degree of differentiation into plasma cells and isotype switching. Each such focus may produce 100 to 200 antibody-secreting plasma cells. In the spleen, extrafollicular foci develop in the outer portions of the T cell–rich periarteriolar lymphoid sheath (PALS) or between the T cell zone and the red pulp, and these collections of cells are also called PALS foci. Similar T-dependent foci are observed in the medullary cords of lymph nodes. Isotype switching first occurs in these extrafollicular foci. Some somatic hypermutation of Ig genes, which underlies the process of affinity maturation, also occurs, but this is much less than the magnitude of somatic hypermutation seen in germinal center responses described later. The circulating antibody-secreting cells, called plasmablasts, and tissue plasma cells that are generated in extrafollicular foci are mostly short-lived, and these cells do not acquire the ability to migrate to distant sites such as the bone marrow. The small amount of antibody produced in these foci may contribute to the formation of immune complexes (containing antigen, antibody, and perhaps complement) that are trapped by follicular dendritic cells in lymphoid follicles. It has been speculated that this deposition of immune complexes may be a necessary prelude to the release of chemokines from follicular dendritic cells that draw in a few (perhaps only one or two) activated B cells from the extrafollicular focus into the follicle to initiate the germinal center reaction.

The Germinal Center B Cell Reaction and the Function of Follicular Helper T Cells

The characteristic events of helper T cell–dependent antibody responses, including affinity maturation, isotype switching, generation of memory B cells, and long-lived plasma cell differentiation, occur primarily in the germinal centers of lymphoid follicles. Within 4 to 7 days after antigen exposure, some of the activated helper T cells that migrate to meet activated B cells are triggered by these B cells to differentiate into **follicular helper T cells** (T_{FH} cells), which express high levels of the chemokine receptor CXCR5 and are drawn into lymphoid follicles by the ligand for CXCR5, which is produced only in follicles. These T cells are called follicular helper T (T_{FH}) cells because they are the major $CD4^+$ T cells present within follicles and they serve critical roles in the germinal center reaction. At the same time, a few activated B cells migrate back into the follicle and begin to proliferate rapidly, forming a lightly staining central region of the follicle called the germinal center (Figs. 11-11 and 11-12). Each fully formed germinal center contains cells derived from only one or a few antigen-specific B cell clones. Within the germinal center is a "dark zone" that is densely packed with rapidly proliferating B cells. The doubling time of these proliferating germinal center B cells, also called centroblasts, is estimated to be 6 to 12 hours, so that

TABLE 11–1 Extrafollicular and Germinal Center B Cell Responses

Feature	Follicular/Germinal Center	Extrafollicular
Localization	Secondary follicles	Medullary cords of lymph nodes and at junctions between T cell zone and red pulp of spleen
CD40 signals	Required	Required
Specialized T cell help	T_{FH} cells in germinal center	Extrafollicular T helper cells
AID expression	Yes	Yes
Class switching	Yes	Yes
Somatic hypermutation	High rate	Low rate
Antibody affinity	High	Low
Terminally differentiated B cells	Long-lived plasma cells and memory cells	Short-lived plasma cells (life span of ~3 days)
Fate of plasma cells	Bone marrow or local MALT	Most die by apoptosis in secondary lymphoid tissues where they were produced
B cell transcription factors	Bcl-6	Blimp-1

AID, activation-induced cytidine deaminase; Bcl-6, B cell lymphoma 6; Blimp-1, B lymphocyte–induced maturation protein 1; IL-21R, interleukin-21 receptor; MALT, mucosa-associated lymphoid tissue; T_{FH}, follicular helper T cell.
Data from Vinusa CG, I Sanz, and MC Cook. Dysregulation of germinal centres in autoimmune disease. Nature Reviews Immunology 9:845-857, 2009.

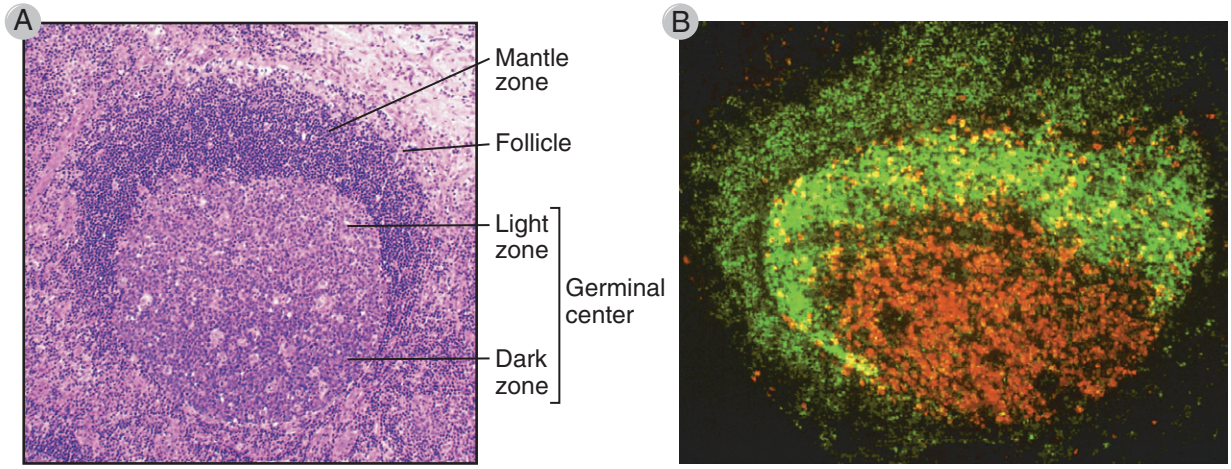


FIGURE 11–11 Germinal centers in secondary lymphoid organs. A, Histology of a secondary follicle with a germinal center in a lymph node. The germinal center is contained within the follicle and includes a basal dark zone and an adjacent light zone. The mantle zone is the parent follicle within which the germinal center has formed. *(Courtesy of Dr. James Gulizia, Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts.)* **B,** Cellular components of the germinal center. A secondary follicle has been stained with an anti-CD23 antibody (green), which brightly stains follicular dendritic cells in the light zone and dimly stains naive B cells in the mantle zone. Anti-Ki67 (red), which detects cycling cells, stains mitotically active B cell blasts in the dark zone. *(Modified from Liu YJ, GD Johnson, J Gordon, and IC MacLennan. Germinal centres in T-cell–dependent antibody responses. Immunology Today 13:17-21, Copyright 1992, with permission from Elsevier.)*

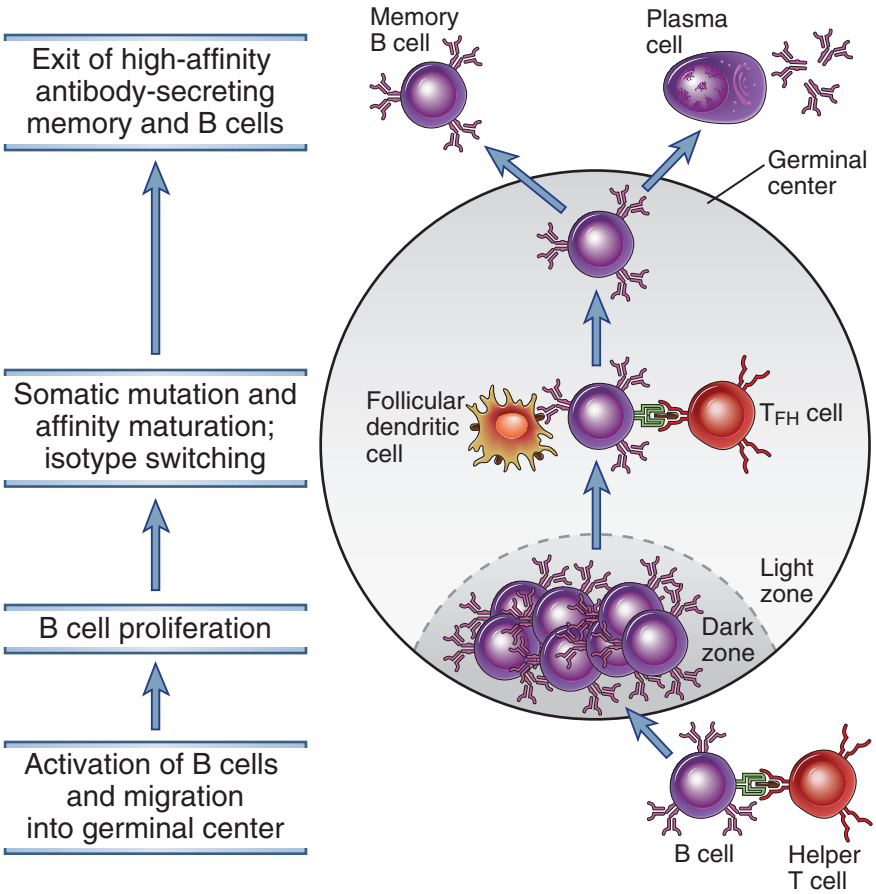


FIGURE 11–12 The germinal center reaction in a lymph node. B cells that have been activated by T helper cells at the edge of a primary follicle migrate into the follicle and proliferate, forming the dark zone of the germinal center. Germinal center B cells undergo extensive isotype switching. Somatic hypermutation of Ig V genes occur in these B cells, and they migrate into the light zone, where they encounter follicular dendritic cells displaying antigen and T_{FH} cells. B cells with the highest affinity Ig receptors are selected to survive, and they differentiate into antibody-secreting or memory B cells. The antibody-secreting cells leave and reside in the bone marrow as long-lived plasma cells, and the memory B cells enter the recirculating pool.

within 5 days, a single lymphocyte may give rise to almost 5000 progeny. The progeny of the proliferating B cells in the germinal center are smaller cells, sometimes called centrocytes, that undergo differentiation and selection processes in the "light zone," described later. B cells in germinal centers express a transcriptional repressor known as **Bcl-6** (for B cell lymphoma gene 6), whose role is described later when we consider the transcriptional regulation of B cell fate.

In addition to the chemokine receptor CXCR5, T_{FH} cells are characterized by the expression of ICOS (inducible costimulator), the cytokine IL-21, and the transcription factor Bcl-6. T_{FH} cells have a phenotype that makes them distinct from the T_H1, T_H2, T_H17, and Treg subsets described in Chapters 9 and 10. It is possible that T_{FH} cells can develop from naive $CD4^+$ T cells or from polarized T cell subsets that retain developmental plasticity. The "signature" cytokine secreted by T_{FH} cells is IL-21. It is required for germinal center development and also contributes to the generation of plasma cells in the germinal center reaction. In addition to IL-21, T_{FH} cells secrete other cytokines, including IFN-γ and IL-4 (but at lower levels than differentiated T_H1 and T_H2 cells, respectively), and all these play important roles in isotype switching.

The mechanisms that drive the development of T_{FH} cells from $CD4^+$ cells and the mechanisms by which T_{FH} cells activate B cells are not fully understood. A number of molecules on B cells and helper T cells are known to play key roles in these processes (Fig. 11-13). The costimulator ICOS, which is related to CD28 and is expressed on T_{FH} cells, is essential for the germinal center reaction. The interaction of ICOS with ICOS ligand on B cells promotes the differentiation of T cells into T_{FH} cells. The interactions between B cells and helper T cells are mediated by integrins and by members of the SLAM family of costimulators. A signaling molecule that associates with these SLAM family proteins in T_{FH} cells is called SAP, and SAP signaling activates transcriptional regulators, particularly Bcl-6, that are required for T_{FH} cell development. SAP is mutated in patients with a disease known as the X-linked lymphoproliferative syndrome, which is associated with defects in antibody and cytotoxic T cell responses (see Chapter 20). IL-21 secreted by T_{FH} cells may facilitate germinal center B cell selection events and differentiation of activated B cells into plasmablasts. These helper T cells can secrete other cytokines that may be characteristic of T_H1, T_H2, and T_H17 cells, and these cytokines can contribute to isotype switching. Germinal center formation is also dependent on CD40L:CD40 interactions. These may be critical for B cell proliferation, which is required for expansion of B cells in germinal centers, and also for isotype switching and affinity maturation. Therefore, germinal centers are defective in humans and in mice with genetic defects in T cell development or activation or with mutations of either CD40 or its ligand (see Chapter 20).

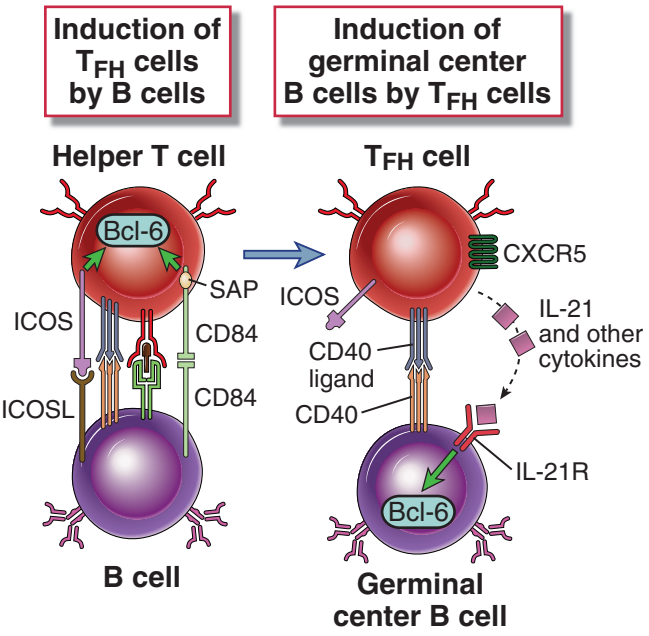


FIGURE 11–13 Molecular events in T follicular helper cell generation and function. Activated B cells express ICOSL and signal to T helper cells. Triggering of ICOS and homotypic activation of SLAM family proteins on T cells result in differentiation into T follicular helper (T_{FH}) cells. SLAM-associated protein (SAP) is a signaling molecule required for T_{FH} cell differentiation. T_{FH} cells express the Bcl-6 transcription factor, secrete IL-21 and other cytokines, and activate B cells in the germinal center reaction.

The architecture of lymphoid follicles and the germinal center reaction within follicles depend on the presence of **follicular dendritic cells (FDCs)**. FDCs are found only in lymphoid follicles and express complement receptors (CR1, CR2, and CR3) and Fc receptors. These molecules are involved in displaying antigens for the selection of germinal center B cells, as described later. FDCs do not express class II MHC molecules and are not derived from progenitors in the bone marrow. In spite of their name, they are distinct from the class II MHC–expressing dendritic cells that capture antigens in tissues and transport them to lymphoid organs where they present peptides to T lymphocytes. The long cytoplasmic processes of FDCs form a meshwork around which germinal centers are formed. Proliferating B cells accumulate in the histologically identifiable dark zone of the germinal center, which has few FDCs. The small nondividing progeny of the B cells migrate to the adjacent light zone, where they come into close contact with the processes of the abundant FDCs and also form intimate contacts with T_{FH} cells, and this is where subsequent selection events occur (see Fig. 11-12). The rim of naive B cells in the follicle, surrounding the germinal center, is called the mantle zone.

Heavy Chain Isotype (Class) Switching

In response to CD40 engagement and cytokines, some of the progeny of activated IgM- and IgD-expressing B cells undergo the process of heavy chain isotype (class) switching, leading to the production of antibodies with heavy chains of different classes, such as γ, α, and ε (Fig. 11-14). Isotype switching is observed in B cells in extrafollicular foci, driven by extrafollicular helper T cells, and much more in germinal centers, driven by T_{FH} cells. The capacity of B cells to produce different antibody isotypes provides a remarkable plasticity in humoral immune responses by generating antibodies that perform distinct effector functions and are involved in defense against different types of infectious agents.

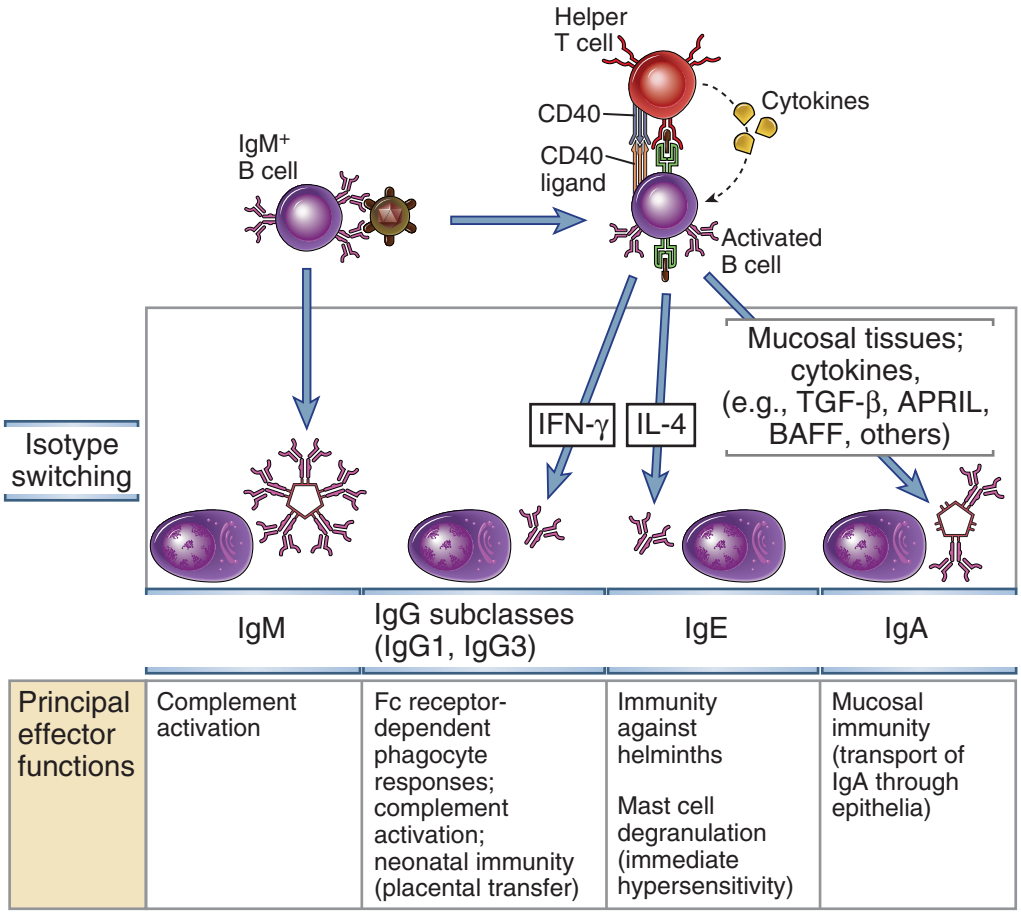


FIGURE 11–14 Ig heavy chain isotype switching. B cells activated by helper T cell signals (CD40L, cytokines) undergo switching to different Ig isotypes, which mediate distinct effector functions. Selected examples of switched isotypes are shown. The role of IFN-γ in directing specific isotype switching events has been established only in rodents.

Isotype switching in response to different types of microbes is regulated by cytokines produced by the helper T cells that are activated by these microbes. For instance, the major protective humoral immune response to bacteria with polysaccharide-rich capsules consists of IgM antibodies, which bind to the bacteria, activate the complement system, and induce phagocytosis of the opsonized bacteria. Polysaccharide antigens, which do not elicit T cell help, stimulate mainly IgM antibodies, with little if any isotype switching to some IgG subclasses. The response to many viruses and bacteria involves production of IgG antibodies, which block entry of the microbes into host cells and also promote phagocytosis by macrophages. Viruses and many bacteria activate helper T cells of the T_H1 subset, which produce the cytokine IFN-γ. In mice, IFN-γ is the main inducer of B cell switching to opsonizing and complement-fixing IgG subclasses; it is still not clear which cytokines serve this role in humans. The antibody response to many helminthic parasites is mainly IgE, which participates in eosinophil- and mast cell–mediated elimination of the helminths (see Chapters 12 and 15); IgE antibodies also mediate immediate hypersensitivity (allergic) reactions (see Chapter 19). Helminths activate the T_H2 subset of helper T cells, which produces IL-4, the cytokine that induces switching to IgE. In the germinal center reaction, these cytokines might be produced not by classical T_H1 and T_H2 effector cells (which tend to migrate to peripheral sites of infection and inflammation) but by T_{FH} cells that retain the capacity to produce T_H1 or T_H2 cytokines. In addition, B cells in different anatomic sites switch to different isotypes. Specifically, B cells in mucosal tissues switch to IgA, which is the antibody class that is most efficiently transported through epithelia into mucosal secretions, where it defends against microbes that try to enter through the epithelia (see Chapter 13). Switching to IgA is stimulated by transforming growth factor-β (TGF-β), which is produced by many cell types, including helper T cells, in mucosal and other tissues. Cytokines of the TNF family, BAFF and APRIL, also stimulate switching to IgA. Because these cytokines are produced by myeloid cells, they can stimulate IgA responses in the absence of T cell help. Some individuals who inherit mutant versions of the *TACI* gene, which encodes a receptor for these cytokines, have a selective deficiency of IgA production (see Chapter 20).

CD40 signals work together with cytokines to induce isotype switching. CD40 engagement induces the enzyme activation-induced deaminase (AID), which, as we shall see later, is crucial for both isotype switching and somatic mutation. The requirement for CD40 signaling and AID to promote isotype switching in B cells is well

documented by analysis of mice and humans lacking CD40, its ligand, or AID. In all these cases, the antibody response to protein antigens is dominated by IgM antibodies, and there is limited switching to other isotypes. Cytokines, as described later, identify the specific Ig heavy chain loci that will participate in the switching process.

The molecular mechanism of isotype switching is a process called switch recombination, in which the rearranged VDJ exon that encodes an Ig heavy chain V domain recombines with a downstream C region gene and the intervening DNA is deleted. An overview of the process is provided in Figure 11-15. These DNA recombination events involve nucleotide sequences called **switch regions**, which are located in the introns between the J and C segments at the 5′ ends of each C_H locus. Switch regions are 1 to 10 kilobases long, contain numerous tandem repeats of GC-rich DNA sequences, and are found upstream of every heavy chain gene. Upstream of each switch region is a small exon called the I exon (for initiator of transcription) preceded by an I region promoter. Signals from cytokines and CD40 induce transcription from a particular I region promoter reading through the I exon, switch region, and adjacent C_H exons. These transcripts are known as germline transcripts. They do not encode specific proteins but are required for

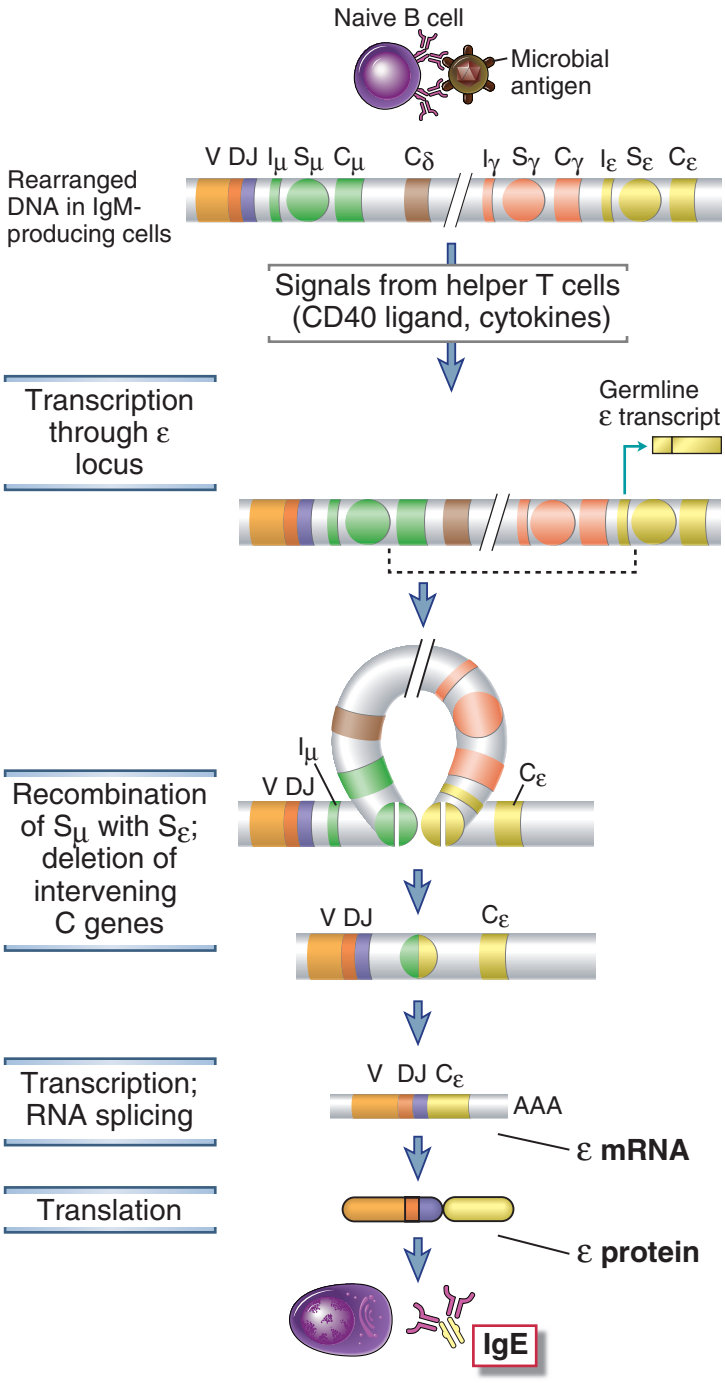


FIGURE 11–15 Mechanisms of heavy chain isotype switching. In the absence of helper T cell signals, B cells produce IgM. When antigen-activated B cells encounter helper T cell signals (CD40L and, in this example, IL-4), the B cells undergo switching to other Ig isotypes (in this example, IgE). These stimuli initiate germline transcription through the I_ε-S_ε-C_ε locus. The proximal C_H genes are deleted in a circle of DNA, leading to recombination of the VDJ exon with the C_ε gene. Switch regions are indicated by circles labeled S_μ or S_γ. Although a switch region is not shown for the δ gene, in humans a switch-like region upstream of the δ gene is functional. I_μ and I_ε represent initiation site for germline transcription. (Note that there are multiple C_γ genes located between C_δ and C_ε, but these are not shown.)

isotype switching to proceed. Germline transcripts are found at both the μ locus and the downstream heavy chain locus to which an activated B cell is being induced to switch. At each participating switch region, the germline transcript facilitates the generation of DNA double-stranded breaks, as described later. The DNA break in the upstream (μ) switch region is joined to the break in the downstream selected switch region. As a result, the rearranged VDJ exon just upstream of the μ switch region in the IgM-producing B cell recombines with the transcriptionally active downstream switch region. Cytokines determine which C_H region will undergo germline transcription. For instance, IL-4 induces germline transcription through the I_ε-S_ε-C_ε locus (see Fig. 11-15). This leads first to the production of germline ε transcripts in an IgM-expressing B cell and then to recombination of the S_μ switch region with the S_ε switch region. The intervening DNA is lost, and the VDJ exon is thus brought adjacent to C_ε. The end result is the production of IgE with the same V region as that of the original IgM produced by that B cell.

The key enzyme required for isotype switching (and somatic mutation, described later) is **activation-induced deaminase (AID)**. Humans and knockout mice lacking this enzyme have profound defects in isotype switching and affinity maturation. AID expression is activated mainly by CD40 signals. The enzyme deaminates cytosines in single-stranded DNA templates, converting cytosine (C) residues to uracil (U) residues (Fig. 11-16). Switch regions are rich in G and C bases, and switch region transcripts tend to form stable DNA-RNA hybrids involving the coding (top) strand of DNA, thus freeing up the bottom or nontemplate strand, which forms an open single-stranded DNA loop called an R-loop. The R-loop is where a large number of C residues in the switch DNA sequence are converted to U residues by AID. An enzyme called uracil N-glycosylase removes the U residues, leaving abasic sites. The *Ape*I endonuclease and probably other endonucleases cleave these abasic sites, generating a nick at each position. Some nicks are generated on the upper strand as well in an AID-dependent manner, but it is less clear how that happens. Nicks on both strands contribute to double-stranded breaks both at the S_μ region and at the downstream switch locus that is involved in a particular isotype switch event. The existence of double-stranded breaks in two switch regions results in the deletion of the intervening DNA and joining together of the two broken switch regions by use of the machinery involved in double-stranded break repair by nonhomologous end joining. This machinery is also used to repair double-stranded breaks during V(D)J recombination (see Chapter 8).

Affinity Maturation: Somatic Mutation of Ig Genes and Selection of High-Affinity B Cells

Affinity maturation is the process that leads to increased affinity of antibodies for a particular antigen as a T-dependent humoral response progresses and is the result of somatic mutation of Ig genes followed by selective survival of the B cells producing the antibodies with the highest affinities. The process of affinity maturation generates antibodies with an increasing capacity to bind antigens and thus to more efficiently bind to, neutralize, and eliminate microbes (Fig. 11-17). Helper T cells and CD40:CD40L interactions are required for somatic mutation to be initiated, and as a result, affinity maturation is observed only in antibody responses to T-dependent protein antigens. Some somatic mutation occurs in B cells in extrafollicular foci, but extensive somatic mutation occurs in germinal centers. As discussed earlier, the need for CD40 reflects the ability of this receptor to induce AID as well as extensive proliferation in B cells.

In proliferating germinal center B cells in the dark zone, Ig V genes undergo point mutations at an extremely high rate. This rate is estimated to be 1 in 10^3 V gene base pairs per cell division, which is about a thousand times higher than the spontaneous rate of mutation in other mammalian genes. (For this reason, mutation in Ig V genes is also called **hypermutation**.) The V genes of expressed heavy and light chains in each B cell contain a total of about 700 nucleotides; this implies that mutations will accumulate in expressed V regions at an average rate of almost one per cell division. Ig V gene mutations continue to occur in the progeny of individual B cells. As a result, any B cell clone can accumulate more and more mutations during its life in the germinal center. It is estimated that as a consequence of somatic mutations, the nucleotide sequences of IgG antibodies derived from one clone of B cells can diverge as much as 5% from the original germline sequence. This usually translates to up to 10 amino acid substitutions. Several features of these mutations are noteworthy. First, the mutations are clustered in the V regions, mostly in the antigen-binding complementarity-determining regions (Fig. 11-18). Second, there are far more mutations in IgG than in IgM antibodies. Third, the presence of mutations correlates with increasing affinities of the antibodies for the antigen that induced the response.

The mechanisms underlying somatic mutation in Ig genes are partially understood. It is clear that the rearranged Ig VDJ exon becomes highly susceptible to mutation, suggesting enhanced susceptibility of this region to DNA-binding factors that identify rearranged V regions for mutation. The enzyme AID, discussed before in the context of isotype switching, plays an essential role in affinity maturation. Its DNA deaminase activity converts C residues to U residues at hotspots for mutation. The U's may be changed to T's when DNA replication occurs, thus generating a common type of C to T mutation, or the U may be excised by uracil N-glycosylase, and the abasic site thus generated is repaired by an error-prone repair process, eventually generating all types of substitutions at each site of AID-induced cytidine deamination. These error-prone repair processes extend mutations to residues beyond the C residues that are targeted by AID.

Repeated stimulation by T cell–dependent protein antigens leads to increasing numbers of mutations in the Ig genes of antigen-specific germinal center B cells. Some of these mutations are likely to be useful because they will generate high-affinity antibodies. However, many of the mutations may result in a decline or even in a loss of antigen binding. Therefore, the next and crucial step

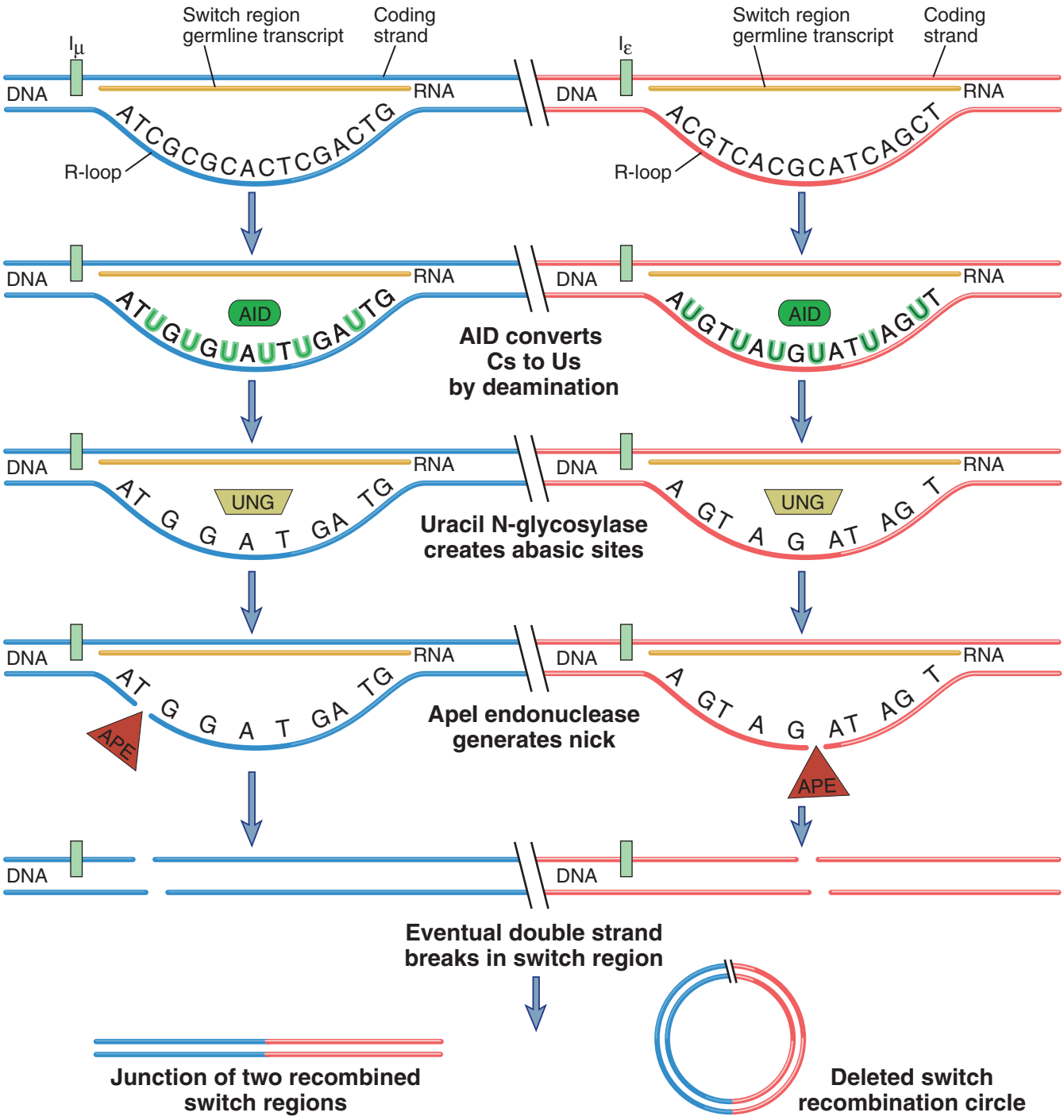


FIGURE 11–16 Mechanism by which AID and germline transcription collaborate to generate double-stranded breaks at switch regions. Two different switch regions for μ and ε are shown. Germline transcripts form DNA-RNA hybrids in the switch region, freeing up the nontemplate strand as an R-loop of single-stranded DNA. This is a particularly good template for AID, which deaminates C residues to generate U residues in single-stranded DNA. Uracil N-glycosylase (UNG) removes U residues to generate abasic sites that can be sites of nick generation after the action of the *Apel* endonuclease. Two nicks roughly opposite each other contribute to a double-stranded break. The mechanism of generation of the nick in the template strand is less well understood. Double-stranded breaks are made in each switch region, and these are recombined while the intervening DNA is deleted as a circle.

in the process of affinity maturation is the selection of the most useful, high-affinity B cells.

B cells that bind antigens in germinal centers with high affinity are selected to survive (Fig. 11-19). The early response to antigen results in the production of antibodies, some of which form complexes with residual antigen and may activate complement. FDCs express receptors for the Fc portions of antibodies and for products of complement activation, including C3b and C3d. These receptors bind and display antigens that are complexed with antibodies and complement products. Antigen may also be displayed in free form in the germinal center. Meanwhile, germinal center B cells that have undergone somatic mutation migrate into the FDC-rich light zone of the germinal center. In germinal center B cells, IL-21 secreted by T_{FH} cells induces the expression of proteins that induce apoptosis and reduces the expression of proteins that prevent apoptosis. Therefore, these B cells die by apoptosis unless they are rescued by recognition of antigen. B cells with high-affinity receptors for the antigen are best able to bind the antigen when it is present at low concentrations, and these B cells survive preferentially because of several mechanisms. First, antigen recognition by itself induces expression of anti-apoptotic proteins of the Bcl-2 family. Second, high-affinity B cells will preferentially endocytose and present the antigen and interact stably with the limiting numbers of T_{FH} cells in the germinal center. These helper T cells may use CD40L to promote the survival of the B cells they interact with. Third, some T_{FH} cells express Fas ligand, which can recognize the death receptor Fas on germinal center B cells and deliver an apoptotic signal. High-affinity B cells, which are best able to recognize and respond to antigen, may activate endogenous inhibitors of Fas when their BCRs recognize antigen and thus be protected from death while low-affinity B cells are killed.

As more antibody is produced, more of the antigen is eliminated and less is available in the germinal centers. Therefore, the B cells that will be able to specifically bind

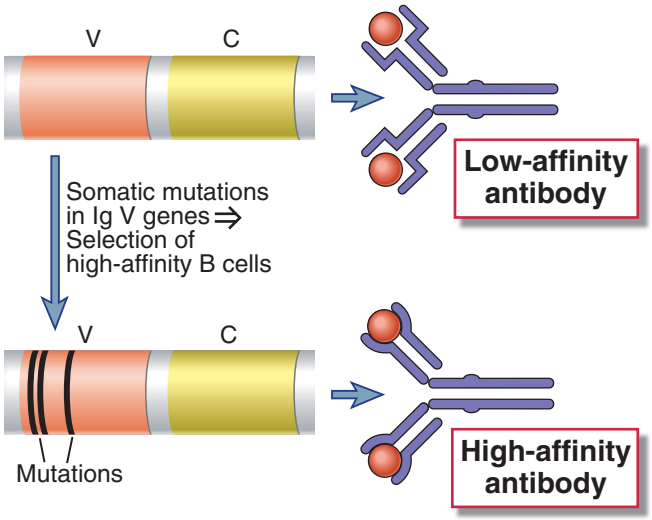


FIGURE 11–17 An overview of affinity maturation. Early in the immune response, low-affinity antibodies are produced. During the germinal center reaction, somatic mutation of Ig V genes and selection of mutated B cells with high-affinity antigen receptors result in the production of antibodies with high affinity for antigen.

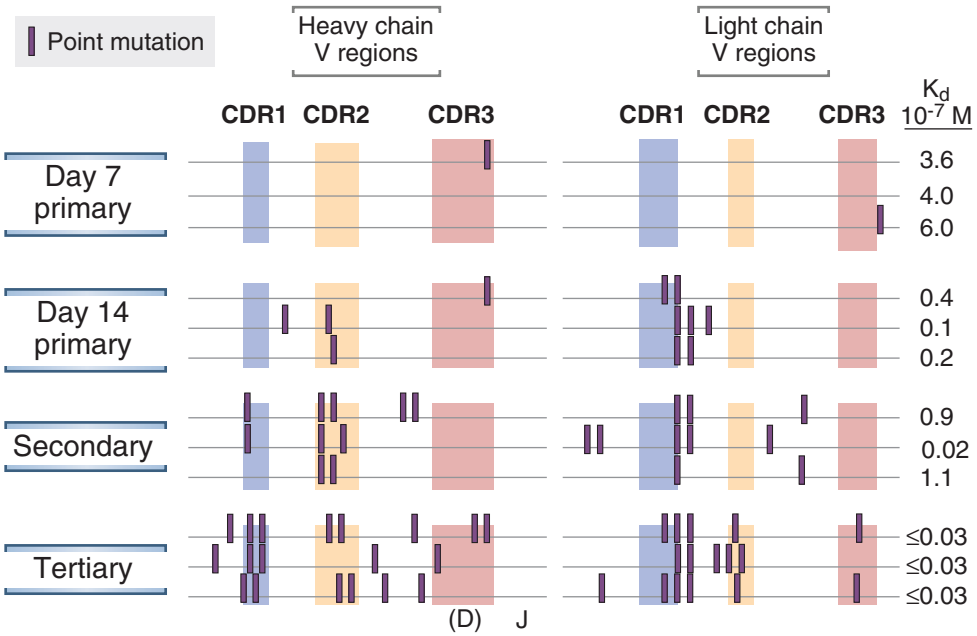


FIGURE 11–18 Somatic mutations in Ig V genes. Hybridomas were produced from spleen cells of mice immunized 7 or 14 days previously with a hapten, oxazolone, coupled to a protein and from spleen cells obtained after secondary and tertiary immunizations with the same antigen. Hybridomas producing oxazolone-specific monoclonal antibodies were isolated, and the nucleotide sequences of the V genes encoding the Ig heavy and light chains were determined. Mutations in V genes increase with time after immunization and with repeated immunizations and are clustered in the complementarity-determining regions (CDRs). The location of CDR3 in the heavy chains is approximate. The affinities of the antibodies produced also tend to increase with more mutations, as indicated by the lower dissociation constants (K_d) for hapten binding. *(Modified from Berek C, and C Milstein. Mutation drift and repertoire shift in maturation of the immune response. Immunological Reviews 96:23-41, 1987, Blackwell Publishing.)*

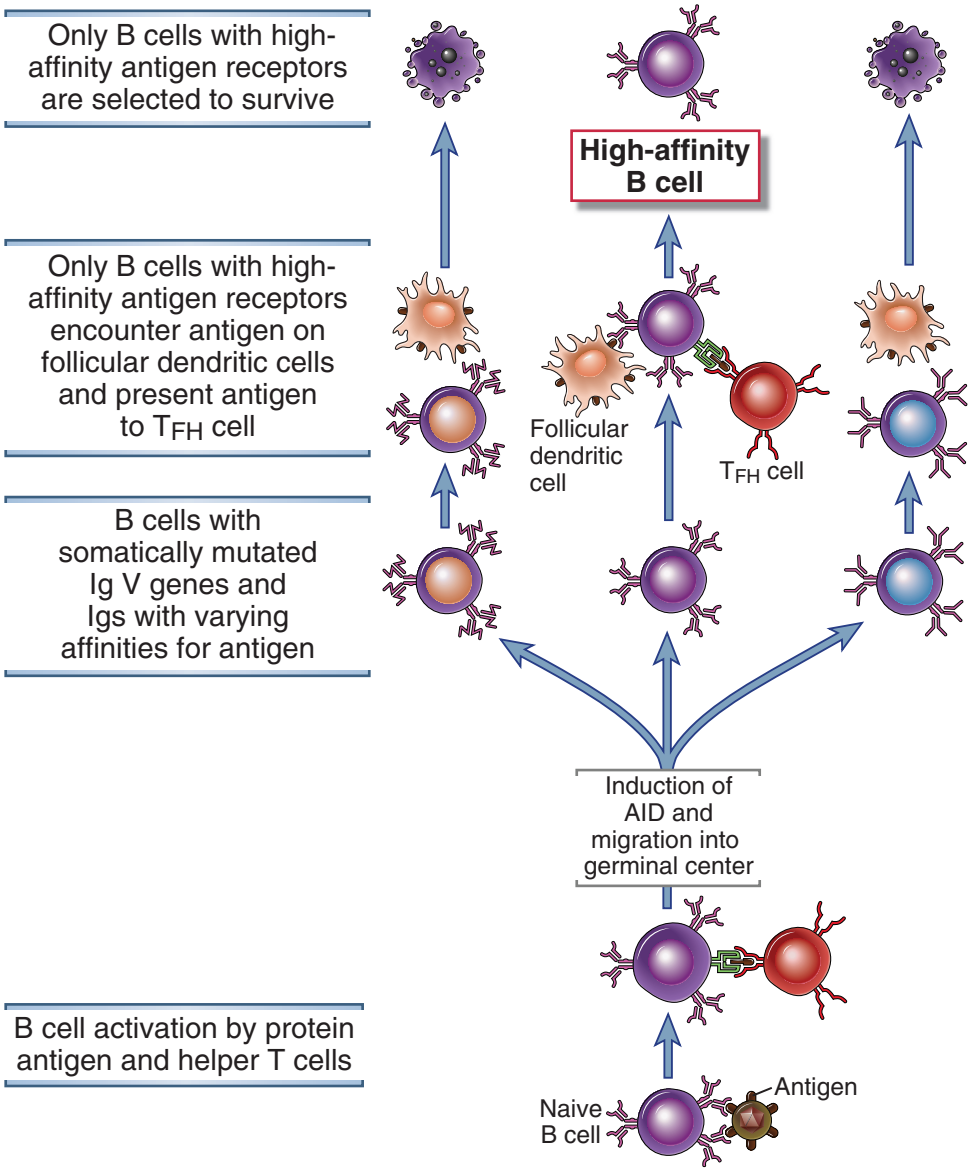


FIGURE 11–19 B cell selection in germinal centers. Somatic mutation of V region genes in germinal center B cells generates antibodies with different affinities for antigen. Subsequently, binding of the B cells to antigen displayed on follicular dendritic cells is necessary to rescue the B cells from programmed cell death. B cells may also present antigen to germinal center T_{FH} cells, which may promote B cell survival. The B cells with the highest affinity for antigen will have a selective advantage for survival as the amount of available antigen decreases during an immune response. This leads to an average increase in the affinity of antibodies for antigen as the humoral immune response progresses.

this antigen and to be rescued from death need to express antigen receptors with higher and higher affinity for the antigen. As a result, as the antibody response to an antigen progresses, the B cells that are selected in germinal centers produce Ig of increasing affinity for the antigen. This selection process results in affinity maturation of the antibody response. Because somatic mutation also generates many B cells that do not express high-affinity receptors for antigen and cannot therefore be selected to survive, the germinal centers are sites of tremendous apoptosis.

Somatic mutation occurs in the basal dark zone of germinal centers in B cells called centroblasts, which contain nuclear AID, and these mutated cells may repeatedly cycle between the basal dark zone and the apical light zone, where they differentiate into morphologically distinct cells called centrocytes. Eventually, high-affinity centrocytes may be selected in the light zone by antigen, with help from T_{FH} cells, and may undergo additional isotype switching. The selected cells then either differentiate into memory B cells or into high-affinity antibody-secreting plasma cells that exit the germinal center.

Given the extraordinary rate of mutation in germinal centers, it is not surprising that human lymphoid malignant neoplasms develop most often from B cells in this location because the DNA breaks associated with mutation and isotype switching set the stage for chromosomal translocations of various oncogenes into Ig gene loci, producing tumors of B cells (lymphomas). Germinal centers also assume an important role in the

pathogenesis of autoimmunity because T cell tolerance mechanisms can be subverted if somatic mutation drives a B cell clone in the germinal center to become strongly self-reactive. In fact, it is known that dysregulation of B cell selection in germinal centers contributes to autoantibody production.

B Cell Differentiation into Antibody-Secreting Plasma Cells

Some of the progeny of the B cells that have proliferated in response to antigen and T cell help differentiate into antibody-secreting plasma cells. Plasma cells are morphologically distinct, terminally differentiated B cells committed to abundant antibody production (see Chapter 2). They are generated after the activation of B cells through signals from the BCR, CD40, TLRs, and other receptors including cytokine receptors. There are two types of plasma cells. Short-lived plasma cells are generated during T-independent responses and early during T cell–dependent responses in extrafollicular B cell foci, described earlier. These cells are generally found in secondary lymphoid organs and in peripheral nonlymphoid tissues. Long-lived plasma cells are generated in T-dependent germinal center responses to protein antigens. Signals from the B cell antigen receptor and IL-21 cooperate in the generation of plasma cells. Plasma cells (and their precursors, plasmablasts, which may be found in the circulation) generated in germinal centers acquire the ability to home to the bone marrow, where they are maintained by cytokines of the BAFF family which bind to a plasma cell membrane receptor called BCMA, thus allowing the plasma cells to survive for long periods, often as long as the life span of the host. Typically 2 to 3 weeks after immunization with a T cell–dependent antigen, the bone marrow becomes a major site of antibody production. Plasma cells in the bone marrow may continue to secrete antibodies for months or even years after the antigen is no longer present. These antibodies can provide immediate protection if the antigen is encountered later. It is estimated that almost half the antibody in the blood of a healthy adult is produced by long-lived plasma cells and is specific for antigens that were encountered in the past. Secreted antibodies enter the circulation and mucosal secretions, but mature plasma cells do not recirculate.

The differentiation of activated B cells into antibody-secreting plasma cells involves major structural alterations, especially of components of the endoplasmic reticulum and secretory pathway, and increased Ig production as well as a change in Ig heavy chains from the membrane to the secreted form. Although a plasma cell is derived from a B cell, it undergoes a remarkable transformation as it differentiates from an activated B cell to the plasmablast stage. Most strikingly, the cell enlarges dramatically, and the ratio of cytoplasm to nucleus also undergoes a striking increase. The endoplasmic reticulum becomes prominent, and the cell is transformed into a secretory cell that bears little or no resemblance to a B cell. The transcription factors that regulate the development of plasma cells are described later.

The change in Ig production from the membrane form (characteristic of B cells) to the secreted form (in plasma cells) is the result of changes in the carboxyl terminal of the Ig heavy chain (Fig. 11-20). For instance, in

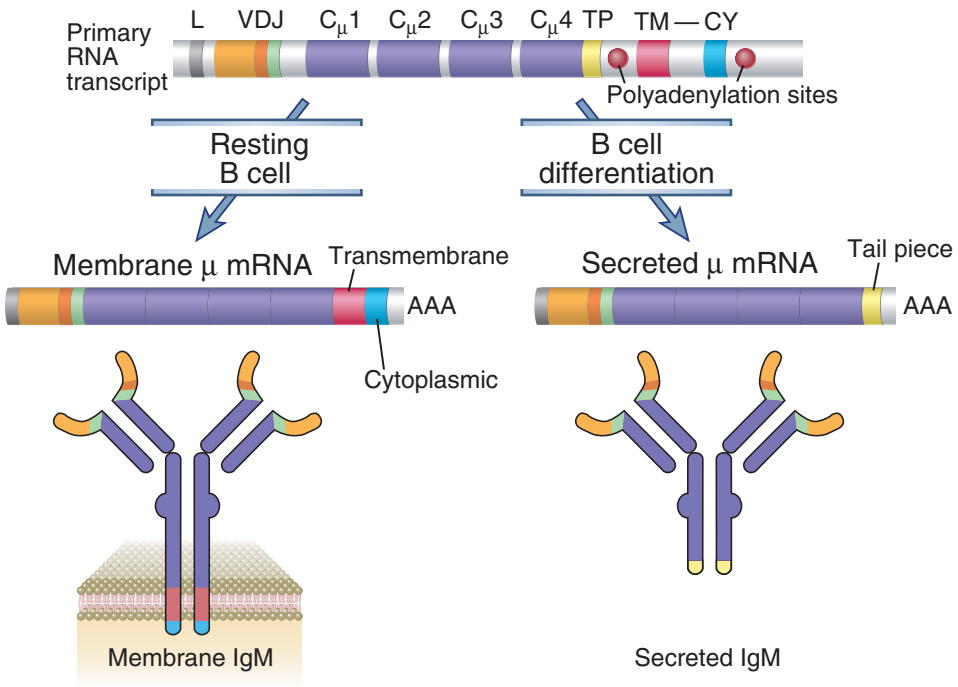


FIGURE 11–20 Production of membrane and secreted μ chains in B lymphocytes. Alternative processing of a primary RNA transcript results in the formation of mRNA for the membrane or secreted form of the μ heavy chain. B cell differentiation results in an increasing fraction of the μ protein produced as the secreted form. TP, TM, and CY refer to tail piece, transmembrane, and cytoplasmic segments, respectively. $C_\mu1$, $C_\mu2$, $C_\mu3$, and $C_\mu4$ are four exons of the C_μ gene.

membrane μ, $C_\mu 4$ is followed by a short spacer, 26 hydrophobic transmembrane residues, and a cytoplasmic tail of three amino acids (lysine, valine, and lysine). In secreted IgM, on the other hand, the $C_\mu 4$ domain is followed by a tail piece containing polar amino acids. This transition from membrane to secreted Ig is caused by altered RNA processing of the heavy chain messenger RNA (mRNA). The primary RNA transcript in all IgM-producing B cells contains the rearranged VDJ cassette, the four C_μ exons coding for the constant (C) region domains, and the two exons encoding the transmembrane and cytoplasmic domains. Alternative processing of this transcript, which is regulated by RNA cleavage and the choice of polyadenylation sites, determines whether or not the transmembrane and cytoplasmic exons are included in the mature mRNA. If they are included, the μ chain produced contains the amino acids that make up the transmembrane and cytoplasmic segments and is therefore anchored in the lipid bilayer of the plasma membrane. If, on the other hand, the transmembrane segment is excluded from the μ chain, the carboxyl terminus consists of about 20 amino acids constituting the tail piece. Because this protein does not have a stretch of hydrophobic amino acids or a positively charged cytoplasmic tail, it cannot remain anchored in the endoplasmic reticulum membrane, resides initially in the luminal space of the secretory pathway, and is secreted. Thus, each B cell can synthesize both membrane and secreted Ig. Most of the Ig heavy chain mRNA in a plasma cell is cleaved at the upstream polyadenylation site, so most of this mRNA is of the secretory form. All C_H genes contain similar membrane exons, and all heavy chains can be potentially expressed in membrane-bound and secreted forms. The secretory form of the δ heavy chain is rarely made, however, so that IgD is usually present only as a membrane-bound protein.

Generation of Memory B Cells and Secondary Humoral Immune Responses

Some of the antigen-activated B cells emerging from germinal centers acquire the ability to survive for long periods, apparently without continuing antigenic stimulation. These are memory cells, capable of mounting rapid responses to subsequent introduction of antigen. Because memory cells are generated mainly in germinal centers, they are seen in T-dependent immune responses and usually emerge in parallel with memory helper T cells. Memory B cells express high levels of the antiapoptotic protein Bcl-2, which contributes to the long life span of these cells. Some memory B cells may remain in the lymphoid organ where they were generated, whereas others exit germinal centers and recirculate between the blood and lymphoid organs. Memory cells typically bear high-affinity (mutated) antigen receptors and Ig molecules of switched isotypes more commonly than do naive B lymphocytes. The production of large quantities of isotype-switched, high-affinity antibodies is greatly accelerated after secondary exposure to antigens, and this can be attributed to the activation of memory cells in germinal centers.

Many of the features of secondary antibody responses to protein antigens, and their differences from primary responses (see Fig. 11-2), reflect the differences between responses of memory and naive B cells, respectively. Because memory B cell generation is optimal in the presence of T cell help, secondary responses are also best seen after exposure to T-dependent protein antigens. Thus, heavy chain class switching, which is typical of secondary responses, is stimulated by helper T cells. Affinity maturation, which increases with repeated antigenic stimulation, is also a consequence of helper T cell–induced B cell activation and somatic mutation. High-affinity antibodies are required to neutralize the infectivity of many microbes and the pathogenicity of microbial toxins, and memory B cells mount large, rapid and high-affinity responses to repeated infections. Therefore, effective vaccines against these microorganisms must induce both affinity maturation and memory B cell formation, and these events will occur only if the vaccines are able to activate helper T cells. This concept has been applied to the design of vaccines for some bacterial infections in which the target antigen is a capsular polysaccharide, which is incapable of stimulating T cells. In these cases, the polysaccharide is covalently linked to a foreign protein to form the equivalent of a hapten-carrier conjugate, which does activate helper T cells. Such vaccines, which are called **conjugate vaccines**, more readily induce high-affinity antibodies and memory than do polysaccharide vaccines without linked proteins. Such vaccines have proved particularly effective at inducing protective immunity in infants and young children, who are less able to make strong T-independent responses to polysaccharides than are adults.

Role of Transcriptional Regulators in Determining the Fate of Activated B Cells

The outcome of B cell differentiation is regulated by the induction and activation of different transcription factors. It is clear from the discussion so far that activated B cells can follow several fates. They can develop into short-lived or long-lived plasma cells, which secrete large amounts of antibodies, or into long-lived memory cells, which do not secrete antibodies but survive for prolonged periods and respond rapidly to antigen challenge. In Chapter 9, we discussed the concept that T cell fates are determined in large part by the expression of various transcriptional activators and repressors. The same general principle applies to the fates of activated B cells.

In germinal center B cells, signals delivered through CD40 and the IL-21 receptor induce the expression of a transcriptional repressor called **Bcl-6**. Bcl-6 antagonizes another repressor called Blimp-1, which is required for plasma cell development (see later) and thus prevents cells in the germinal center from differentiating into plasma cells during the massive proliferation that is characteristic of the germinal center reaction. B cells in extrafollicular foci express lower levels of Bcl-6 and more readily develop into short-lived plasma cells. Bcl-6 and other transcriptional regulators help orchestrate rapid

cell cycle entry of germinal center B cells. They also make germinal center B cells sensitive to apoptosis by increasing levels of proapoptotic factors and reducing the levels of antiapoptotic proteins.

Two key steps in becoming a plasma cell are the loss of expression of a transcription factor, **Pax-5**, which is required for the development and maintenance of a mature B cell (see Chapter 8), and the repression of Bcl-6. During plasma cell differentiation, a transcriptional activator called **IRF4** and a transcriptional repressor called **Blimp-1** are induced. IRF4 and Blimp-1 together induce the expression and splicing of XBP-1, a transcription factor that plays a critical role in the unfolded protein response. XBP-1 may protect developing plasma cells from the injurious consequences of unfolded proteins (which are produced as a side effect of the massive increase in protein synthesis), or it may contribute to the maturation of plasma cells and the enhanced synthesis of Ig seen in these cells.

How exactly the decision is made for a given germinal center B cell to choose between becoming either a memory B cell or a long-lived plasma cell is unclear. Indeed, transcription factors that delineate memory B cell development remain to be identified. It appears, however, that some of the progeny of an antigen-stimulated B cell clone express low levels of IRF4, and these become functionally quiescent, self-renewing, long-lived memory cells. Whereas high levels of IRF4 lead to plasma cell differentiation, lower levels of IRF4 are insufficient to drive an activated B cell toward plasma cell differentiation and thus may be permissive for memory B cell generation.

ANTIBODY RESPONSES TO T CELL–INDEPENDENT ANTIGENS

Many nonprotein antigens, such as polysaccharides and lipids, stimulate antibody production in the absence of helper T cells, and these antigens and the responses they elicit are termed thymus independent or T independent (TI). These antibody responses differ in several respects from responses to T cell–dependent protein antigens (Table 11-2). The antibodies that are produced in the absence of T cell help are generally of low affinity and consist mainly of IgM with limited isotype switching to some IgG subtypes and also to IgA.

Nature of B Cells That Respond to T-Independent Antigens

The marginal zone and B-1 subsets of B cells are especially important for antibody responses to TI antigens. Whereas responses to T-dependent protein antigens are largely mediated by follicular B cells, other B cell subsets may be the primary responders to TI antigens (see Fig. 11-3). **Marginal zone B cells** are a distinct population of B cells that mainly respond to polysaccharides. After activation, these cells differentiate into short-lived plasma cells that produce mainly IgM. In humans these cells are also called IgM memory cells. **B-1 cells** represent another lineage of B cells that responds readily to TI antigens mainly in the peritoneum and in mucosal sites.

TI responses may be initiated in the spleen, bone marrow, peritoneal cavity, and mucosal sites. Macrophages located in the marginal zones surrounding lymphoid follicles in the spleen are particularly efficient at trapping polysaccharides when these antigens are injected intravenously. TI antigens may persist for prolonged periods on the surfaces of marginal zone macrophages, where they are recognized by specific B cells.

Mechanisms of T-Independent Antibody Responses

The most important TI antigens are polysaccharides, glycolipids, and nucleic acids, all of which induce specific antibody production in T cell–deficient animals. These antigens cannot be processed and presented in association with MHC molecules, and therefore they cannot be recognized by $CD4^+$ helper T cells. Most TI antigens are multivalent, being composed of repeated identical antigenic epitopes. Such multivalent antigens may induce maximal cross-linking of the BCR complex on specific B cells, leading to activation without a requirement for cognate T cell help. In addition, many polysaccharides activate the complement system by the alternative pathway, generating C3d, which binds to the antigen and is recognized by CR2, thus augmenting B cell activation (see Fig. 11-5 and Chapter 7). Membrane proteins at a high density on a microbial surface may be functionally multivalent and may function in a T-independent as well as in a T-dependent manner. As mentioned earlier, TI responses may also be facilitated by additional signals derived from microbial products that activate TLRs on B cells.

TABLE 11–2 Properties of Thymus-Dependent and Thymus-Independent Antigens

	Thymus-Dependent Antigen	Thymus-Independent Antigen
Chemical nature	Proteins	Polymeric antigens, especially polysaccharides; also glycolipids, nucleic acids
Features of Antibody Response		
Isotype switching	Yes; IgG, IgE, and IgA	Little or no; may be some IgG and IgA
Affinity maturation	Yes	No
Secondary response (memory B cells)	Yes	Only seen with some antigens (e.g., polysaccharides)

Although TI responses typically show little isotype switching, some T-independent nonprotein antigens do induce Ig isotypes other than IgM. In humans, the dominant antibody class induced by pneumococcal capsular polysaccharide is IgG2. In mice engineered to lack CD40, IgE and many IgG subclasses are barely detectable in the serum, but levels of IgG3 (which resembles human IgG2) and IgA in the serum are reduced to only about half their normal levels. Cytokines produced by non-T cells may stimulate isotype switching in TI responses. As described earlier, in the absence of T cells, BAFF and APRIL produced by cells of myeloid origin, such as dendritic cells and macrophages, can induce the synthesis of AID in antigen-activated B cells through a receptor of the BAFF receptor family called TACI. This may be further facilitated by the activation of TLRs on these B cells. In addition, cytokines such as TGF-β that help mediate the IgA switch are secreted by many nonlymphoid cells at mucosal sites and may contribute to the generation of IgA antibodies directed against nonprotein antigens.

Functions of T-Independent Antibody Responses

The practical significance of TI antigens is that many bacterial cell wall polysaccharides belong to this category, and humoral immunity is the major mechanism of host defense against infections by such encapsulated bacteria. For this reason, individuals with congenital or acquired deficiencies of humoral immunity are especially susceptible to life-threatening infections with encapsulated bacteria, such as pneumococcus, meningococcus, and *Haemophilus*. In addition, TI antigens contribute to the generation of **natural antibodies**, which are present in the circulation of normal individuals and are apparently produced without overt exposure to pathogens. Most natural antibodies are low-affinity anticarbohydrate antibodies, postulated to be produced by B-1 peritoneal B cells stimulated by bacteria that colonize the gastrointestinal tract and by marginal zone B cells in the spleen. Antibodies to the A and B glycolipid blood group antigens are examples of these natural antibodies (see Chapter 16).

Despite their inability to specifically activate helper T cells, many polysaccharide vaccines, such as the pneumococcal vaccine, induce long-lived protective immunity. Rapid and large secondary responses typical of memory (but without much isotype switching or affinity maturation) do occur on secondary exposure to these carbohydrate antigens. The phenomenon of IgM memory has been clearly demonstrated in the mouse, and in both mice and adult humans, IgM memory B cells can be identified by the expression of specific cell surface markers. In humans, these memory-like cells express high levels of CD27 and IgM and IgD and they are also called marginal zone B cells.

ANTIBODY FEEDBACK: REGULATION OF HUMORAL IMMUNE RESPONSES BY Fc RECEPTORS

Secreted antibodies inhibit continuing B cell activation by forming antigen-antibody complexes that simultaneously bind to antigen receptors and inhibitory Fcγ receptors on antigen-specific B cells (Fig. 11-21). This is the explanation for a phenomenon called **antibody feedback**, which refers to the downregulation of antibody production by secreted IgG antibodies. IgG antibodies inhibit B cell activation by forming complexes with the antigen, and these complexes bind to a B cell receptor for the Fc portions of the IgG, called the Fcγ receptor II (FcγRIIB, or CD32). (The biology of Fc receptors is discussed in Chapter 12.) As discussed in Chapter 7, the cytoplasmic tail of FcγRIIB contains a six–amino acid (isoleucine-x-tyrosine-x-x-leucine) motif shared by other receptors in the immune system that mediate negative signals, including inhibitory receptors on NK cells. By analogy to ITAMs, this inhibitory motif is called an immunoreceptor tyrosine-based inhibition motif (ITIM). When the Fcγ receptor of B cells is engaged, the ITIM on the cytosolic tail of the receptor is phosphorylated on tyrosine residues, and it forms a docking site for the inositol 5-phosphatase SHIP (SH2 domain–containing inositol phosphatase). The recruited SHIP hydrolyses a phosphate on the signaling lipid intermediate phosphatidylinositol trisphosphate (PIP3) and inactivates this molecule. By this mechanism, engagement of FcγRII terminates the B cell response to antigen. The antigen-antibody complexes simultaneously interact with the antigen receptor (through the antigen) and with FcγRIIB (through the antibody), and this brings the inhibitory phosphatases close to the antigen receptors whose signaling is blocked. In addition to B cells, FcγRIIB binds and sends inhibitory signals to myeloid cells, including macrophages and dendritic cells, and perhaps also plasma cells.

Fc receptor–mediated antibody feedback is a physiologic control mechanism in humoral immune responses because it is triggered by secreted antibody and blocks further antibody production. We have stated earlier in the chapter that antibodies can also amplify antibody production by activating complement and generating C3d. It is not clear under which circumstances secreted antibodies provide complement-mediated amplification or Fc receptor–mediated inhibition. A likely scenario is that early in humoral immune responses, IgM antibodies (which activate complement but do not bind to the Fcγ receptor) are involved in amplification, whereas increasing production of IgG leads to feedback inhibition.

The importance of FcγRIIB-mediated inhibition is demonstrated by the uncontrolled antibody production seen in mice in which the gene encoding this receptor has been knocked out. A polymorphism in the *FcγRIIB* gene has been linked to susceptibility to the autoimmune disease systemic lupus erythematosus in humans.

B cells express another inhibitory receptor called CD22. CD22 is a sialic acid–binding lectin; its natural ligand is not known, and we do not know exactly how it is engaged during physiologic B cell responses. However, knockout mice lacking CD22 show greatly enhanced B cell activation. The cytoplasmic tail of this molecule contains ITIM tyrosine residues, which, when phosphorylated by the Src family kinase Lyn, bind the SH2 domain of the tyrosine phosphatase SHP-1. SHP-1 removes phosphates from the tyrosine residues of several enzymes and adaptor proteins involved in BCR signaling and thus

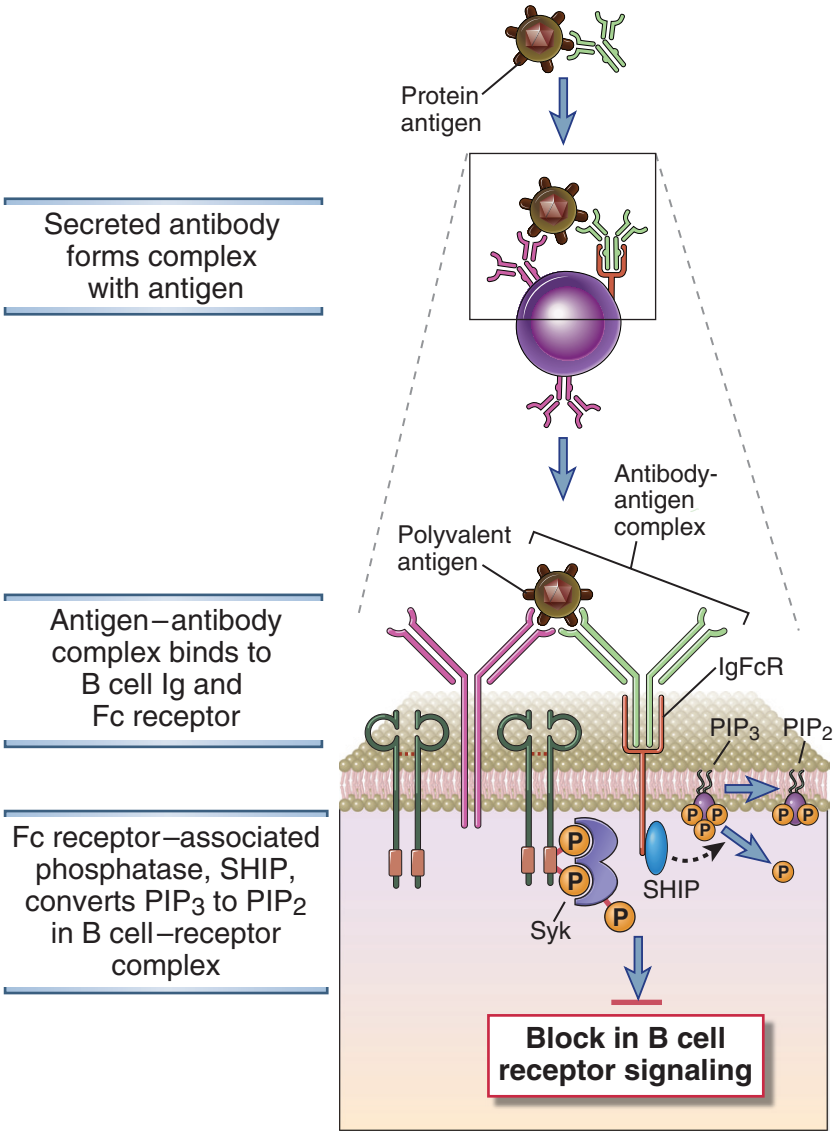


FIGURE 11–21 Regulation of B cell activation by FcγRIIB. Antigen-antibody complexes can simultaneously bind to membrane Ig (through antigen) and the FcγRIIB receptor through the Fc portion of the antibody. As a consequence of this simultaneous ligation of receptors, phosphatases associated with the cytoplasmic tail of the FcγRIIB inhibit signaling by the BCR complex and block B cell activation.

abrogates B cell activation. A mouse strain called *motheaten,* which develops severe autoimmunity with uncontrolled B cell activation and autoantibody production, has a naturally occurring mutation in SHP-1. Conditional deletion of SHP-1 as well as the engineered loss of Lyn in B cells leads to a breakdown of peripheral B cell tolerance and the development of autoimmunity.

SUMMARY

- In humoral immune responses, B lymphocytes are activated by antigen and secrete antibodies that act to eliminate the antigen. Both protein and nonprotein antigens can stimulate antibody responses. B cell responses to protein antigens require the contribution of $CD4^+$ helper T cells specific for the antigen.
- Helper T cell–dependent B cell responses to protein antigens require initial activation of naive T cells in the T cell zones and of B cells in lymphoid follicles in lymphoid organs. The activated lymphocytes migrate toward one another and interact at the edges of follicles, where the B cells present the antigen to helper T cells.
- Activated helper T cells express CD40L, which engages CD40 on the B cells, and the T cells secrete cytokines that bind to cytokine receptors on the B cells. The combination of CD40 and cytokine signals stimulates initial B cell proliferation and differentiation.
- Stimulation of activated B cells at extrafollicular sites by helper T cells leads to the formation of extrafollicular foci where some isotype switching occurs and short-lived plasma cells are generated.
- Some activated helper T cells differentiate into specialized T_{FH} cells that express high levels of ICOS and CXCR5 and secrete IL-21. T_{FH} cells and activated B cells migrate into the follicle, and T_{FH} cells activate these specific B cells to initiate the

formation of germinal centers. The late events in T cell–dependent antibody responses, including extensive isotype switching, somatic mutation, affinity maturation, generation of memory B cells, and induction of long-lived plasma cells, take place within germinal centers.

- Helper T cell–derived signals, including CD40L and cytokines, induce isotype switching in B cells by a process of switch recombination, leading to the production of various Ig isotypes. Isotype switching requires the induction of AID, a cytidine deaminase that converts cytosine to uracil in single-stranded DNA, and different cytokines allow AID to access distinct downstream heavy chain loci.
- Affinity maturation occurs in germinal centers and leads to increased affinity of antibodies during the course of a T cell–dependent humoral response. Affinity maturation is a result of somatic mutation of Ig heavy and light chain genes induced by AID, followed by selective survival of the B cells that produce the high-affinity antibodies and bind to antigen displayed by FDCs in the germinal centers. T_{FH} cells also participate in selection of high-affinity B cells.
- Some of the progeny of germinal center B cells differentiate into antibody-secreting plasma cells that migrate to the bone marrow. Other progeny become memory B cells that live for long periods, recirculate between lymph nodes and spleen, and respond rapidly to subsequent exposures to antigen by differentiating into high-affinity antibody secretors. The differentiation of activated B cells into plasma cells or memory cells is controlled by the expression of various transcription factors.
- TI antigens are generally nonprotein antigens that induce humoral immune responses without the involvement of helper T cells. Many TI antigens, including polysaccharides, membrane glycolipids, and nucleic acids, are multivalent, can cross-link multiple membrane Ig molecules on a B cell, and activate complement, thereby activating the B cells without T cell help. TLR activation on B cells by microbial products facilitates T-independent B cell activation. TI antigens stimulate antibody responses in which there is limited heavy chain class switching, affinity maturation, or memory B cell generation because these features are largely dependent on helper T cells, which are not activated by nonprotein antigens. However, some T-independent isotype switching can be induced by TLR stimulation by microbes, which may lead to the production of cytokines of the TNF family that activate B cells to induce AID.
- Antibody feedback is a mechanism by which humoral immune responses are downregulated when enough antibody has been produced and soluble antibody-antigen complexes are present. B cell membrane Ig and the receptor on B cells for the Fc portions of IgG, called FcγRIIB, are clustered together by antibody-antigen complexes. This activates an inhibitory signaling cascade through the cytoplasmic tail of FcγRIIB that terminates the activation of the B cell.

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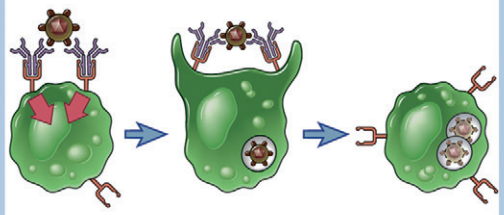
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CHAPTER

12

Effector Mechanisms of Humoral Immunity

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Humoral immunity is mediated by secreted antibodies, and its physiologic function is defense against extracellular microbes and microbial toxins. This type of immunity contrasts with cell-mediated immunity, the other effector arm of the adaptive immune system, which is mediated by T lymphocytes and functions to eradicate microbes that infect and live within host cells (see Chapter 10). Humoral immunity against microbial toxins was discovered by von Behring and Kitasato in 1890 as a form of immunity that could be conveyed from immunized to naive individuals by the transfer of serum. The types of microorganisms that are combated by humoral immunity are extracellular bacteria, fungi, and even obligate intracellular microbes such as viruses, which are targets of antibodies before they infect cells or when they are released from infected cells. Defects in antibody production result in increased susceptibility to infection with many microbes, including bacteria, fungi, and viruses. Currently used vaccines induce protection primarily by stimulating the production of antibodies. Apart from their crucial protective roles, in allergic individuals and in certain autoimmune diseases, some specific antibodies can be harmful and mediate tissue injury. In this chapter, we discuss the effector mechanisms that are used by antibodies to eliminate antigens. The structure of antibodies is described in Chapter 5 and the process of antibody production in Chapter 11.

OVERVIEW OF HUMORAL IMMUNITY

Before we discuss the principal mechanisms by which antibodies provide protection against microbes, we will summarize some of the salient features of antibody-mediated host defense.

- ***The main functions of antibodies are to neutralize and eliminate infectious microbes and microbial toxins*** (Fig. 12-1). As we shall see later, antibody-mediated elimination of antigens involves a number of effector mechanisms and requires the participation of various cellular and humoral components of the immune system, including phagocytes and complement proteins.
- ***Antibodies are produced by plasma cells in the lymphoid organs and bone marrow***, but antibodies perform their effector functions at sites distant from their production. Antibodies produced in the lymph nodes, spleen, and bone marrow may enter the blood and then circulate throughout the body. Antibodies produced in mucosa-associated lymphoid tissues are transported across epithelial barriers into the lumens of mucosal organs, such as the intestine and the airways, where these secreted antibodies block the entry of ingested and inhaled microbes (see Chapter 13). Antibodies are also actively transported across the placenta

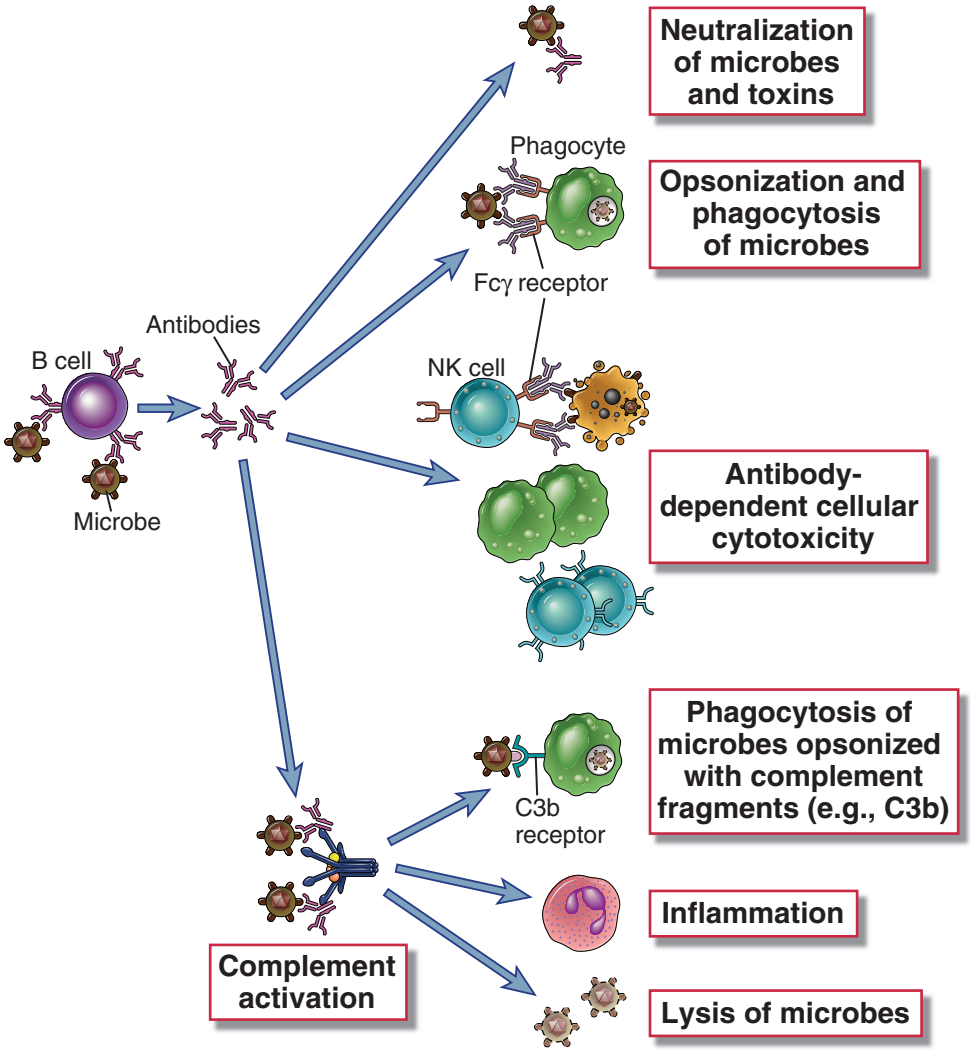


FIGURE 12–1 Effector functions of antibodies. Antibodies against microbes (and their toxins, not shown here) neutralize these agents, opsonize them for phagocytosis, sensitize them for antibody-dependent cellular cytotoxicity, and activate the complement system. These various effector functions may be mediated by different antibody isotypes.

into the circulation of the developing fetus. In cell-mediated immunity, activated T lymphocytes are able to migrate to peripheral sites of infection and inflammation, but they are not transported into mucosal secretions or across the placenta.

- ***The antibodies that mediate protective immunity may be derived from short-lived or long-lived antibody-producing plasma cells that are generated by the activation of naive or memory B cells***. The first exposure to an antigen, either by infection or by vaccination, leads to the activation of naive B lymphocytes and their differentiation into antibody-secreting plasma cells and memory cells (see Chapter 11). Subsequent exposure to the same antigens leads to the activation of memory B cells and a larger and more rapid antibody response. Plasma cells generated early in an immune response or from marginal zone or B-1 B cells tend to be short-lived. In contrast, germinal center–derived, class-switched antibody-secreting plasma cells migrate to the bone marrow and persist at this site, where they continue to produce antibodies for years after the antigen is eliminated. Much of the immunoglobulin G (IgG) found in the serum of normal individuals is derived from these long-lived plasma cells, which were induced by the exposure of naive and memory B cells to various antigens throughout the life of the individual. If an immune individual is exposed to a previously encountered microbe, the level of circulating antibody produced by the long-lived plasma cells provides immediate protection against the infection. At the same time, activation of memory B cells generates a larger burst of antibody that provides a second and more effective wave of protection.
- ***Many of the effector functions of antibodies are mediated by the heavy chain constant regions of Ig molecules, and different Ig heavy chain isotypes serve distinct effector functions*** (Table 12-1). For instance, some IgG subclasses bind to phagocyte Fc receptors and promote the phagocytosis of antibody-coated particles, IgM and some subclasses of IgG activate the complement system, and IgE binds to the Fc receptors of mast cells and triggers their activation. Each of these

TABLE 12–1 Functions of Antibody Isotypes

Antibody Isotype	Isotype-Specific Effector Functions
IgG	Opsonization of antigens for phagocytosis by macrophages and neutrophils Activation of the classical pathway of complement Antibody-dependent cell-mediated cytotoxicity mediated by natural killer cells Neonatal immunity: transfer of maternal antibody across the placenta and gut Feedback inhibition of B cell activation
IgM	Activation of the classical pathway of complement Antigen receptor of naive B lymphocytes*
IgA	Mucosal immunity: secretion of IgA into the lumens of the gastrointestinal and respiratory tracts Activation of complement by the lectin pathway or by the alternative pathway
IgE	Mast cell degranulation (immediate hypersensitivity reactions)
IgD	Antigen receptor of naive B lymphocytes*

*These functions are mediated by membrane-bound and not secreted antibodies.

effector mechanisms will be discussed later in this chapter. The humoral immune system is specialized in such a way that different microbes or antigen exposures stimulate B cell switching to the Ig isotypes that are best for combating these microbes. The major stimuli for isotype switching during the process of B cell activation are helper T cell–derived cytokines together with CD40 ligand expressed by activated helper T cells (see Chapter 11). As we discussed in Chapters 10 and 11, T_H1-stimulated antibody isotypes are induced by and particularly effective at clearing viruses and bacteria, and T_H2-dependent antibodies are induced by and especially effective against helminthic parasites. Neutralization is the only function of antibodies that is mediated entirely by binding of antigen and does not require participation of the Ig constant regions.

- ***Although many effector functions of antibodies are mediated by the Ig heavy chain constant regions, all these functions are triggered by the binding of antigens to the variable regions.*** The binding of antibodies to a multivalent antigen, such as a polysaccharide or a repeated epitope on a microbial surface, brings the Fc regions of antibodies close together, and this clustering of antibody molecules leads to complement activation and allows the antibodies to bind to and activate Fc receptors on phagocytes. The requirement for antigen binding ensures that antibodies activate various effector mechanisms only when they are needed, that is, when the antibodies encounter and specifically bind antigens, not when the antibodies are circulating in an antigen-free form.

With this introduction to humoral immunity, we proceed to a discussion of the various functions of antibodies in host defense.

NEUTRALIZATION OF MICROBES AND MICROBIAL TOXINS

Antibodies against microbes and microbial toxins block the binding of these microbes and toxins to cellular receptors (Fig. 12-2). In this way, antibodies inhibit, or "neutralize," the infectivity of microbes as well as the potential injurious effects of infection. Many microbes enter host cells by the binding of particular microbial surface molecules to membrane proteins or lipids on the surface of host cells. For example, influenza viruses use their envelope hemagglutinin to infect respiratory epithelial cells, and gram-negative bacteria use pili to attach to and infect a variety of host cells. Antibodies that bind to these microbial structures interfere with the ability of the microbes to interact with cellular receptors by means of steric hindrance and may thus prevent infection. In some cases, very few antibody molecules may bind to a microbe and induce conformational changes in surface molecules that prevent the microbe from interacting with cellular receptors; such interactions are examples of the allosteric effects of antibodies. Many microbial toxins mediate their pathologic effects also by binding to specific cellular receptors. For instance, tetanus toxin binds to receptors in the motor end plate of neuromuscular junctions and inhibits neuromuscular transmission, which leads to paralysis, and diphtheria toxin binds to cellular receptors and enters various cells, where it inhibits protein synthesis. Antibodies against such toxins sterically hinder the interactions of toxins with host cells and thus prevent the toxins from causing tissue injury and disease.

Antibody-mediated neutralization of microbes and toxins requires only the antigen-binding regions of the antibodies. Therefore, such neutralization may be mediated by antibodies of any isotype in the circulation and in mucosal secretions and can experimentally also be mediated by Fab or $F(ab')_2$ fragments of specific antibodies, which lack the Fc regions of the heavy chains. Most neutralizing antibodies in the blood are of the IgG isotype; in mucosal organs, they are largely of the IgA isotype. The most effective neutralizing antibodies are those with high affinities for their antigens. High-affinity antibodies are produced by the process of affinity maturation (see Chapter 11). Many prophylactic vaccines work by stimulating the production of high-affinity neutralizing antibodies (Table 12-2). A mechanism that microbes have developed to evade host immunity is to mutate the genes encoding surface antigens that are the targets of neutralizing antibodies (see Chapter 15).

ANTIBODY-MEDIATED OPSONIZATION AND PHAGOCYTOSIS

Antibodies of the IgG isotype coat (opsonize) microbes and promote their phagocytosis by binding to Fc receptors on phagocytes. Mononuclear phagocytes and

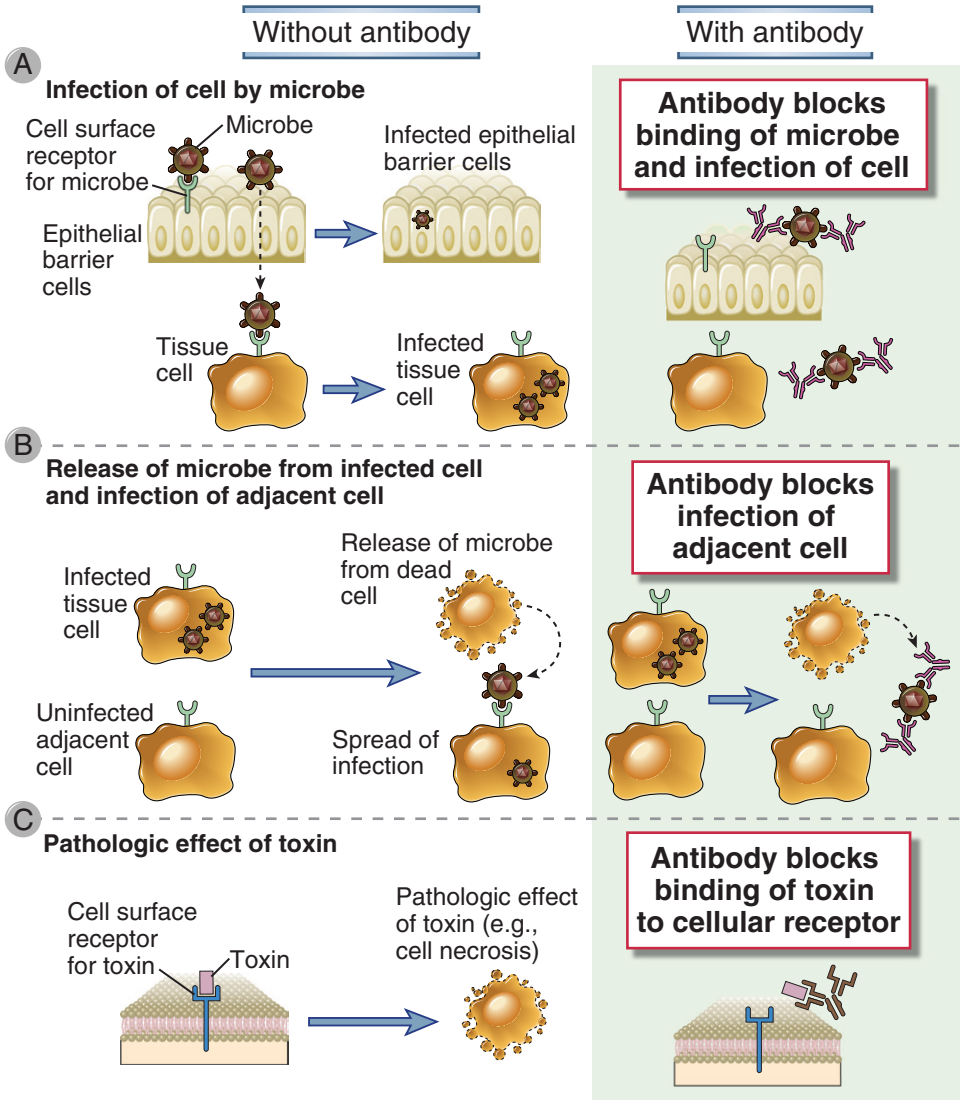


FIGURE 12–2 Neutralization of microbes and toxins by antibodies. A, Antibodies prevent the binding of microbes to cells and thus block the ability of the microbes to infect host cells. **B,** Antibodies inhibit the spread of microbes from an infected cell to an adjacent uninfected cell. **C,** Antibodies block the binding of toxins to cells and thus inhibit the pathologic effects of the toxins.

TABLE 12–2 Vaccine-Induced Humoral Immunity

Infectious Disease	Vaccine	Mechanism of Protective Immunity
Polio	Oral attenuated poliovirus	Neutralization of virus by mucosal IgA antibody
Tetanus, diphtheria	Toxoids	Neutralization of toxin by systemic IgG antibody
Hepatitis, A or B	Recombinant viral envelope proteins	Neutralization of virus by systemic IgG antibody
Pneumococcal pneumonia, *Haemophilus*	Conjugate vaccines composed of bacterial capsular polysaccharide attached to a carrier protein	Opsonization and phagocytosis mediated by IgM and IgG antibodies, directly or secondary to complement activation

Selected examples of vaccines that work by stimulating protective humoral immunity are listed.

neutrophils ingest microbes as a prelude to intracellular killing and degradation. These phagocytes express a variety of surface receptors that directly bind microbes and ingest them, even without antibodies, providing one mechanism of innate immunity (see Chapter 4). The efficiency of this process is markedly enhanced if the phagocyte can bind the particle with high affinity. Mononuclear phagocytes and neutrophils express receptors for the Fc portions of IgG antibodies that specifically bind antibody-coated (opsonized) particles. Microbes may also be opsonized by a product of complement activation called C3b and are phagocytosed by binding to a leukocyte receptor for C3b (described later in this chapter). The process of coating particles to promote phagocytosis is called **opsonization**, and substances that perform this function, including antibodies and complement proteins, are called **opsonins**.

Leukocyte Fc Receptors

Leukocytes express Fc receptors that bind to the constant regions of antibodies, and thereby promote the phagocytosis of Ig-coated particles and deliver signals that stimulate the microbicidal activities of the leukocytes and induce inflammation. Fc receptors for different Ig heavy chain isotypes are expressed on many leukocyte populations and serve diverse functions in immunity. Of these Fc receptors, the ones that are most important for phagocytosis of opsonized particles are receptors for the heavy chains of IgG antibodies, called Fcγ receptors, and these are the receptors that will primarily be considered in this chapter. The Fc receptors that bind to IgE are discussed in Chapter 19. We have already considered the neonatal Fc receptor (FcRn), which is expressed in the placenta, on intestinal epithelial cells, and on vascular endothelium, in Chapter 5. The poly-Ig receptor, which is involved in the transcytosis of IgA and IgM, is discussed in Chapter 13.

There are a number of different Fcγ receptors that have different affinities for the heavy chains of different IgG subclasses and are expressed on different cell types. Most Fc receptors result in cellular activation when triggered except for FcγRII, which is an inhibitory receptor. All Fcγ receptors contain a ligand-binding chain, called the α chain, that recognizes their IgG ligands. Differences in specificities or affinities of each FcγR for the various IgG isotypes are based on differences in the structure of these α chains. All Fc receptors are optimally activated by antibodies bound to their antigens and not by free, circulating antibodies. In all the FcRs except FcγRII, the α chain is associated with one or more additional polypeptide chains involved in signal transduction (Fig. 12-3). Signaling functions of FcγRII are mediated by the cytoplasmic tail of the α chain.

Fcγ receptors have been classified into three groups based on their affinities for heavy chains of different IgG subclasses (Table 12-3). Some of these Fc receptors have multiple isoforms that may differ in structure and function.

- **FcγRI** (CD64) is the major phagocyte Fcγ receptor. It is expressed on macrophages and neutrophils and is a high-affinity receptor that binds IgG1 and IgG3, with a K_d of 10^{-8} to 10^{-9} M. (In mice, FcγRI preferentially binds IgG2a and IgG2b antibodies.) The large extracellular amino-terminal region of the Fc-binding α chain folds into three tandem Ig-like domains. The α chain of FcγRI is associated with a disulfide-linked homodimer of a signaling protein called the FcR γ chain. This γ chain is also found in the signaling complexes associated with FcγRIII, FcαR, and FcεRI. The γ chain has

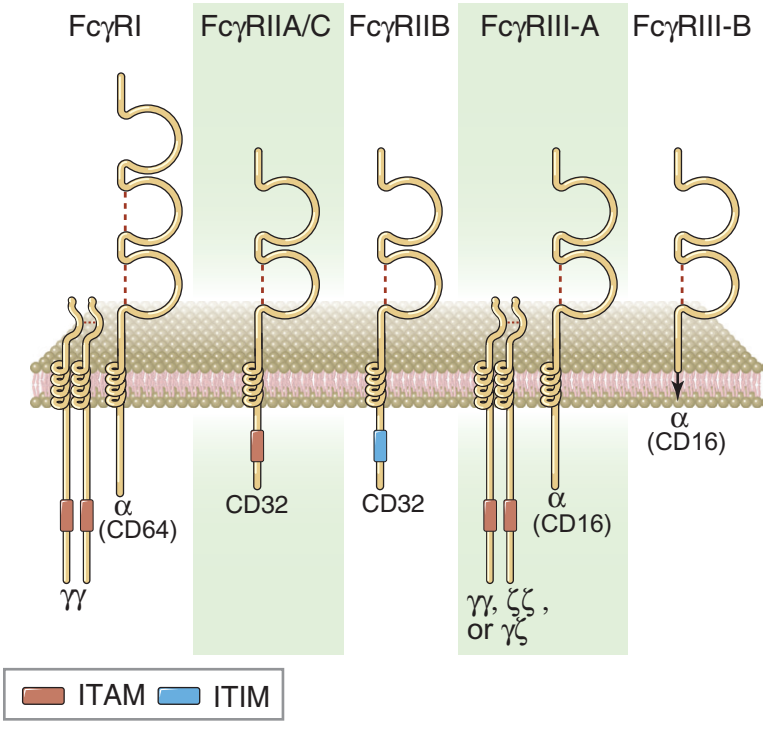


FIGURE 12–3 Subunit composition of Fcγ receptors. Schematic models of the different human Fc receptors illustrate the Fc-binding α chains and the signaling subunits. FcγRIII-B is a glycophosphatidylinositol anchored membrane protein with no known signaling functions. FcγRIIA and IIC are structurally similar low-affinity activating receptors with slightly different patterns of expression.

TABLE 12–3 Fc Receptors

FcR	Affinity for Immunoglobulin	Cell Distribution	Function
FcγRI (CD64)	High ($K_d < 10^{-9}$ M); binds IgG1 and IgG3, can bind monomeric IgG	Macrophages, neutrophils; also eosinophils	Phagocytosis; activation of phagocytes
FcγRIIA (CD32)	Low ($K_d > 10^{-7}$ M)	Macrophages, neutrophils; eosinophils, platelets	Phagocytosis; cell activation (inefficient)
FcγRIIB (CD32)	Low ($K_d > 10^{-7}$ M)	B lymphocytes	Feedback inhibition of B cells
FcγRIIC (CD32)	Low ($K_d > 10^{-7}$ M)	Macrophages, neutrophils, NK cells	Phagocytosis, cell activation
FcγRIIIA (CD16)	Low ($K_d > 10^{-6}$ M)	NK cells	Antibody-dependent cell-mediated cytotoxicity
FcγRIIIB (CD16)	Low ($K_d > 10^{-6}$ M); GPI-linked protein	Neutrophils	Phagocytosis (inefficient)
FcεRI	High ($K_d > 10^{-10}$ M); binds monomeric IgE	Mast cells, basophils, eosinophils	Cell activation (degranulation)
FcεRII (CD23)	Low ($K_d > 10^{-7}$ M)	B lymphocytes, eosinophils, Langerhans cells	Unknown
FcαR (CD89)	Low ($K_d > 10^{-6}$ M)	Neutrophils, eosinophils, monocytes	Cell activation?

GPI, glycophosphatidylinositol; NK, natural killer.

only a short extracellular amino terminus but a large cytoplasmic carboxyl terminus, which is structurally homologous to the ζ chain of the T cell receptor (TCR) complex. Like the TCR ζ chain, the FcR γ chain contains an immunoreceptor tyrosine-based activation motif (ITAM) that couples receptor clustering to activation of protein tyrosine kinases. FcγRI, like the high-affinity receptor for IgE (see Chapter 19), is constantly saturated with its IgG ligands. Triggering of Fc receptors requires that receptors be clustered in the plane of the membrane, and clustering and consequent receptor activation of FcγRI is mediated by multivalent antigen crosslinking receptor-bound IgG molecules.

Transcription of the FcγRI gene and expression of FcγRI on macrophages is stimulated by interferon-γ (IFN-γ). The antibody isotypes that bind best to Fcγ receptors (such as IgG2a/2c in mice) are also produced in part as a result of IFN-γ–mediated isotype switching of B cells. In addition, IFN-γ directly stimulates the microbicidal activities of phagocytes (see Chapter 10).

- **FcγRII** (CD32) binds human IgG subtypes (IgG1 and IgG3) with a low affinity (K_d 10^{-6} M). In humans, gene duplication and diversification has resulted in the generation of three forms, called FcγRII A, B, and C. These isoforms have similar extracellular domains and ligand specificities but differ in cytoplasmic tail structure, cell distribution, and functions. FcγRIIA is expressed by neutrophils and mononuclear phagocytes and participates in the phagocytosis of opsonized particles, while FcγRIIC is expressed in mononuclear phagocytes, neutrophils, and NK cells. The cytoplasmic tails of FcγRIIA and FcγRIIC contain ITAMs and, on clustering by IgG1- or IgG3-coated particles or cells, can deliver an activation signal to phagocytes. FcγRIIB is an inhibitory receptor expressed on all immune cells other than NK cells and is the only Fc receptor on B cells. Its function is described later.
- **FcγRIII** (CD16) is also a low-affinity receptor for IgG. The extracellular ligand-binding portion of FcγRIII is similar to FcγRII in structure, affinity, and specificity for IgG. This receptor exists in two forms, each encoded by a separate gene. The FcγRIIIA isoform is a transmembrane protein expressed mainly on NK cells. FcγRIIIA associates with homodimers of the FcR γ chain, homodimers of the TCR ζ chain, or heterodimers composed of the FcR γ chain and the ζ chain. This association is necessary for the cell surface expression and function of these FcRs because intracellular activating signals are delivered through the ITAMs in these signaling chains. The FcγRIIIB isoform is a glycophosphatidylinositol (GPI)–linked protein expressed on neutrophils; it does not mediate phagocytosis or trigger neutrophil activation, and its function is poorly understood.

Most of the FcRs serve to activate the cells on which they are expressed. FcεRI is described in Chapter 19, in the context of mast cell activation. The function of FcαR is not well established.

Role of Fcγ Receptors in Phagocytosis and Activation of Phagocytes

Phagocytosis of IgG-coated particles is mediated by binding of the Fc portions of opsonizing antibodies to Fcγ receptors on phagocytes. Therefore, the IgG subtypes that bind best to these receptors (IgG1 and IgG3) are the most efficient opsonins for promoting phagocytosis. As discussed before, FcγRI (CD64) is high-affinity Fcγ receptor on phagocytic cells, and it is the most important receptor for phagocytosis of opsonized particles.

Binding of Fc receptors on phagocytes to multivalent antibody-coated particles leads to engulfment of the particles and the activation of phagocytes (Fig. 12-4). The particles are internalized into vesicles known as phagosomes, which fuse with lysosomes, and the phagocytosed particles are destroyed in these phagolysosomes.

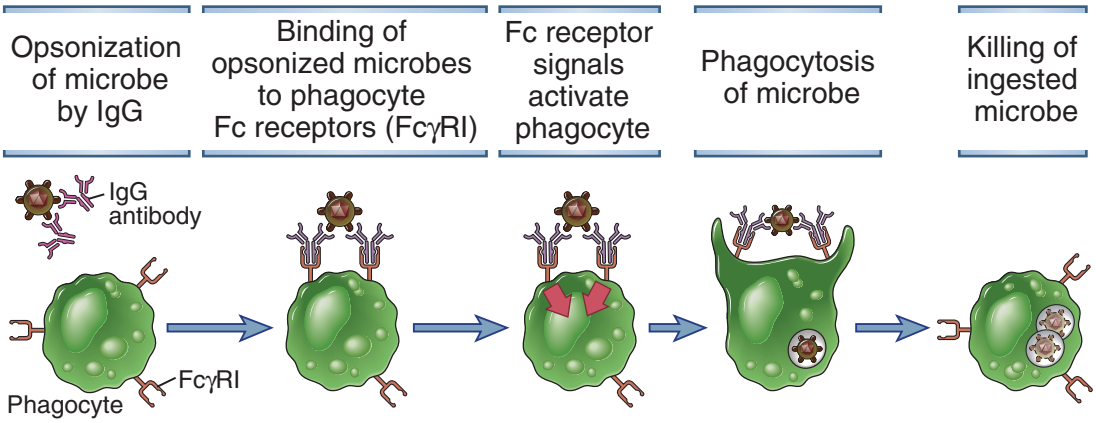


FIGURE 12–4 Antibody-mediated opsonization and phagocytosis of microbes. Antibodies of certain IgG subclasses bind to microbes and are then recognized by Fc receptors on phagocytes. Signals from the Fc receptors promote the phagocytosis of the opsonized microbes and activate the phagocytes to destroy these microbes. The microbicidal mechanisms of phagocytes are described in Chapters 4 (see Fig. 4-13) and 10 (see Fig. 10-7).

Activation requires cross-linking of the FcRs by several adjacent Ig molecules (e.g., on antibody-coated microbes or in immune complexes). Cross-linking of the ligand-binding α chains of an FcR results in signal transduction events that are similar to those that occur after antigen receptor cross-linking in lymphocytes (see Chapter 7). These include Src kinase–mediated tyrosine phosphorylation of the ITAMs in the signaling chains of the FcRs; SH2 domain–mediated recruitment of Syk family kinases to the ITAMs; activation of phosphatidylinositol 3-kinase; recruitment of adaptor molecules, including SLP-76 and BLNK; and recruitment of enzymes such as phospholipase Cγ and Tec family kinases. These events lead to generation of inositol trisphosphate and diacylglycerol and sustained calcium mobilization. Responses to these mediators in leukocytes include transcription of genes encoding cytokines, inflammatory mediators, and microbicidal enzymes and mobilization of the cytoskeleton, leading to phagocytosis, granule exocytosis, and cell migration. One consequence of phagocyte activation is production of the enzyme phagocyte oxidase, which catalyzes the intracellular generation of reactive oxygen species that are cytotoxic for phagocytosed microbes. This process is called the respiratory burst. Another consequence of FcγRI activation is the activation of an enzyme called inducible nitric oxide synthase (iNOS), which triggers the production of nitric oxide that also contributes to the killing of pathogens. In addition, leukocytes that are activated by their Fc receptors secrete hydrolytic enzymes and reactive oxygen intermediates into the external milieu that are capable of killing extracellular microbes too large to be phagocytosed. The same toxic products may damage tissues; this mechanism of antibody-mediated tissue injury is important in hypersensitivity diseases (see Chapter 18). Knockout mice lacking the ligand-binding α chain of FcγRI or the signal-transducing FcR γ chain are defective in antibody-mediated defense against microbes and do not develop some forms of IgG antibody-mediated tissue injury, thus demonstrating the essential role of Fc receptors in these processes.

Inhibitory Signaling by the FcγRIIB Receptor

The FcγRIIB receptor is an inhibitory Fc receptor that was described earlier in the context of inhibitory signaling in B cells and the phenomenon of antibody feedback (see Chapter 11). FcγRIIB is the only Fc receptor that has an ITIM motif in its cytoplasmic tail. When antibodies are produced during an immune response, these antibodies bind to remaining antigen, and the complex is simultaneously recognized by the antigen receptor and FcγRIIB on antigen-specific B cells. Immune complex–mediated cross-linking of the inhibitory FcγRIIB leads to tyrosine phosphorylation of the ITIM in the cytoplasmic tail, recruitment and activation of the SHIP inositol phosphatase, and subsequent inhibition of B cell receptor–mediated, ITAM-dependent activation pathways. FcγRIIB is also expressed on dendritic cells, neutrophils, macrophages, and mast cells and may play a role in regulating the responses of these cells to activating Fc receptors and other stimuli. One somewhat empirical but often useful treatment of many autoimmune diseases is the intravenous administration of pooled human IgG (IVIG). IVIG may engage FcγRIIB to deliver inhibitory signals to B lymphocytes and other cells, thus reducing antibody production and dampening inflammation. Another mechanism by which IVIG may ameliorate disease is by competing with circulating autoantibodies for the neonatal Fc receptor, which results in enhanced clearance of the antibodies (see Chapter 5).

Antibody-Dependent Cell-Mediated Cytotoxicity

Natural killer (NK) cells and other leukocytes bind to antibody-coated cells by Fc receptors and destroy these cells. This process is called antibody-dependent cellular cytotoxicity (ADCC) (Fig. 12-5). It was first described as a function of NK cells, which use their Fc receptor, FcγRIIIA, to bind to antibody-coated cells. FcγRIIIA (CD16) is a low-affinity receptor that binds clustered IgG molecules displayed on cell surfaces but does not bind circulating monomeric IgG. Therefore, ADCC occurs only when the target cell is coated with antibody molecules,

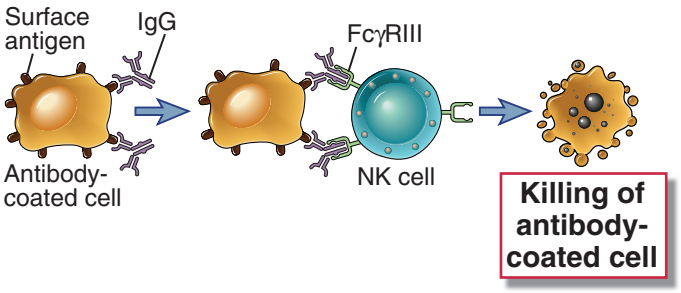


FIGURE 12–5 Antibody-dependent cell-mediated cytotoxicity. Antibodies of certain IgG subclasses bind to cells (e.g., infected cells), and the Fc regions of the bound antibodies are recognized by an Fcγ receptor on NK cells. The NK cells are activated and kill the antibody-coated cells. Presumably, NK cells can lyse even class I MHC–expressing targets when these target cells are opsonized because the Fc receptor–mediated stimulation may overcome the inhibitory actions of class I MHC–recognizing NK cell inhibitory receptors (see Chapter 12).

and free IgG in plasma neither activates NK cells nor competes effectively with cell-bound IgG for binding to FcγRIII. Engagement of FcγRIII by antibody-coated target cells activates the NK cells to synthesize and secrete cytokines such as IFN-γ as well as to discharge the contents of their granules, which mediate the killing functions of this cell type (see Chapter 4). ADCC can be readily demonstrated in vitro, but its role in host defense against microbes is not definitively established. It is likely an important mechanism for the elimination of cells that are coated by specific therapeutic monoclonal antibodies, such as B cells and B cell–derived tumor cells that are targeted by anti-CD20 antibody.

Antibody-Mediated Clearance of Helminths

Antibodies, mast cells, and eosinophils function with antibodies to mediate the expulsion and killing of some helminthic parasites. Helminths (worms) are too large to be engulfed by phagocytes, and their integuments are relatively resistant to the microbicidal products of neutrophils and macrophages. They can, however, be killed by a toxic cationic protein, known as the major basic protein, present in the granules of eosinophils. IgE, IgG, and IgA antibodies that coat helminths can bind to Fc receptors on eosinophils and cause the degranulation of these cells, releasing the basic protein and other eosinophil granule contents that kill the parasites. The high-affinity Fcε receptor of eosinophils (FcεRI) lacks the signaling β chain and can only signal relatively weakly through the associated γ chain. In addition, IgE antibodies that recognize antigens on the surface of the helminths may initiate local mast cell degranulation through the high-affinity IgE receptor (see Chapter 19). Mast cell mediators may induce bronchoconstriction and increased local motility, contributing to the expulsion of worms from sites such as the airways and the lumen of the gastrointestinal tract. Chemokines and cytokines released by activated mast cells may attract eosinophils and cause their degranulation as well.

THE COMPLEMENT SYSTEM

The complement system is one of the major effector mechanisms of humoral immunity and is also an important effector mechanism of innate immunity. We briefly discussed the role of complement in innate immunity in Chapter 4. Here we describe the activation and regulation of complement in more detail.

The name "complement" is derived from experiments performed by Jules Bordet shortly after the discovery of antibodies. He demonstrated that if fresh serum containing an antibacterial antibody is added to the bacteria at physiologic temperature (37°C), the bacteria are lysed. If, however, the serum is heated to 56°C or more, it loses its lytic capacity. This loss of lytic capacity is not due to decay of antibody activity because antibodies are relatively heat stable, and even heated serum is capable of agglutinating the bacteria. Bordet concluded that the serum must contain another heat-labile component that assists, or *complements,* the lytic function of antibodies, and this component was later given the name complement.

The complement system consists of serum and cell surface proteins that interact with one another and with other molecules of the immune system in a highly regulated manner to generate products that function to eliminate microbes. Complement proteins are plasma proteins that are normally inactive; they are activated only under particular conditions to generate products that mediate various effector functions of complement. Several features of complement activation are essential for its normal function.

- ***The complement system is activated by microbes and by antibodies that are attached to microbes and other antigens.*** The mechanisms of initial activation are described later.
- ***Activation of complement involves the sequential proteolysis of proteins to generate enzyme complexes with proteolytic activity.*** Proteins that acquire proteolytic enzymatic activity by the action of other proteases are called zymogens. The process of sequential zymogen activation, a defining feature of a proteolytic enzyme cascade, is also characteristic of the coagulation and kinin systems. Proteolytic cascades allow tremendous amplification because each enzyme molecule activated at one step can generate multiple activated enzyme molecules at the next step.
- ***The products of complement activation become covalently attached to microbial cell surfaces or to antibodies bound to microbes and to other antigens.*** In the fluid phase, complement proteins are inactive or only transiently active (for seconds), and they become stably activated after they are attached to microbes or to antibodies. Many of the biologically active cleavage products of complement proteins also bind covalently to microbes, antibodies, and tissues in which the complement is activated. This characteristic ensures that

the full activation and therefore the biologic functions of the complement system are limited to microbial cell surfaces or to sites of antibodies bound to antigens and do not occur in the blood.

- ***Complement activation is inhibited by regulatory proteins that are present on normal host cells and absent from microbes.*** The regulatory proteins are an adaptation of normal cells that minimize complement-mediated damage to host cells. Microbes lack these regulatory proteins, which allows complement activation to occur on microbial surfaces.

Pathways of Complement Activation

There are three major pathways of complement activation: the classical pathway, which is activated by certain isotypes of antibodies bound to antigens; the alternative pathway, which is activated on microbial cell surfaces in the absence of antibody; and the lectin pathway, which is activated by a plasma lectin that binds to mannose residues on microbes (Fig. 12-6). The names *classical* and *alternative* arose because the classical pathway was discovered and characterized first, but the alternative

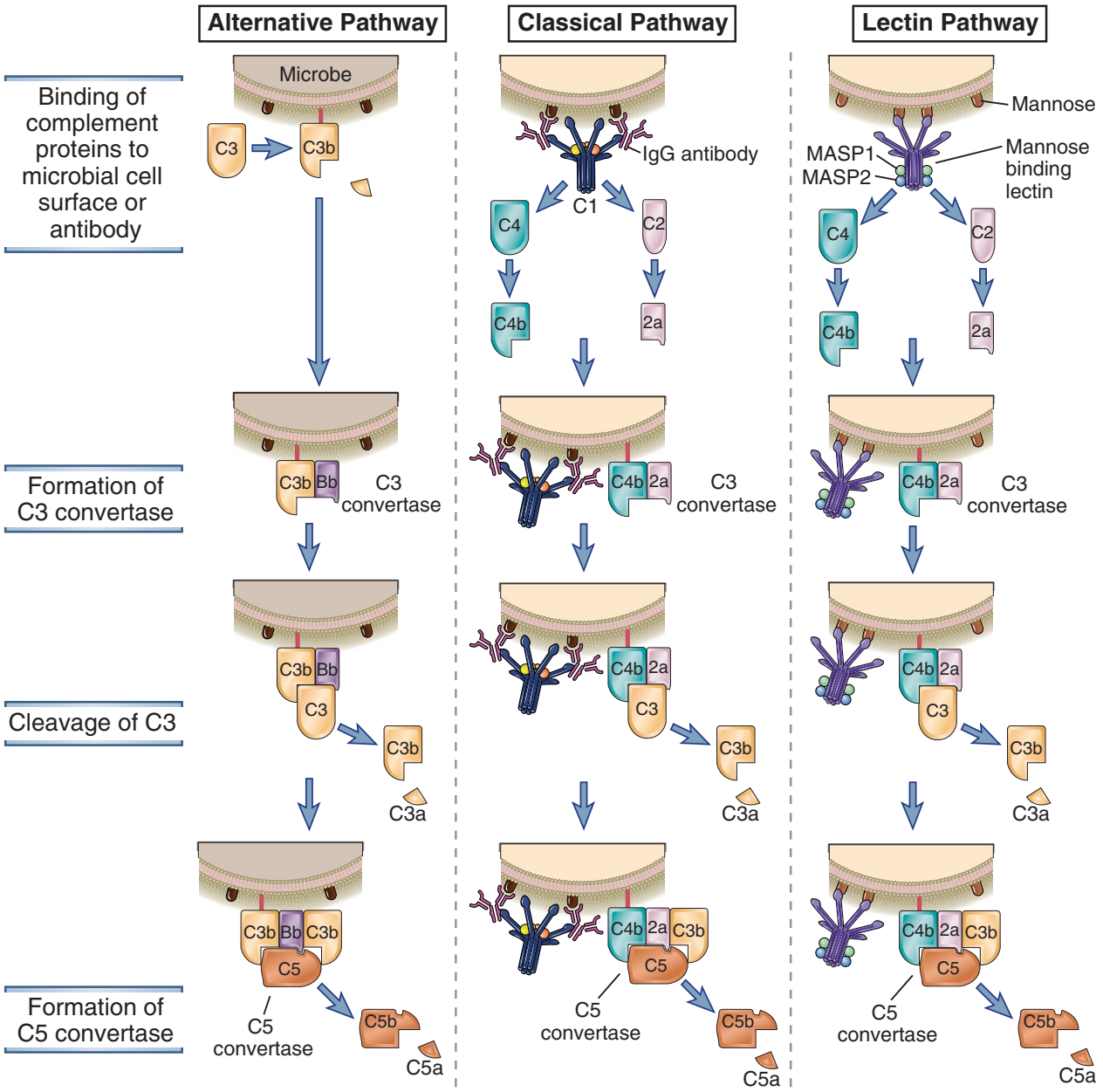


FIGURE 12–6 The early steps of complement activation by the alternative, classical, and lectin pathways. The alternative pathway is activated by C3b binding to various activating surfaces, such as microbial cell walls; the classical pathway is initiated by C1 binding to antigen-antibody complexes; and the lectin pathway is activated by binding of a plasma lectin to microbes. The C3b that is generated by the action of the C3 convertase binds to the microbial cell surface or the antibody and becomes a component of the enzyme that cleaves C5 (C5 convertase) and initiates the late steps of complement activation. The late steps of all three pathways are the same (not shown here), and complement activated by all three pathways serves the same functions.

pathway is phylogenetically older. Although the pathways of complement activation differ in how they are initiated, all of them result in the generation of enzyme complexes that are able to cleave the most abundant complement protein, C3. The alternative and lectin pathways are effector mechanisms of innate immunity, whereas the classical pathway is a major mechanism of adaptive humoral immunity.

The central event in complement activation is proteolysis of the complement protein C3 to generate biologically active products and the subsequent covalent attachment of a product of C3, called C3b, to microbial cell surfaces or to antibody bound to antigen (see Fig. 12-6). Complement activation depends on the generation of two proteolytic complexes: the C3 convertase, which cleaves C3 into two proteolytic fragments called C3a and C3b; and the C5 convertase, which cleaves C5 into C5a and C5b. By convention, the proteolytic products of each complement protein are identified by lowercase letter suffixes, *a* referring to the smaller product and *b* to the larger one. C3b becomes covalently attached to the microbial cell surface or to the antibody molecules at the site of complement activation. All the biologic functions of complement are dependent on the proteolytic cleavage of C3. For example, complement activation promotes phagocytosis because phagocytes (neutrophils and macrophages) express receptors for C3b. Peptides produced by proteolysis of C3 (and other complement proteins) stimulate inflammation. The C5 convertase assembles after the prior generation of C3b, and this convertase contributes both to inflammation (by the generation of the C5a fragment) and to the formation of pores in the membranes of microbial targets. The pathways of complement activation differ in how C3b is produced but follow a common sequence of reactions after the cleavage of C5.

With this background, we proceed to more detailed descriptions of the alternative, classical, and lectin pathways.

The Alternative Pathway

The alternative pathway of complement activation results in the proteolysis of C3 and the stable attachment of its breakdown product C3b to microbial surfaces, without a role for antibody (Fig. 12-7 and Table 12-4). The C3 protein contains a reactive thioester bond that is buried in a region of the protein known as the thioester domain. When C3 is cleaved, the C3b molecule undergoes a dramatic conformational change and the thioester domain flips out (a massive shift of about 85 Å), exposing the previously hidden reactive thioester bond. Normally, C3 in plasma is being continuously cleaved at a low rate to generate C3b in a process that is called C3 tickover. A small amount of the C3b may become covalently attached to the surfaces of cells, including microbes, through the thioester domain, which reacts with the amino or hydroxyl groups of cell surface proteins or polysaccharides to form amide or ester bonds (Fig. 12-8). If these bonds are not formed, the C3b remains in the fluid phase, and the exposed and reactive thioester bond is quickly hydrolyzed, rendering the protein inactive. As a result, further complement activation cannot proceed.

When C3b undergoes its post-cleavage conformational change, a binding site for a plasma protein called **factor B** is also exposed. Factor B then binds to the C3b protein that is now covalently tethered to the surface of a microbial or host cell. Bound factor B is in turn cleaved by a plasma serine protease called **factor D**, releasing a small fragment called Ba and generating a larger fragment called Bb that remains attached to C3b. The C3bBb complex is the **alternative pathway C3 convertase**, and it functions to cleave more C3 molecules, thus setting up an amplification sequence. Even when C3b is generated by the classical or lectin pathways, it can form a complex with Bb, and this complex is able to cleave more C3. Thus, the alternative pathway C3 convertase functions to amplify complement activation when it is initiated by the alternative, classical, or lectin pathways. When C3 is broken down, C3b remains attached to cells and C3a is released. This soluble fragment has several biologic activities that are discussed later.

Alternative pathway activation readily occurs on microbial cell surfaces and not on mammalian cells. If the C3bBb complex is formed on mammalian cells, it is rapidly degraded and the reaction is terminated by the action of several regulatory proteins present on these cells (discussed later). Lack of the regulatory proteins on microbial cells allows binding and activation of the alternative pathway C3 convertase. In addition, another protein of the alternative pathway, called **properdin**, can bind to and stabilize the C3bBb complex, and the attachment of properdin is favored on microbial as opposed to normal host cells. Properdin is the only known positive regulator of complement.

Some of the C3b molecules generated by the alternative pathway C3 convertase bind to the convertase itself. This results in the formation of a complex containing one Bb moiety and two molecules of C3b, which functions as the **alternative pathway C5 convertase**, which will cleave C5 and initiate the late steps of complement activation.

The Classical Pathway

The classical pathway is initiated by binding of the complement protein C1 to the C_H2 domains of IgG or the C_H3 domains of IgM molecules that have bound antigen (Fig. 12-9 and Table 12-5). Among IgG antibodies, IgG3 and IgG1 (in humans) are more efficient activators of complement than are other subclasses. C1 is a large, multimeric protein complex composed of C1q, C1r, and C1s subunits; C1q binds to the antibody, and C1r and C1s are proteases. The C1q subunit is made up of an umbrella-like radial array of six chains, each of which has a globular head connected by a collagen-like arm to a central stalk. This hexamer performs the recognition function of the molecule and binds specifically to the Fc regions of μ and some γ heavy chains (Fig. 12-10). Each Ig Fc region has a single C1q-binding site, and each C1q molecule must bind to at least two Ig heavy chains to be activated. This requirement explains why antibodies bound to antigens, and not free circulating antibodies, can initiate classical pathway activation (Fig. 12-11). Because each IgG molecule has only one Fc region, multiple IgG molecules must be brought close together before C1q can bind, and

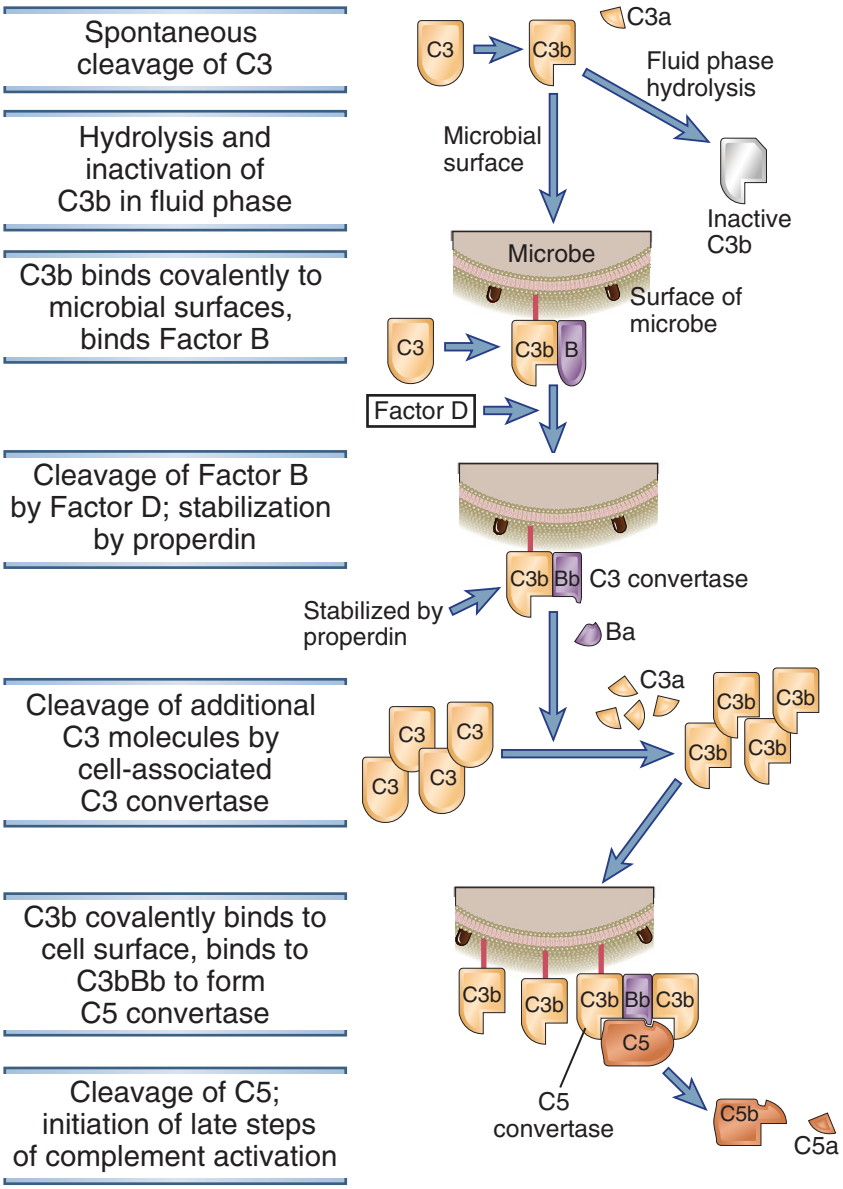


FIGURE 12–7 The alternative pathway of complement activation. Soluble C3 in plasma undergoes slow spontaneous hydrolysis of its internal thioester bond, which leads to the formation of a fluid-phase C3 convertase (not shown) and the generation of C3b. If the C3b is deposited on the surfaces of microbes, it binds factor B and forms the alternative pathway C3 convertase. This convertase cleaves C3 to produce more C3b, which binds to the microbial surface and participates in the formation of a C5 convertase. The C5 convertase cleaves C5 to generate C5b, the initiating event in the late steps of complement activation.

TABLE 12–4 Proteins of the Alternative Pathway of Complement

Protein	Structure	Serum Concentration (μg/mL)	Function
C3	185 kD (α subunit, 110 kD; β subunit, 75 kD)	1000-1200	C3b binds to the surface of the microbe, where it functions as an opsonin and as a component of C3 and C5 convertases C3a stimulates inflammation (anaphylatoxin)
Factor B	93-kD monomer	200	Bb is a serine protease and the active enzyme of the C3 and C5 convertases
Factor D	25-kD monomer	1-2	Plasma serine protease, cleaves factor B when it is bound to C3b
Properdin	Composed of up to four 56-kD subunits	25	Stabilizes C3 convertases (C3bBb) on microbial surfaces

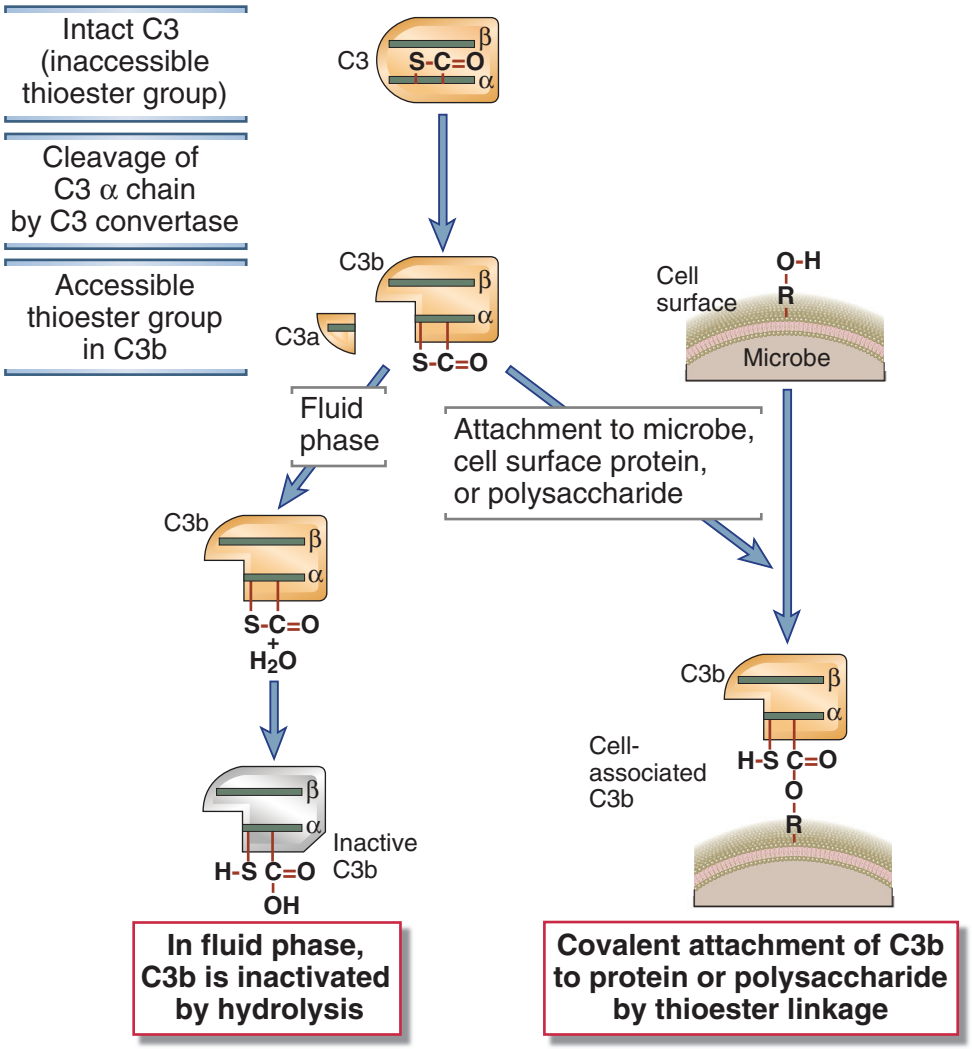


FIGURE 12–8 Internal thioester bonds of C3 molecules. A schematic view is shown of the internal thioester groups in C3 and their role in forming covalent bonds with other molecules. Proteolytic cleavage of the α chain of C3 converts it into a metastable form in which the internal thioester bonds are exposed and susceptible to nucleophilic attack by oxygen (as shown) or nitrogen atoms. The result is the formation of covalent bonds with proteins or carbohydrates on the cell surfaces. C4 is structurally homologous to C3 and has an identical thioester group.

TABLE 12–5 Proteins of the Classical Pathway of Complement

Protein	Structure	Serum Concentration (μg/mL)	Function
C1 ($C1qr_2s_2$)	750 kD		Initiates the classical pathway
C1q	460 kD; hexamer of three pairs of chains (22, 23, 24 kD)	75-150	Binds to the Fc portion of antibody that has bound antigen, to apoptotic cells, and to cationic surfaces
C1r	85-kD dimer	50	Serine protease, cleaves C1s to make it an active protease
C1s	85-kD dimer	50	Serine protease, cleaves C4 and C2
C4	210 kD, trimer of 97-, 75-, and 33-kD chains	300-600	C4b covalently binds to the surface of a microbe or cell, where antibody is bound and complement is activated C4b binds C2 for cleavage by C1s C4a stimulates inflammation (anaphylatoxin)
C2	102-kD monomer	20	C2a is a serine protease and functions as the active enzyme of C3 and C5 convertases to cleave C3 and C5
C3	See Table 12-4		

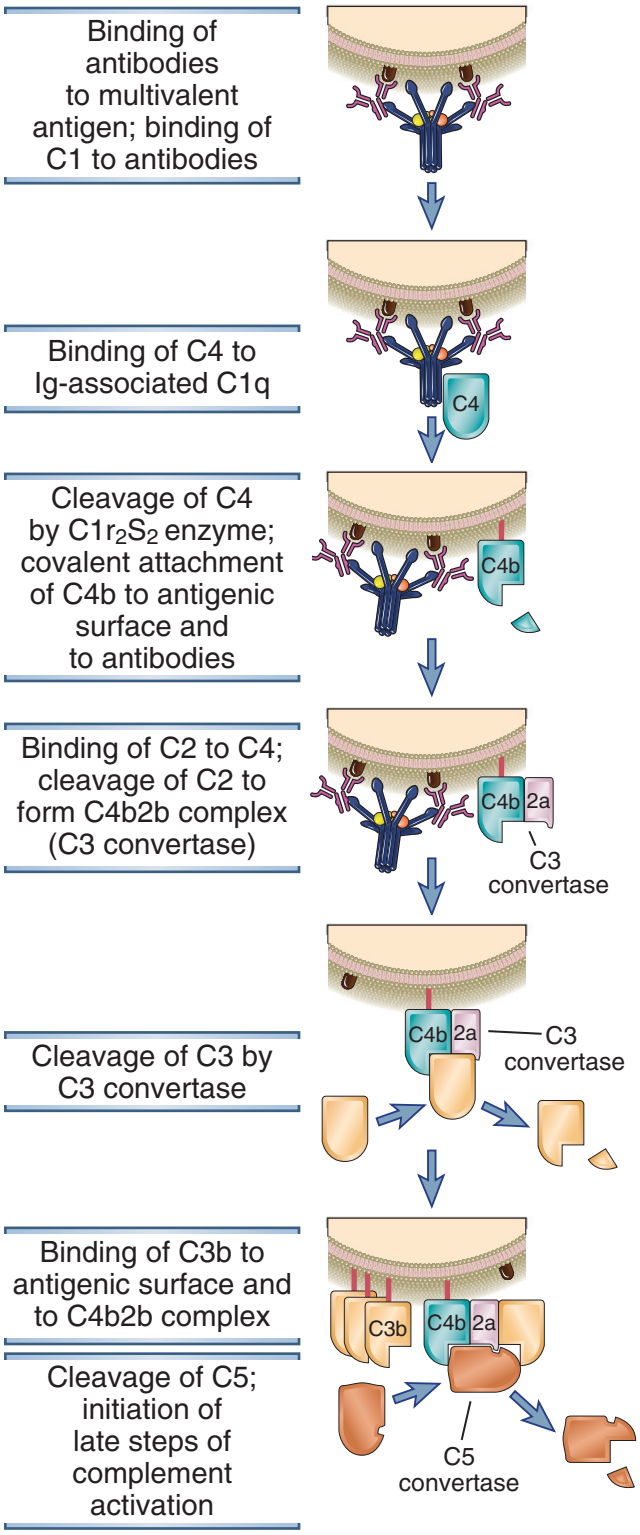


FIGURE 12–9 The classical pathway of complement activation. Antigen-antibody complexes that activate the classical pathway may be soluble, fixed on the surface of cells (as shown), or deposited on extracellular matrices. The classical pathway is initiated by the binding of C1 to antigen-complexed antibody molecules, which leads to the production of C3 and C5 convertases attached to the surfaces where the antibody was deposited. The C5 convertase cleaves C5 to begin the late steps of complement activation.

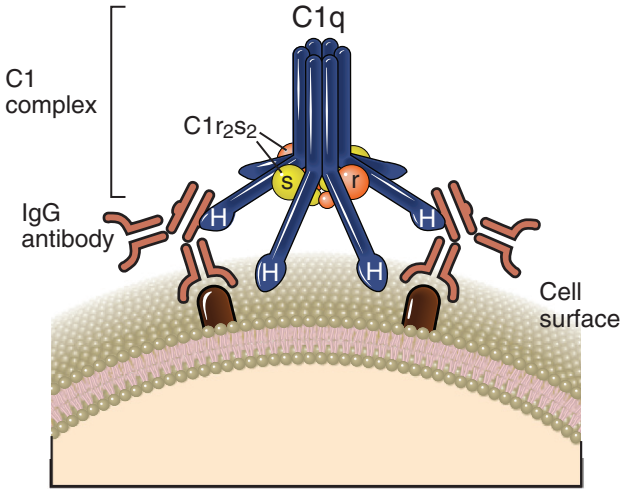


FIGURE 12–10 Structure of C1. C1q consists of six identical subunits arranged to form a central core and symmetrically projecting radial arms. The globular heads at the end of each arm, designated H, are the contact regions for immunoglobulin. C1r and C1s form a tetramer composed of two C1r and two C1s molecules. The ends of C1r and C1s contain the catalytic domains of these proteins. One $C1r_2s_2$ tetramer wraps around the radial arms of the C1q complex in a manner that juxtaposes the catalytic domains of C1r and C1s.

multiple IgG antibodies are brought together only when they bind to a multivalent antigen. Even though free (circulating) IgM is pentameric, it does not bind C1q because the Fc regions of free IgM are in a planar configuration that is inaccessible to C1q. Binding of the IgM to an antigen induces a conformational change into a "staple" form that exposes the C1q binding sites in the Fc regions and allows C1q to bind. Because of its pentameric structure, a single molecule of IgM can bind two C1q molecules, and this is one reason that IgM is a more efficient complement-binding (also called complement-fixing) antibody than IgG is.

C1r and C1s are serine proteases that form a tetramer containing two molecules of each protein. Binding of two or more of the globular heads of C1q to the Fc regions of IgG or IgM leads to enzymatic activation of the associated C1r, which cleaves and activates C1s (see Fig. 12-9). Activated C1s cleaves the next protein in the cascade, C4, to generate C4b. (The smaller C4a fragment is released and has biologic activities that are described later.) C4 is homologous to C3, and C4b contains an internal thioester bond, similar to that in C3b, that forms covalent amide or ester linkages with the antigen-antibody complex or with the adjacent surface of a cell to which the antibody is bound. This attachment of C4b ensures that classical pathway activation proceeds on a cell surface or immune complex. The next complement protein, C2, then complexes with the cell surface–bound C4b and is cleaved by a nearby C1s molecule to generate a soluble C2b fragment of unknown importance and a larger C2a fragment that remains physically associated with C4b on the cell surface. (Note that the nomenclature of C2 fragments that is now accepted is different from the other complement proteins because the attached fragment is called the *a* piece and the released part is the *b* fragment. This is because, for C2, the attached fragment

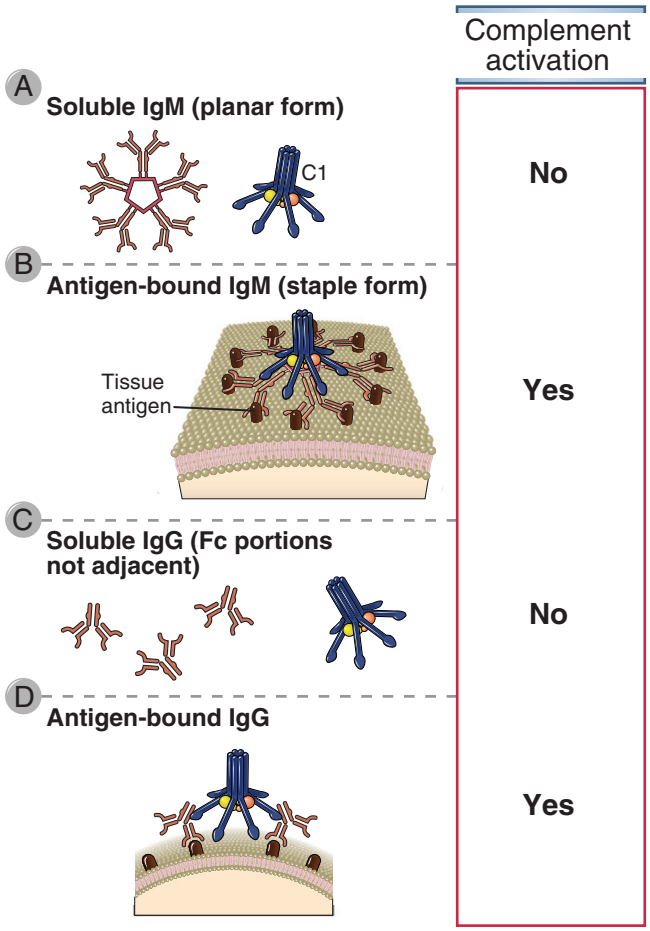


FIGURE 12–11 C1 binding to the Fc portions of IgM and IgG. C1 must bind to two or more Fc portions to initiate the complement cascade. The Fc portions of soluble pentameric IgM are not accessible to C1 **(A)**. After IgM binds to surface-bound antigens, it undergoes a shape change that permits C1 binding and activation **(B)**. Soluble IgG molecules will also not activate C1 because each IgG has only one Fc region **(C)**, but after binding to cell surface antigens, adjacent IgG Fc portions can bind and activate C1 **(D)**.

is larger.) The resulting C4b2a complex is the **classical pathway C3 convertase**; it has the ability to bind to and proteolytically cleave C3. Binding of this enzyme complex to C3 is mediated by the C4b component, and proteolysis is catalyzed by the C2a component. Cleavage of C3 results in the removal of the small C3a fragment, and C3b can form covalent bonds with cell surfaces or with the antibody where complement activation was initiated. Once C3b is deposited, it can bind factor B and generate more C3 convertase by the alternative pathway, as discussed earlier. The net effect of the multiple enzymatic steps and amplification is that a single molecule of C3 convertase can lead to the deposition of hundreds or thousands of molecules of C3b on the cell surface where complement is activated. The key early steps of the alternative and classical pathways are analogous: C3 in the alternative pathway is homologous to C4 in the classical pathway, and factor B is homologous to C2.

Some of the C3b molecules generated by the classical pathway C3 convertase bind to the convertase (as in the alternative pathway) and form a C4b2a3b complex. This complex functions as the **classical pathway C5 convertase**; it cleaves C5 and initiates the late steps of complement activation.

An unusual antibody-independent variant form of the classical pathway, which is activated by carbohydrates binding to a cell surface lectin, occurs in pneumococcal infections. Splenic marginal zone macrophages express a cell surface C-type lectin called SIGN-R1 that can recognize the pneumococcal polysaccharide and can also bind C1q. Multivalent binding of whole bacteria or the polysaccharide to SIGN-R1 activates the classical pathway and permits the eventual coating of the pneumococcus with C3b. This is an example of a cell surface lectin that mediates activation of the classical pathway but without a requirement for antibody.

The Lectin Pathway

The **lectin pathway** of complement activation is triggered in the absence of antibody by the binding of microbial polysaccharides to circulating lectins, such as plasma mannose (or mannan)–binding lectin (MBL), or to ficolins (Table 12-6). These soluble lectins are collagen-like proteins that structurally resemble C1q (see Fig. 4-10, Chapter 4). MBL, L-ficolin, and H-ficolin are plasma proteins; M-ficolin is mainly secreted by activated macrophages in tissues. MBL is a member of the collectin family and has an N-terminal collagen-like domain and a C-terminal carbohydrate recognition domain. The ficolins have a similar structure with an N-terminal collagen-like domain and a C-terminal fibrinogen-like domain. The collagen-like domains help assemble basic triple-helical structures that can form higher order oligomers. MBL binds to mannose residues on polysaccharides; the ficolin fibrinogen-like domain binds *N*-acetylglucosamine–containing glycans. MBL and ficolins associate with MBL-associated serine proteases (MASPs) including MASP1, MASP2, and MASP3 (see Table 12-6). The MASP proteins are structurally homologous to the C1r and C1s proteases and serve a similar function, namely, the cleavage of C4 and C2 to activate the complement pathway. Higher order oligomers of MBL associate with MASP1 and MASP2, although MASP3/MASP2 complexes may also be found. MASP1 (or MASP3) can form a tetrameric complex with MASP2 similar to the one formed by C1r and C1s, and MASP2 is the protease that cleaves C4 and C2. Subsequent events in this pathway are identical to those that occur in the classical pathway.

Late Steps of Complement Activation

C5 convertases generated by the alternative, classical, or lectin pathway initiate activation of the late components of the complement system, which culminates in formation of the cytocidal membrane attack complex (MAC) (Table 12-7 and Fig. 12-12). C5 convertases cleave C5 into a small C5a fragment that is released and a two-chain C5b fragment that remains bound to the complement proteins deposited on the cell surface. C5a has potent biologic effects on several cells that are discussed later in this chapter. The remaining components of the complement

TABLE 12–6 Proteins of the Lectin Pathway of Complement

Protein	Structure	Serum Concentration (μg/mL)	Function
Mannose-binding lectin	Helical trimer of 32-kD chain and dimers to hexamers of this triple helix	1-8	Agglutinin, opsonin, complement fixing Lectin of collectin family
M-ficolin (ficolin-1)	Helical trimer of 34-kD chain and a tetramer of this triple helix	Undetectable	Agglutinin, opsonin, complement fixing Protein secreted by activated macrophages
L-ficolin (ficolin-2)	Helical trimer of 34-kD chain and a tetramer of this triple helix	1-7	Agglutinin, opsonin, complement fixing Plasma protein
H-ficolin (ficolin-3)	Helical trimer of 34-kD chain and a tetramer of this triple helix	6-83	Agglutinin, opsonin, complement fixing Plasma protein
MASP1	90-kD homodimer; homology to C1r/C1s	2-13*	Forms complex with MASP2 and collectins or ficolins and activates MASP3
MASP2	110-kD homodimer; homology to C1r/C1s	2-13	Forms complex with lectins, especially ficolin-3
MASP3	76-kD homodimer; homology to C1r/C1s	0.02-1.0	Associates with collectins or ficolins and MASP1 and cleaves C4

*Published concentrations may have been influenced by cross-reactivity of antibodies with MASP3; concentrations of the latter are derived by use of specific monoclonal antibodies.

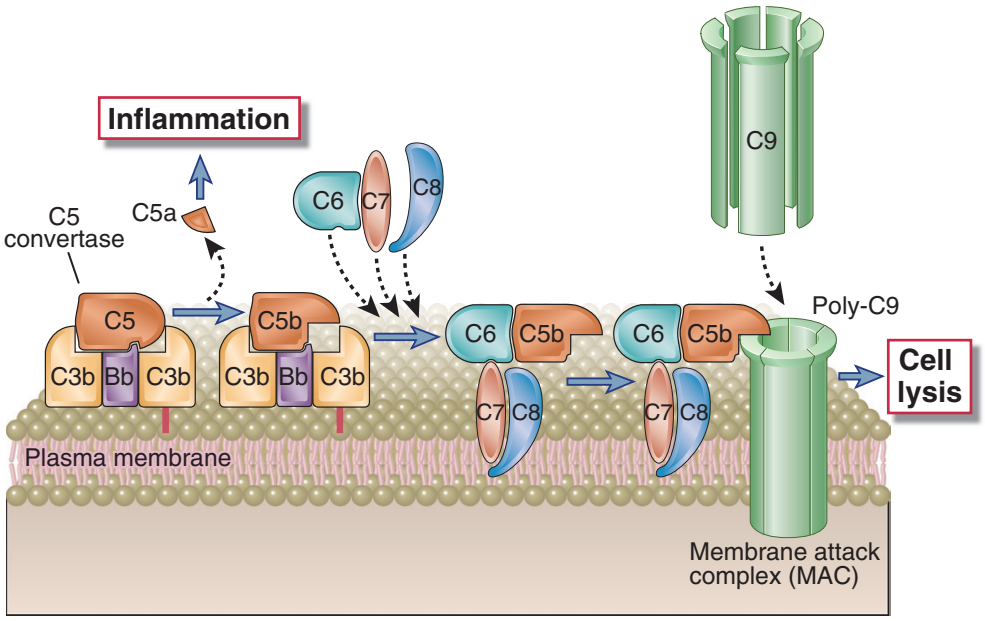


FIGURE 12–12 Late steps of complement activation and formation of the MAC. A schematic view of the cell surface events leading to formation of the MAC is shown. Cell-associated C5 convertase cleaves C5 and generates C5b, which becomes bound to the convertase. C6 and C7 bind sequentially, and the C5b,6,7 complex becomes directly inserted into the lipid bilayer of the plasma membrane, followed by stable insertion of C8. Up to 15 C9 molecules may then polymerize around the complex to form the MAC, which creates pores in the membrane and induces cell lysis. C5a released on proteolysis of C5 stimulates inflammation.

TABLE 12–7 Proteins of the Late Steps of Complement Activation

Protein	Structure	Serum Concentration (μg/mL)	Function
C5	190-kD dimer of 115- and 75- kD chains	80	C5b initiates assembly of the membrane attack complex C5a stimulates inflammation (anaphylatoxin)
C6	110-kD monomer	45	Component of the MAC: binds to C5b and accepts C7
C7	100-kD monomer	90	Component of the MAC: binds to C5b,6 and inserts into lipid membranes
C8	155-kD trimer of 64-, 64-, 22-kD chains	60	Component of the MAC: binds to C5b,6,7 and initiates the binding and polymerization of C9
C9	79-kD monomer	60	Component of the MAC: binds to C5b,6,7,8 and polymerizes to form membrane pores

cascade, C6, C7, C8, and C9, are structurally related proteins without enzymatic activity. C5b transiently maintains a conformation capable of binding the next proteins in the cascade, C6 and C7. The C7 component of the resulting C5b,6,7 complex is hydrophobic, and it inserts into the lipid bilayer of cell membranes, where it becomes a high-affinity receptor for the C8 molecule. The C8 protein is a trimer composed of three distinct chains, one of which binds to the C5b,6,7 complex and forms a covalent heterodimer with the second chain; the third chain inserts into the lipid bilayer of the membrane. This stably inserted C5b,6,7,8 complex (C5b-8) has a limited ability to lyse cells. The formation of a fully active MAC is accomplished by the binding of C9, the final component of the complement cascades, to the C5b-8 complex. C9 is a serum protein that polymerizes at the site of the bound C5b-8 to form pores in plasma membranes. These pores are about 100 Å in diameter, and they form channels that allow free movement of water and ions. The entry of water results in osmotic swelling and rupture of the cells on whose surface the MAC is deposited. The pores formed by polymerized C9 are similar to the membrane pores formed by perforin, the cytolytic granule protein found in cytotoxic T lymphocytes and NK cells (see Chapter 10), and C9 is structurally homologous to perforin.

Receptors for Complement Proteins

Many of the biologic activities of the complement system are mediated by the binding of complement fragments to membrane receptors expressed on various cell types. The best characterized of these receptors are specific for fragments of C3 and are described here (Table 12-8). Other receptors include those for C3a, C4a, and C5a, which stimulate inflammation, and some that regulate complement activation.

- ***The type 1 complement receptor (CR1, or CD35) functions mainly to promote phagocytosis of C3b- and C4b-coated particles and clearance of immune complexes from the circulation.*** CR1 is a high-affinity receptor for C3b and C4b. It is expressed mainly on bone marrow–derived cells, including erythrocytes, neutrophils, monocytes, macrophages, eosinophils, and T and B lymphocytes; it is also found on follicular dendritic cells in the follicles of peripheral lymphoid organs. Phagocytes use this receptor to bind and internalize particles opsonized with C3b or C4b. The binding of C3b- or C4b-coated particles to CR1 also transduces signals that activate the microbicidal mechanisms of the phagocytes, especially when the Fcγ receptor is simultaneously engaged by antibody-coated particles. CR1 on erythrocytes binds circulating immune complexes with attached C3b and C4b and transports the complexes to the liver and spleen. Here, the immune complexes are removed from the erythrocyte surface by phagocytes, and the erythrocytes continue to circulate. CR1 is also a regulator of complement activation (see next section).
- ***The type 2 complement receptor (CR2, or CD21) functions to stimulate humoral immune responses by enhancing B cell activation by antigen and by promoting the trapping of antigen-antibody complexes in germinal centers.*** CR2 is present on B lymphocytes, follicular dendritic cells, and some epithelial cells. It specifically binds the cleavage products of C3b, called C3d, C3dg, and iC3b (*i* referring to inactive), which are generated by factor I–mediated proteolysis (discussed later). On B cells, CR2 is expressed as part of a trimolecular complex that includes two other noncovalently attached proteins called CD19 and target of antiproliferative antibody 1 (TAPA-1, or CD81). This complex delivers signals to B cells that enhance the responses of B cells to antigen (see Fig. 7-20, Chapter

TABLE 12–8 Receptors for Fragments of C3

Receptor	Structure	Ligands	Cell Distribution	Function
Type 1 complement receptor (CR1, CD35)	160-250 kD; multiple CCPRs	C3b > C4b > iC3b	Mononuclear phagocytes, neutrophils, B and T cells, erythrocytes, eosinophils, FDCs	Phagocytosis Clearance of immune complexes Promotes dissociation of C3 convertases by acting as cofactor for cleavage of C3b, C4b
Type 2 complement receptor (CR2, CD21)	145 kD; multiple CCPRs	C3d, C3dg > iC3b	B lymphocytes, FDCs, nasopharyngeal epithelium	Coreceptor for B cell activation Trapping of antigens in germinal centers Receptor for EBV
Type 3 complement receptor (CR3, Mac-1, CD11bCD18)	Integrin, with 165-kD α chain and 95-kD β2 chain	iC3b, ICAM-1; also binds microbes	Mononuclear phagocytes, neutrophils, NK cells	Phagocytosis Leukocyte adhesion to endothelium (via ICAM-1)
Type 4 complement receptor (CR4, p150,95, CD11cCD18)	Integrin, with 150-kD α chain and 95-kD β2 chain	iC3b	Mononuclear phagocytes, neutrophils, NK cells	Phagocytosis, cell adhesion?

CCPRs, complement control protein repeats; EBV, Epstein-Barr virus; FDCs, follicular dendritic cells; ICAM-1, intercellular adhesion molecule 1.

7). On follicular dendritic cells, CR2 serves to trap iC3b- and C3dg-coated antigen-antibody complexes in germinal centers. The functions of complement in B cell activation are described later.

In humans, CR2 is the cell surface receptor for Epstein-Barr virus, a herpesvirus that causes infectious mononucleosis and is also linked to several malignant tumors. Epstein-Barr virus infects B cells and can remain latent in these cells for life.

- ***The type 3 complement receptor, also called Mac-1 (CR3, CD11bCD18), is an integrin that functions as a receptor for the iC3b fragment generated by proteolysis of C3b.*** Mac-1 is expressed on neutrophils, mononuclear phagocytes, mast cells, and NK cells. It is a member of the integrin family of cell surface receptors (see Chapter 3) and consists of an α chain (CD11b) noncovalently linked to a β chain (CD18) that is identical to the β chains of two closely related integrin molecules, leukocyte function-associated antigen 1 (LFA-1) and p150,95. Mac-1 on neutrophils and monocytes promotes phagocytosis of microbes opsonized with iC3b. In addition, Mac-1 may directly recognize bacteria for phagocytosis by binding to some unknown microbial molecules (see Chapter 4). It also binds to intercellular adhesion molecule 1 (ICAM-1) on endothelial cells and promotes stable attachment of the leukocytes to endothelium, even without complement activation. This binding leads to the recruitment of leukocytes to sites of infection and tissue injury (see Chapter 3).
- The ***type 4 complement receptor*** (CR4, p150,95, CD11c/CD18) is another integrin with a different α chain (CD11c) and the same β chain as Mac-1. It also binds iC3b, and the function of this receptor is probably similar to that of Mac-1. CD11c is also abundantly expressed on dendritic cells and is used as a marker for this cell type.
- The ***complement receptor of the immunoglobulin family*** (CRIg) is expressed on the surface of macrophages in the liver known as Kupffer cells. CRIg is an integral membrane protein with an extracellular region made up of Ig domains. It binds the complement fragments C3b and iC3b and is involved in the clearance of opsonized bacteria and other blood-borne pathogens.

Regulation of Complement Activation

Activation of the complement cascade and the stability of active complement proteins are tightly regulated to prevent complement activation on normal host cells and to limit the duration of complement activation even on microbial cells and antigen-antibody complexes. Regulation of complement is mediated by several circulating and cell membrane proteins (Table 12-9). Many of these proteins as well as several proteins of the classical and alternative pathways belong to a family called regulators of complement activity (RCA) and are encoded by homologous genes that are located adjacent to one another in the genome.

Complement activation needs to be regulated for two reasons. First, low-level complement activation goes on spontaneously, and if such activation is allowed to proceed, the result can be damage to normal cells and tissues. Second, even when complement is activated where needed, such as on microbial cells or antigen-antibody complexes, it needs to be controlled because degradation products of complement proteins can diffuse to adjacent cells and injure them. Different regulatory mechanisms inhibit the formation of C3 convertases in the early steps of complement activation, break down

TABLE 12–9 Regulators of Complement Activation

Receptor	Structure	Distribution	Interacts with	Function
C1 inhibitor (C1 INH)	104 kD	Plasma protein; conc. 200 μg/mL	C1r, C1s	Serine protease inhibitor; binds to C1r and C1s and dissociates them from C1q
Factor I	88-kD dimer of 50- and 38- kD subunits	Plasma protein; conc. 35 μg/mL	C4b, C3b	Serine protease; cleaves C3b and C4b by using factor H, MCP, C4BP, or CR1 as cofactors
Factor H	150 kD; multiple CCPRs	Plasma protein; conc. 480 μg/mL	C3b	Binds C3b and displaces Bb Cofactor for factor I–mediated cleavage of C3b
C4-binding protein (C4BP)	570 kD; multiple CCPRs	Plasma protein; conc. 300 μg/mL	C4b	Binds C4b and displaces C2 Cofactor for factor I–mediated cleavage of C4b
Membrane cofactor for protein (MCP CD46)	45-70 kD; four CCPRs	Leukocytes, epithelial cells, endothelial cells	C3b, C4b	Cofactor for factor I–mediated cleavage of C3b and C4b
Decay-accelerating factor (DAF)	70 kD; GPI linked, four CCPRs	Blood cells, endothelial cells, epithelial cells	C4b2a, C3bBb	Displaces C2b from C4b and Bb from C3b (dissociation of C3 convertases)
CD59	18 kD; GPI linked	Blood cells, endothelial cells, epithelial cells	C7, C8	Blocks C9 binding and prevents formation of the MAC

CCPRs, complement control protein repeats; conc., concentration; GPI, glycophosphatidylinositol; MAC, membrane attack complex.

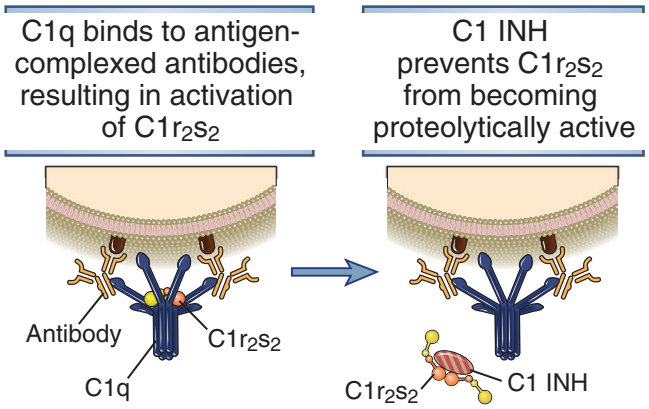


FIGURE 12–13 Regulation of C1 activity by C1 INH. C1 INH displaces $C1r_2s_2$ from C1q and terminates classical pathway activation.

and inactivate C3 and C5 convertases, and inhibit formation of the MAC in the late steps of the complement pathway.

- ***The proteolytic activity of C1r and C1s is inhibited by a plasma protein called C1 inhibitor (C1 INH).*** C1 INH is a serine protease inhibitor (serpin) that mimics the normal substrates of C1r and C1s. If C1q binds to an antibody and begins the process of complement activation, C1 INH becomes a target of the enzymatic activity of the bound $C1r_2$-$C1s_2$. C1 INH is cleaved by and becomes covalently attached to these complement proteins, and as a result, the $C1r_2$-$C1s_2$ tetramer dissociates from C1q, thus stopping activation by the classical pathway (Fig. 12-13). In this way, C1 INH prevents the accumulation of enzymatically active $C1r_2$-$C1s_2$ in the plasma and limits the time for which active $C1r_2$-$C1s_2$ is available to activate subsequent steps in the complement cascade. An autosomal dominant inherited disease called **hereditary angioneurotic edema** is due to a deficiency of C1 INH. Clinical manifestations of the disease include intermittent acute accumulation of edema fluid in the skin and mucosa, which causes abdominal pain, vomiting, diarrhea, and potentially life-threatening airway obstruction. In these patients, the plasma levels of C1 INH protein are sufficiently reduced (<20% to 30% of normal) that activation of C1 by immune complexes is not properly controlled and increased breakdown of C4 and C2 occurs. The mediators of edema formation in patients with hereditary angioneurotic edema include a proteolytic fragment of C2, called C2 kinin, and bradykinin. C1 INH is an inhibitor of other plasma serine proteases besides C1, including kallikrein and coagulation factor XII, and both activated kallikrein and factor XII can promote increased formation of bradykinin.
- ***Assembly of the components of C3 and C5 convertases is inhibited by the binding of regulatory proteins to C3b and C4b deposited on cell surfaces*** (Fig. 12-14). If C3b is deposited on the surfaces of normal mammalian cells, it may be bound by several membrane proteins, including membrane cofactor protein (MCP, or CD46), type 1 complement receptor (CR1), decay-accelerating factor (DAF), and a plasma protein called factor H. C4b deposited on cell surfaces is similarly bound by DAF, CR1, and another plasma protein called C4-binding protein (C4BP). By binding to C3b or C4b, these proteins competitively inhibit the binding of other components of the C3 convertase, such as Bb of the alternative pathway and C2b of the classical pathway, thus blocking further progression of the complement cascade. (Factor H inhibits binding of only Bb to C3b and is thus a regulator of the alternative but not the classical pathway.) MCP, CR1, and DAF are produced by mammalian cells but not by microbes. Therefore, these regulators of complement selectively inhibit complement activation on host cells and allow complement activation to proceed on microbes. In addition, cell surfaces rich in sialic acid favor binding of the regulatory protein factor H over the alternative pathway protein factor B. Mammalian cells express higher levels of sialic acid than most microbes do, which is another reason that complement activation is prevented on normal host cells and permitted on microbes.

DAF is a glycophosphatidylinositol-linked membrane protein expressed on endothelial cells and erythrocytes. Genetic deficiency of an enzyme required to form such protein-lipid linkages results in failure to express many glycophosphatidylinositol-linked membrane proteins, including DAF and CD59 (see following), and causes a disease called **paroxysmal nocturnal hemoglobinuria**. This disease is characterized by recurrent bouts of intravascular hemolysis,

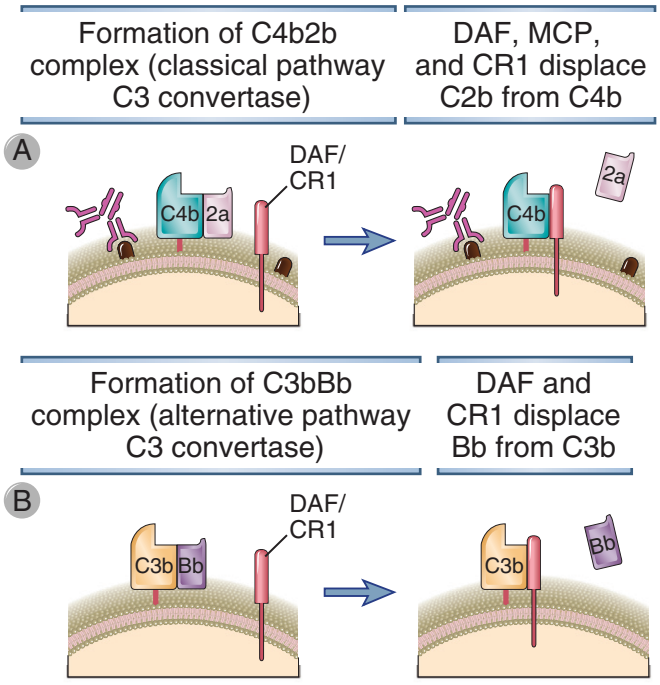


FIGURE 12–14 Inhibition of the formation of C3 convertases. Several membrane proteins present on normal cells displace either C2a from the classical pathway C3 convertase **(A)** or Bb from the alternative pathway C3 convertase **(B)** and stop complement activation.

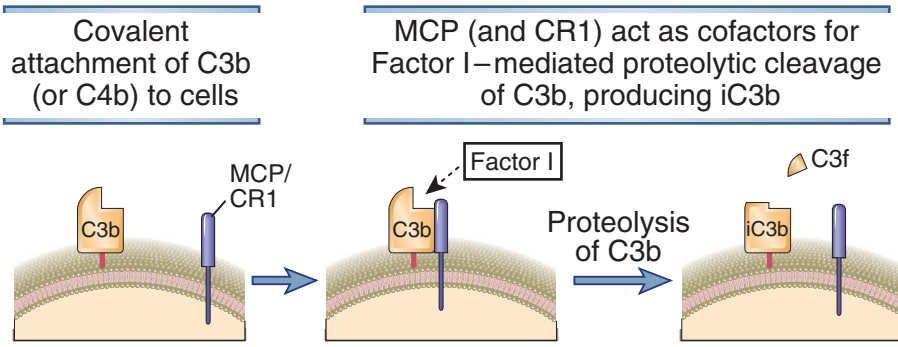


FIGURE 12–15 Factor I–mediated cleavage of C3b. In the presence of cell membrane–bound cofactors (MCP or CR1), plasma factor I proteolytically cleaves C3b attached to cell surfaces, leaving an inactive form of C3b (iC3b). Factor H and C4-binding protein can also serve as cofactors for factor I–mediated cleavage of C3b. The same process is involved in the proteolysis of C4.

at least partly attributable to unregulated complement activation on the surface of erythrocytes. Recurrent intravascular hemolysis in turn leads to chronic hemolytic anemia and venous thrombosis. An unusual feature of this disease is that the mutation in the defective gene is not inherited but represents an acquired mutation in hematopoietic stem cells.

- ***Cell-associated C3b is proteolytically degraded by a plasma serine protease called factor I, which is active only in the presence of regulatory proteins*** (Fig. 12-15). MCP, factor H, C4BP, and CR1 all serve as cofactors for factor I–mediated cleavage of C3b (and C4b). Thus, these regulatory host cell proteins promote proteolytic degradation of complement proteins; as discussed earlier, the same regulatory proteins cause dissociation of C3b (and C4b)–containing complexes. Factor I–mediated cleavage of C3b generates the fragments called iC3b, C3d, and C3dg, which do not participate in complement activation but are recognized by receptors on phagocytes and B lymphocytes.
- ***Formation of the MAC is inhibited by a membrane protein called CD59.*** CD59 is a glycophosphatidylinositol-linked protein expressed on many cell types. It works by incorporating itself into assembling MACs after the membrane insertion of C5b-8, thereby inhibiting the subsequent addition of C9 molecules (Fig. 12-16). CD59 is present on normal host cells, where it limits MAC formation, but it is not present on microbes. Formation of the MAC is also inhibited by plasma proteins such as S protein, which functions by binding to soluble C5b,6,7 complexes and thereby preventing their insertion into cell membranes near the site where the complement cascade was initiated. Growing MACs can insert into any neighboring cell membrane besides the membrane on which they were generated. Inhibitors of the MAC in the plasma and in host cell membranes ensure that lysis of innocent bystander cells does not occur near the site of complement activation.

Much of the analysis of the function of complement regulatory proteins has relied on in vitro experiments, and most of these experiments have focused on assays that measure MAC-mediated cell lysis as an endpoint. On the basis of these studies, a hierarchy of importance for inhibiting complement activation is believed to be CD59 > DAF > MCP; this hierarchy may reflect the relative abundance of these proteins on cell surfaces.

The function of regulatory proteins may be overwhelmed by excessive activation of complement pathways. We have emphasized the importance of these regulatory proteins in preventing complement activation on normal cells. However, complement-mediated phagocytosis and damage to normal cells are important pathogenic mechanisms in many immunologic diseases (see Chapter 18). In these diseases, large amounts of antibodies may be deposited on host cells, generating enough active complement proteins that the regulatory molecules are unable to control complement activation.

Functions of Complement

The principal effector functions of the complement system in innate immunity and specific humoral immunity are to promote phagocytosis of microbes on which complement is activated, to stimulate inflammation, and to induce the lysis of these microbes. In addition, products of complement activation facilitate the activation of B lymphocytes and the production of antibodies. Phagocytosis, inflammation, and stimulation of humoral immunity are all mediated by the binding of proteolytic fragments of complement proteins to various cell surface receptors, whereas cell lysis is mediated by the MAC. In the following section, we describe each of these functions of the complement system and their role in host defense.

Opsonization and Phagocytosis

Microbes on which complement is activated by the alternative or classical pathway become coated with C3b, iC3b, or C4b and are phagocytosed by the binding of these proteins to specific receptors on macrophages and neutrophils (Fig. 12-17A). As discussed previously, activation of complement leads to the generation of C3b and iC3b covalently bound to cell surfaces. Both C3b and iC3b act as opsonins by virtue of the fact that they specifically bind to receptors on neutrophils and macrophages. C3b and C4b (the latter generated by the classical pathway only) bind to CR1, and iC3b binds to CR3 (Mac-1) and CR4. By itself, CR1 is inefficient at inducing the phagocytosis

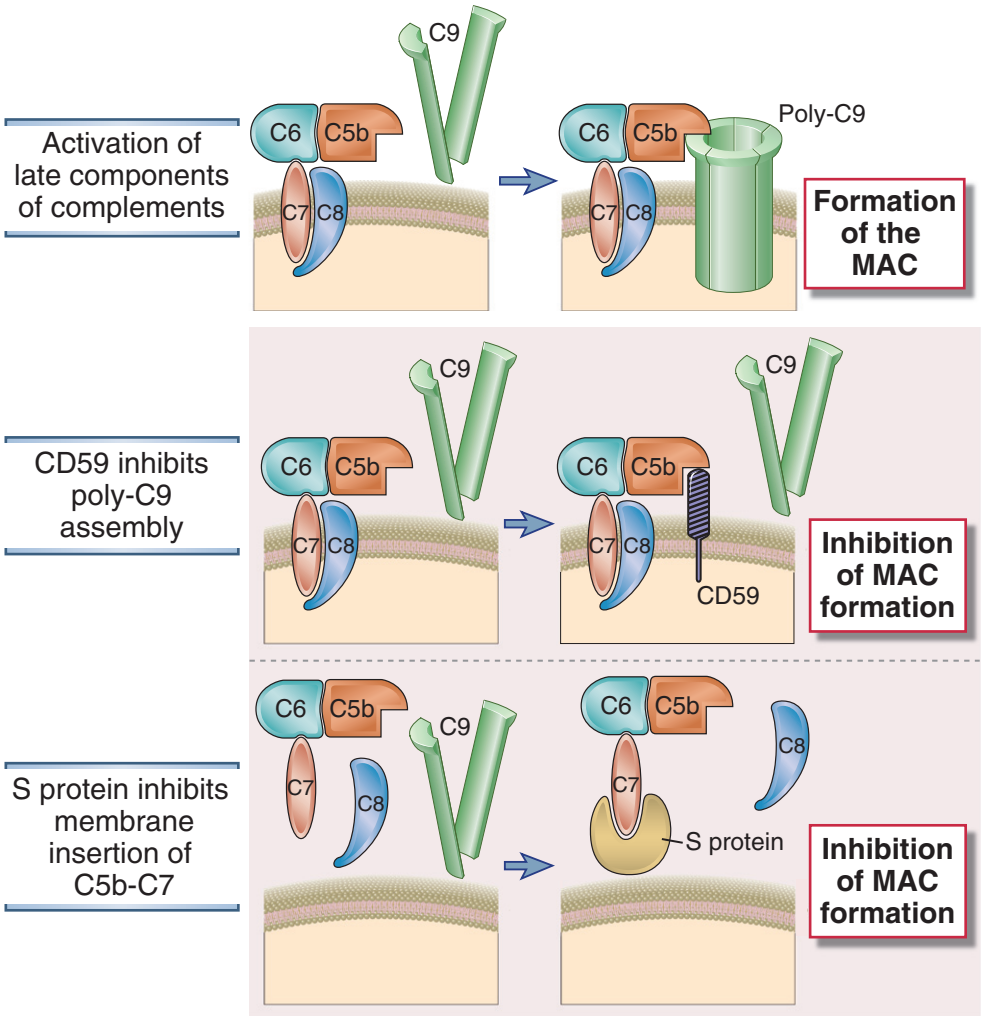


FIGURE 12–16 Regulation of formation of the MAC. The MAC is formed on cell surfaces as an end result of complement activation. The membrane protein CD59 and S protein in the plasma inhibit formation of the MAC.

of C3b-coated microbes, but its ability to do so is enhanced if the microbes are coated with IgG antibodies that simultaneously bind to Fcγ receptors. Macrophage activation by the cytokine IFN-γ also enhances CR1-mediated phagocytosis. C3b- and iC3b-dependent phagocytosis of microorganisms is a major defense mechanism against infections in innate and adaptive immunity. One example of the importance of complement is host defense against bacteria with polysaccharide-rich capsules, such as pneumococci and meningococci, which is mediated primarily by humoral immunity. IgM antibodies against capsular polysaccharides bind to the bacteria, activate the classical pathway of complement, and cause phagocytic clearance of the bacteria in the spleen. In addition, SIGN-R1–expressing marginal zone macrophages may also bind to capsular polysaccharides and activate the classical pathway in the absence of antibody. This is why individuals lacking the spleen (e.g., as a result of surgical removal after traumatic rupture or in patients with autoimmune hemolytic anemia or thrombocytopenia) are susceptible to disseminated pneumococcal and meningococcal septicemia. C3-deficient humans and mice are extremely susceptible to lethal bacterial infections.

Stimulation of Inflammatory Responses

The proteolytic complement fragments C5a, C4a, and C3a induce acute inflammation by activating mast cells and neutrophils (Fig. 12-17B). All three peptides bind to mast cells and induce degranulation, with the release of vasoactive mediators such as histamine. These peptides are also called **anaphylatoxins** because the mast cell reactions they trigger are characteristic of anaphylaxis (see Chapter 19). In neutrophils, C5a stimulates motility, firm adhesion to endothelial cells, and, at high doses, stimulation of the respiratory burst and production of reactive oxygen species. In addition, C5a may act directly on vascular endothelial cells and induce increased vascular permeability and the expression of P-selectin, which promotes neutrophil binding. This combination of C5a actions on mast cells, neutrophils, and endothelial cells contributes to inflammation at sites of complement activation. C5a is the most potent mediator of mast cell degranulation, C3a is about 20-fold less potent, and C4a is about 2500-fold less. The proinflammatory effects of C5a, C4a, and C3a are mediated by binding of the peptides to specific receptors on various cell types. The C5a receptor is the most thoroughly characterized. It is a

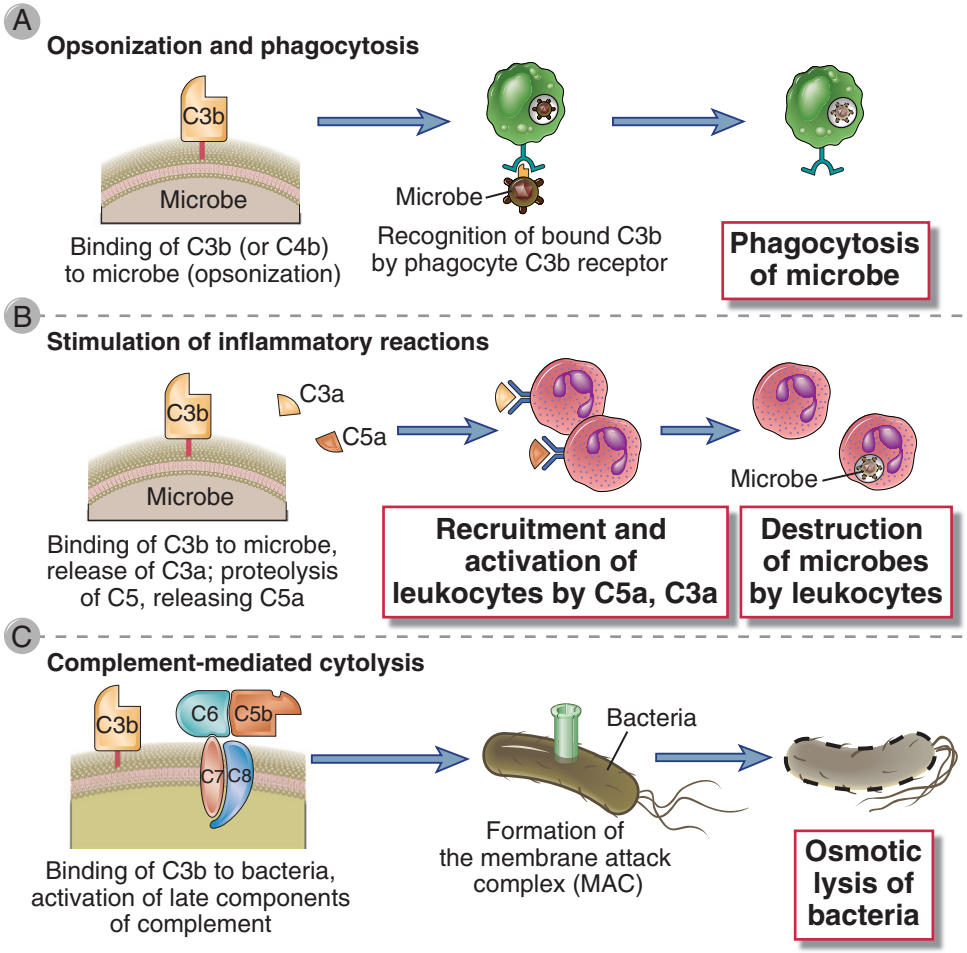


FIGURE 12–17 Functions of complement. The major functions of the complement system in host defense are shown. Cell-bound C3b is an opsonin that promotes phagocytosis of coated cells **(A)**; the proteolytic products C5a, C3a, and (to a lesser extent) C4a stimulate leukocyte recruitment and inflammation **(B)**; and the MAC lyses cells **(C)**.

member of the seven–α-helical transmembrane G protein–coupled receptor family. The C5a receptor is expressed on many cell types, including neutrophils, eosinophils, basophils, monocytes, macrophages, mast cells, endothelial cells, smooth muscle cells, epithelial cells, and astrocytes. The C3a receptor is also a member of the G protein–coupled receptor family.

Complement-Mediated Cytolysis

Complement-mediated lysis of foreign organisms is mediated by the MAC (Fig. 12-17C). Most pathogens have evolved thick cell walls or capsules that impede access of the MAC to their cell membranes. Complement-mediated lysis appears to be critical for defense against only a few pathogens that are unable to resist MAC insertion, such as infections by bacteria of the genus *Neisseria* that have very thin cell walls.

Other Functions of the Complement System

By binding to antigen-antibody complexes, complement proteins promote the solubilization of these complexes and their clearance by phagocytes. Small numbers of immune complexes are frequently formed in the circulation when an individual mounts a vigorous antibody response to a circulating antigen. If the immune complexes accumulate in the blood, they may be deposited in vessel walls and lead to inflammatory reactions that damage the vessels and surrounding tissue. The formation of immune complexes may require not only the multivalent binding of Ig Fab regions to antigens but also noncovalent interactions of Fc regions of juxtaposed Ig molecules. Complement activation on Ig molecules can sterically block these Fc-Fc interactions, thereby promoting dissolution of the immune complexes. In addition, as discussed earlier, immune complexes with attached C3b are bound to CR1 on erythrocytes, and the complexes are cleared by phagocytes in the liver.

The C3d protein generated from C3 binds to CR2 on B cells and facilitates B cell activation and the initiation of humoral immune responses. C3d is generated when complement is activated by an antigen, either directly (e.g., when the antigen is a microbial polysaccharide) or after the binding of antibody. Complement activation results in the covalent attachment of C3b and its cleavage product C3d to the antigen. B lymphocytes can bind the antigen through their Ig receptors and simultaneously bind the attached C3d through CR2, the coreceptor for the B cell antigen receptor, thus enhancing

antigen-induced signaling in B cells (see Chapter 7). Opsonized antigens are also bound by follicular dendritic cells in the germinal centers of lymphoid organs. Follicular dendritic cells display antigens to B cells in the germinal centers, and this process is important for the selection of high-affinity B cells (see Chapter 11, Fig. 11-13). The importance of complement in humoral immune responses is illustrated by the severe impairment in antibody production and germinal center formation seen in knockout mice lacking C3 or C4 or the CR2 protein.

Although our discussion has emphasized the physiologic functions of complement as an effector mechanism of host defense, the complement system is also involved in several pathologic conditions. Some autoimmune diseases are associated with the production of autoantibodies specific for self proteins expressed on cell surfaces (see Chapter 18). Binding of these antibodies results in complement-dependent lysis and phagocytosis of the cells. In other diseases, immune complexes deposit in tissues and induce inflammation by complement-mediated recruitment and activation of leukocytes.

Complement Deficiencies

Genetic deficiencies of complement proteins and regulatory proteins are the causes of various human diseases. Inherited and spontaneous deficiencies in many of the complement proteins have been described in humans.

- Genetic deficiencies in classical pathway components, including C1q, C1r, C4, C2, and C3, have been described; C2 deficiency is the most common human complement deficiency. More than 50% of patients with C2 and C4 deficiencies develop systemic lupus erythematosus. The reason for this association is unknown, but defects in complement activation may lead to failure to clear circulating immune complexes. If normally generated immune complexes are not cleared from the circulation, they may be deposited in blood vessel walls and tissues, where they activate leukocytes by Fc receptor–dependent pathways and produce local inflammation. Complement may also play an important role in the clearance of apoptotic bodies containing fragmented DNA. These apoptotic bodies are likely sources of the nuclear antigens that trigger autoantibody responses in lupus. In addition, complement proteins regulate antigen-mediated signals received by B cells; in their absence, self antigens may not induce B cell tolerance, and autoimmunity results. Somewhat surprisingly, C2 and C4 deficiencies are not usually associated with increased susceptibility to infections, which suggests that the alternative pathway and Fc receptor–mediated effector mechanisms are adequate for host defense against most microbes. Deficiency of C3 is associated with frequent serious pyogenic bacterial infections that may be fatal, illustrating the central role of C3 in opsonization, enhanced phagocytosis, and destruction of these organisms.
- Deficiencies in components of the alternative pathway, including properdin and factor D, result in increased susceptibility to infection with pyogenic bacteria. A mutation of the gene encoding the mannose-binding lectin (MBL) contributes to immunodeficiency in some patients; this is discussed in Chapter 20.
- Deficiencies in the terminal complement components, including C5, C6, C7, C8, and C9, have also been described. Interestingly, as mentioned before, the only consistent clinical problem in these patients is a propensity for disseminated infections by *Neisseria* bacteria, including *Neisseria meningitidis* and *Neisseria gonorrhoeae,* indicating that complement-mediated bacterial lysis is particularly important for defense against these organisms.
- Deficiencies in complement regulatory proteins are associated with abnormal complement activation and a variety of related clinical abnormalities. Deficiencies in C1 inhibitor and decay-accelerating factor are mentioned earlier in the text. In patients with factor I deficiency, plasma C3 is depleted as a result of the unregulated formation of fluid-phase C3 convertase (by the normal tickover mechanism). The clinical consequence is increased infections with pyogenic bacteria. Factor H deficiency is rare and is characterized by excess alternative pathway activation, consumption of C3, and glomerulonephritis caused by inadequate clearance of immune complexes and renal deposition of complement byproducts. An atypical form of the hemolytic-uremic syndrome involves defective complement regulation, and the most common mutations in this condition are in the *factor H* gene. Specific allelic variants of factor H are strongly associated with age-related macular degeneration. The effects of a lack of factor I or factor H are similar to the effects of an autoantibody called C3 nephritic factor (C3NeF), which is specific for alternative pathway C3 convertase (C3bBb). C3NeF stabilizes C3bBb and protects the complex from factor H–mediated dissociation, which results in unregulated consumption of C3. Patients with this antibody often have glomerulonephritis, possibly caused by inadequate clearing of circulating immune complexes.
- Deficiencies in complement receptors include the absence of CR3 and CR4, both resulting from rare mutations in the β chain (CD18) gene common to the CD11CD18 family of integrin molecules. The congenital disease caused by this gene defect is called leukocyte adhesion deficiency (see Chapter 20). This disorder is characterized by recurrent pyogenic infections and is caused by inadequate adherence of neutrophils to endothelium at tissue sites of infection and perhaps by impaired iC3b-dependent phagocytosis of bacteria.

Pathologic Effects of a Normal Complement System

Even when it is properly regulated and appropriately activated, the complement system can cause significant tissue damage. Some of the pathologic effects associated with bacterial infections may be due to complement-mediated acute inflammatory responses to infectious organisms. In some situations, complement activation is associated with intravascular thrombosis and can lead to ischemic injury to tissues. For instance, antiendothelial

antibodies against vascularized organ transplants and the immune complexes produced in autoimmune diseases may bind to vascular endothelium and activate complement, thereby leading to inflammation and generation of the MAC with damage to the endothelial surface, which favors coagulation. There is also evidence that some of the late complement proteins may activate prothrombinases in the circulation that initiate thrombosis independent of MAC-mediated damage to endothelium.

The clearest examples of complement-mediated pathology are immune complex–mediated diseases. Systemic vasculitis and immune complex glomerulonephritis result from the deposition of antigen-antibody complexes in the walls of vessels and kidney glomeruli (see Chapter 18). Complement activated by these deposited immune complexes initiates the acute inflammatory responses that destroy the vessel walls or glomeruli and lead to thrombosis, ischemic damage to tissues, and scarring. Studies with knockout mice lacking the complement proteins C3 or C4 or lacking Fcγ receptors suggest that Fc receptor–mediated leukocyte activation may also cause inflammation and tissue injury as a result of IgG deposition, even in the absence of complement activation.

Evasion of Complement by Microbes

Pathogens have evolved diverse mechanisms for evading the complement system. Some microbes express thick cell walls that prevent the binding of complement proteins, such as the MAC. Gram-positive bacteria and some fungi are examples of microbes that use this relatively nonspecific evasion strategy. A few of the more specific mechanisms employed by a small subset of pathogens will be considered here. These evasion mechanisms may be divided into three groups.

- ***Microbes can evade the complement system by recruiting host complement regulatory proteins.*** Many pathogens, in contrast to non–pathogenic microbes, express sialic acids, which can inhibit the alternative pathway of complement by recruiting factor H, which displaces C3b from Bb. Some pathogens, like schistosomes, *Neisseria gonorrhoeae,* and certain *Haemophilus* species, scavenge sialic acids from the host and enzymatically transfer the sugar to their cell surfaces. Others, including *Escherichia coli K1* and some meningococci, have evolved special biosynthetic routes for sialic acid generation. Some microbes synthesize proteins that can recruit the regulatory protein factor H to the cell surface. GP41 on human immunodeficiency virus (HIV) can bind to factor H, and this property of the virus is believed to contribute to virion protection. Many other pathogens have evolved proteins that facilitate the recruitment of factor H to their cell walls. These include bacteria such as *Streptococcus pyogenes, Borrelia burgdorferi* (the causative agent of Lyme disease), *Neisseria gonorrhoeae, Neisseria meningitidis,* the fungal pathogen *Candida albicans,* and nematodes such as *Echinococcus granulosus*. Other microbes, such as HIV, incorporate multiple host regulatory proteins into their envelopes. For instance, HIV incorporates the GPI-anchored complement regulatory proteins DAF and CD59 when it buds from an infected cell.
- ***A number of pathogens produce specific proteins that mimic human complement regulatory proteins.*** *Escherichia coli* makes a C1q-binding protein (C1qBP) that inhibits the formation of a complex between C1q and C1r and C1s. *Staphylococcus aureus* makes a protein called SCIN (staphylococcal complement inhibitor) that binds to and stably inhibits both the classical and alternative pathway C3 convertases and thus inhibits all three complement pathways. Glycoprotein C-1 of the herpes simplex virus destabilizes the alternative pathway convertase by preventing its C3b component from binding to properdin. GP160, a membrane protein on *Trypanosoma cruzi,* the causative agent of Chagas' disease, binds to C3b and prevents the formation of the C3 convertase and also accelerates its decay. VCP-1 (vaccinia virus complement inhibitory protein 1), a protein made by the vaccinia virus, structurally resembles human C4BP but can bind to both C4b and C3b and accelerate the decay of both C3 and C5 convertases.
- ***Complement-mediated inflammation can also be inhibited by microbial gene products.*** *Staphylococcus aureus* synthesizes a protein called CHIPS (chemokine inhibitory protein of staphylococci), which is an antagonist of the C5a anaphylatoxin.

These examples illustrate how microbes have acquired the ability to evade the complement system, presumably contributing to their pathogenicity.

NEONATAL IMMUNITY

Neonatal mammals are protected from infection by maternally produced antibodies transported across the placenta into the fetal circulation and by antibodies in ingested milk transported across the gut epithelium of newborns by a specialized process known as transcytosis. Neonates lack the ability to mount effective immune responses against microbes, and for several months after birth, their major defense against infection is passive immunity provided by maternal antibodies. Maternal IgG is transported across the placenta, and maternal IgA and IgG in breast milk are ingested by the nursing infant. The transepithelial transport of maternal IgA into breast milk depends on the poly Ig receptor described in Chapter 13. Ingested IgA and IgG can neutralize pathogenic organisms that attempt to colonize the infant's gut, and ingested IgG antibodies are also transported across the gut epithelium into the circulation of the newborn. Thus, a newborn contains essentially the same IgG antibodies as the mother.

Transport of maternal IgG across the placenta and across the neonatal intestinal epithelium is mediated by an IgG-specific Fc receptor called the **neonatal Fc receptor** (FcRn). The FcRn is unique among Fc receptors in that it resembles a class I major histocompatibility complex (MHC) molecule containing a transmembrane heavy chain that is noncovalently associated with β_2-microglobulin. However, the interaction of IgG with

FcRn does not involve the portion of the molecule analogous to the peptide-binding cleft used by class I MHC molecules to display peptides for T cell recognition.

Adults also express the FcRn in the endothelium and in many epithelial tissues. In the post-neonatal period, this receptor functions to protect plasma IgG antibodies from catabolism. This process was described in Chapter 5.

SUMMARY

- Humoral immunity is mediated by antibodies and is the effector arm of the adaptive immune system responsible for defense against extracellular microbes and microbial toxins. The antibodies that provide protection against infection may be produced by long-lived antibody-secreting cells generated by the first exposure to microbial antigen or by reactivation of memory B cells by the antigen.
- The effector functions of antibodies include neutralization of antigens, Fc receptor–dependent phagocytosis of opsonized particles, and activation of the complement system.
- Antibodies block, or neutralize, the infectivity of microbes by binding to the microbes and sterically hindering interactions of the microbes with cellular receptors. Antibodies similarly block the pathologic actions of toxins by preventing binding of the toxins to host cells.
- Antibody-coated (opsonized) particles are phagocytosed by binding of the Fc portions of the antibodies to phagocyte Fc receptors. There are several types of Fc receptors specific for different subclasses of IgG and for IgA and IgE antibodies, and different Fc receptors bind the antibodies with varying affinities. Attachment of antigen-complexed Ig to phagocyte Fc receptors also delivers signals that stimulate the microbicidal activities of phagocytes.
- The complement system consists of serum and membrane proteins that interact in a highly regulated manner to produce biologically active protein products. The three major pathways of complement activation are the alternative pathway, which is activated on microbial surfaces in the absence of antibody; the classical pathway, which is activated by antigen-antibody complexes; and the lectin pathway, initiated by collectins binding to antigens. These pathways generate enzymes that cleave the C3 protein, and cleaved products of C3 become covalently attached to microbial surfaces or antibodies, so subsequent steps of complement activation are limited to these sites. All pathways converge on a common pathway that involves the formation of a membrane pore after the proteolytic cleavage of C5.
- Complement activation is regulated by various plasma and cell membrane proteins that inhibit different steps in the cascades.
- The biologic functions of the complement system include opsonization of organisms and immune complexes by proteolytic fragments of C3, followed by binding to phagocyte receptors for complement fragments and phagocytic clearance, activation of inflammatory cells by proteolytic fragments of complement proteins called anaphylatoxins (C3a, C4a, C5a), cytolysis mediated by MAC formation on cell surfaces, solubilization and clearance of immune complexes, and enhancement of humoral immune responses.
- Protective immunity in neonates is a form of passive immunity provided by maternal antibodies transported across the placenta by a specialized neonatal Fc receptor.

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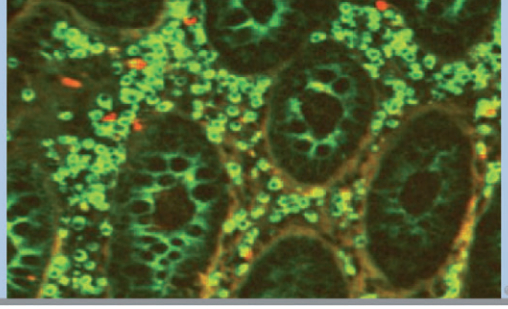
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CHAPTER

13

Regional Immunity: Specialized Immune Responses in Epithelial and Immune Privileged Tissues

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SUMMARY 315

Most of our discussion of innate and adaptive immunity so far in this book has covered features and mechanisms of immune responses in any anatomic location in the mammalian body. However, the immune system has evolved specialized properties in different parts of the body, especially at epithelial surfaces. These features are essential for protection against the types of microbial challenges that are most often encountered at these locations, and they also ensure tolerance to nonpathogenic commensal organisms that live on epithelia and in the lumens of mucosal organs (Table 13-1). The collection of components of the immune system serving specialized functions at a particular anatomic location is called a **regional immune system.** Most of this chapter is devoted to a discussion of these specialized immune systems. We end with a consideration of some tissues that do not normally support immune responses and are said to be immune privileged.

GENERAL FEATURES OF IMMUNITY AT EPITHELIAL BARRIERS

Regional immune systems include the mucosal immune system, which protects the gastrointestinal, bronchopulmonary, and genitourinary mucosal barriers, and the cutaneous (skin) immune system. The gastrointestinal immune system is the largest and most complex. By two simple metrics, including the number of lymphocytes located in the tissue and the amount of antibodies made there, the gastrointestinal system dwarfs all other parts of the immune system combined. The human intestinal mucosa is estimated to contain approximately 50×10^9 lymphocytes (Table 13-2). The dedication of so many immune system resources to the gut reflects the large surface area of the intestinal mucosa, which has evolved to maximize the primary absorptive function of the tissue but must also resist invasion by trillions of bacteria in the lumen. The skin is also a barrier tissue with vast surface area that must be protected from the environmental microbes that have ready access to the external lining. The total number of lymphocytes in the skin is estimated to be about 20×10^9, about twice the total number of circulating lymphocytes (see Table 13-2). The different physical features of the mucosa (soft, wet, and warm) and the skin (tough, dry, and cool) favor colonization and invasion by different types of microbes. Therefore, it is not surprising that the immune system is specialized in different ways in these two types of tissues.

TABLE 13–1 Features of Regional Immunity

Region	Special Challenges	Special Anatomic Features	Specialized Cells or Molecules: Functions
Gastrointestinal tract	Tolerance of food antigens Tolerance to commensal microbiota but responsive to rare pathogens Enormous surface area	Tonsils Peyer's patches, lamina, propria follicles	Intestinal epithelial cells: mucus secretion M cells: luminal antigen sampling Paneth cells: defensin production Secretory IgA, IgM: neutralization of microbes in the lumen DC subsets: luminal antigen sampling; lamina propria antigen sampling; T cell tolerance induction; effector T cell activation; induction of B cell IgA class switching; imprinting gut-homing phenotypes of B and T cells
Respiratory system	Exposure to mix of airborne pathogens and innocuous microbes and particles	Adenoids	Ciliated respiratory epithelial cells: mucus and defensin production and movement of mucus with trapped microbes and particles out of airways Secretory IgA, IgM, IgG: neutralization of microbes outside epithelial barrier
Cutaneous immune system	Large surface area	Keratinizing stratified squamous epithelial barrier	Keratinocyte: keratin production, cytokine and defensin secretion Langerhans cell: epidermal antigen sampling DC subsets: dermal antigen sampling; T cell tolerance induction; effector T cell activation and imprinting skin-homing phenotype

The major regional immune systems share a basic anatomic organization in which there is an outer epithelial barrier that serves essential functions to prevent microbial invasion, underlying connective tissue containing diffusely distributed cells of various types that are important for innate and adaptive immune responses to local microbes, and more distant draining lymph nodes where adaptive immune responses to invading microbes are initiated and amplified. The epithelial barrier may be several layers thick, as in the skin, or a single layer sitting on a basement membrane, as in the intestines. The underlying connective tissue, such as the dermis in the skin or the lamina propria in the gut, contains numerous scattered lymphocytes, dendritic cells (DCs), macrophages, and mast cells that mediate innate immune responses and the effector arm of adaptive immune responses. Mucosal tissues also contain unencapsulated but organized secondary lymphoid tissues just under the epithelial barrier, which include B and T lymphocytes, DCs, and macrophages. These collections of immune cells, often called **mucosa-associated lymphoid tissue (MALT)**, are sites where adaptive immune responses specialized for the particular mucosa are initiated. Adaptive immune responses in regional immune systems are also induced in draining lymph nodes that are located outside the barrier tissues. In skin and mucosal tissues, antigens outside the epithelial barrier are sampled by specialized cells within the epithelium and are delivered to draining lymph nodes or MALT. Remarkably, the effector lymphocytes that are generated in the draining lymph nodes or MALT of a particular regional immune system (e.g., skin, small bowel) will enter the blood and preferentially home back into the subepithelial connective tissue of the same organ (e.g., dermis, lamina propria).

Each regional immune system is defined, in part, by the unique anatomy of the tissues in that region, including secondary lymphoid tissues. For example, the sampling of antigens in the gut and their transport to secondary lymphoid tissues rely on cell types and routes of lymphatic drainage that are fundamentally different from what takes place in the skin or internal organs. Furthermore, the MALT structures in different regions of the gut and in other mucosal organs have distinct features.

Regional immune systems each contain specialized cell types and molecules that may not be abundant in other sites. The cell types that are restricted to one or more regional immune systems but are not present throughout the immune system include subsets of DCs (e.g., Langerhans cells in the skin), antigen transport cells (e.g., M cells in the gut), T lymphocytes (e.g., γδ T cells in epithelia), and subsets of B lymphocytes (e.g., B cells and plasma cells in mucosal tissues that produce IgA). The localization of subsets of lymphocytes to different tissues is in part due to tissue-specific homing mechanisms that direct these subsets from the blood into particular secondary lymphoid organs or peripheral tissues, which we will discuss in detail later in the chapter.

TABLE 13–2 Numbers of Lymphocytes in Different Tissues

Spleen	72×10^9
Bone marrow	50×10^9
Blood	10×10^9
Skin	20×10^9
Gastrointestinal tract	50×10^9

Data from Clark RA, B Chong, N Mirchandani, NK Brinster, K Yamanaka, RK Dowkiert, and TS Kupper. The vast majority of CLA^+ T cells are resident in normal skin. Journal of Immunology 176:4431-4439, 2006; Ganusov AA and De Boer RJ. Do most lymphocytes in humans really reside in the gut? Trends in Immunology 28:514-518, 2007.

Regional immune systems have important regulatory functions that serve to prevent unwanted responses to nonpathogenic microbes and foreign substances that are present at different barriers. The clearest example is the gut-associated immune system, which must suppress responses to commensal bacteria that colonize the intestinal mucosa as well as to foreign food substances but must respond to less frequent pathogenic bacteria. The suppression of immune responses to nonpathogenic organisms and harmless foreign substances is also important in other sites of the body, including the skin, lung, and genitourinary tract, that are not sterile and are constantly exposed to the environment.

With this introduction, we will now discuss the details of these various features in different regional immune systems, beginning with the largest.

IMMUNITY IN THE GASTROINTESTINAL SYSTEM

The gastrointestinal system, like other mucosal tissues, is composed of a tube-like structure lined by a continuous epithelial cell layer sitting on a basement membrane that serves as physical barrier to the external environment. Underlying the epithelium is a layer of loose connective tissue, called the lamina propria in the gut, that contains blood vessels, lymphatic vessels, and mucosa-associated lymphoid tissues (Fig. 13-1). The submucosa is a dense connective tissue layer that connects the mucosa with layers of smooth muscle.

From the perspective of the immunologist, the gastrointestinal tract has two remarkable properties. First, the combined mucosa of the small and large bowel has a total surface area of more than 200 m^2 (the size of a tennis court), made up mostly of small intestinal villi and microvilli. Second, the lumen of the gut is teeming with microbes, many of which are ingested along with food and most of which are continuously growing on the mucosal surface in healthy individuals as commensals. It is estimated that more than 500 different species of bacteria, amounting to approximately 10^{14} cells, live in the mammalian gut. This is 10 times more than the number of all the cells in the body, prompting some microbiologists to point out that we humans are actually only 10% "human" and 90% bacterial! We have evolved to depend on these commensals for several functions, including the degradation of components of our diet that our own cells cannot digest. Although the commensal organisms are beneficial when they are contained on the outside of the gut mucosal barrier, they are potentially lethal if they cross the mucosal barrier and enter the circulation or traverse the bowel wall, especially in immune-compromised individuals. The huge surface area and the high density of mucosal commensals in the gut therefore represent a constant potential danger that must be guarded against. Furthermore, noncommensal pathogenic organisms may become part of the diverse mixture of organisms that make up the gut flora at any time if they are ingested in contaminated food or water. These pathogenic organisms, including bacteria, viruses, protozoa, and helminthic parasites, can cause significant disease, often without invading the epithelial lining and even if they represent a tiny fraction of the microbes in the gut lumen. For health to be maintained, the mucosal immune system must be able to recognize and eliminate these numerically rare pathogens in the presence of overwhelming numbers of nonpathogenic microbes. These challenges have been met by the evolution of a complex set of innate and adaptive immune recognition strategies and effector mechanisms, which we will now describe. Some of these we understand well, and others remain incompletely characterized. Many of the features of the gastrointestinal immune system are shared by other mucosal tissues, and we will point out these common features of mucosal immunity. Unfortunately, intestinal infections by pathogenic organisms are frequently not controlled by mucosal immunity and account for millions of deaths each year throughout the world.

Innate Immunity in the Gastrointestinal Tract

Intestinal epithelial cells lining the small and large bowel are an integral part of the gastrointestinal innate immune system, involved in responses to pathogens, tolerance to commensal organisms, and antigen sampling for delivery to the adaptive immune system in the gut. There are several different types of intestinal epithelial cells, all derived from a common precursor found in the crypts of intestinal glands. Among these are the mucus-secreting goblet cells, which reside at the top of the intestinal villi; cytokine-secreting absorptive epithelial cells; antigen-sampling M cells, found in specialized dome structures overlying lymphoid tissues; and antibacterial peptide-secreting Paneth cells, found at the bottom of the crypts. All these cell types contribute in different ways to the barrier function of the mucosa, as we will discuss later.

Innate immune protection in the gut is mediated in part by the nonspecific physical and chemical barrier provided by the mucosal epithelial cells and their mucus secretions. Adjacent intestinal epithelial cells are held together by proteins that form tight junctions, including zonula occludens 1 and claudins, and these block the movement of bacteria and pathogen-associated molecular patterns (PAMPs) between the cells into the lamina propria. In addition, mucosal epithelial cells produce antimicrobial substances, and several cell types located in the mucosa, including epithelial cells, DCs, and macrophages, are capable of mounting inflammatory and antiviral responses. Most of these responses are induced by pattern recognition receptor engagement of PAMPs, which we discussed in Chapter 4. Interestingly, some innate immune receptors that promote inflammation in other parts of the body have anti-inflammatory actions in the gut. In this section, we will describe features of innate immunity that are unique to the intestines.

Several different extensively glycosylated proteins, called mucins, form a viscous physical barrier that prevents microbes from contacting the cells of the gastrointestinal tract. Mucins contain many different O-linked oligosaccharides and include secreted and cell surface glycoproteins. The secreted mucins, including MUC2, MUC5, and MUC6, form a hydrated gel ranging from 300 to 700 μm in thickness that can prevent microbial contact

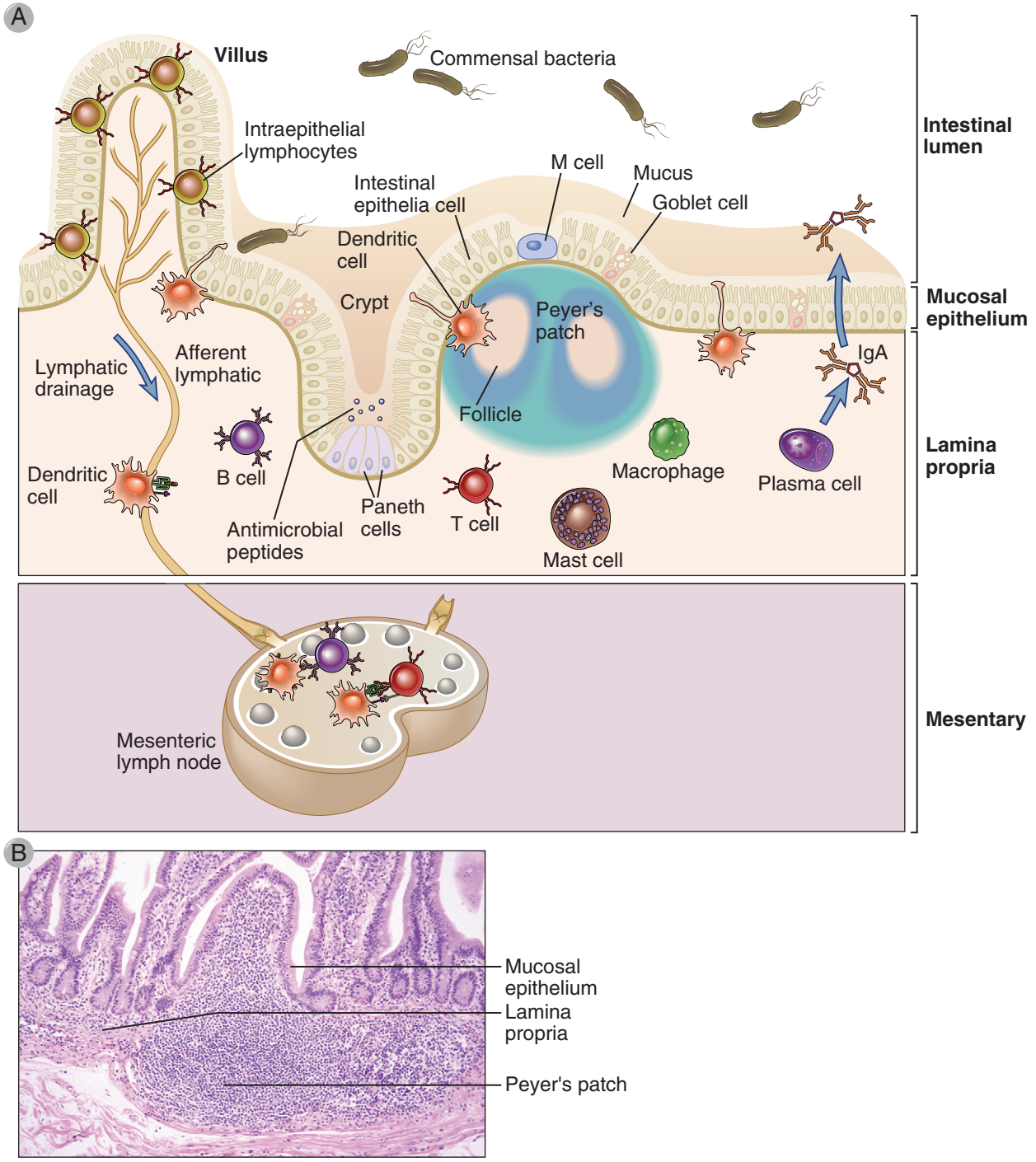


FIGURE 13–1 The gastrointestinal immune system. A, Schematic diagram of the cellular components of the mucosal immune system in the intestine. **B,** Photomicrograph of mucosal lymphoid tissue in the human intestine. Similar aggregates of lymphoid tissue are found throughout the gastrointestinal tract.

with the epithelial lining cells and also serves as a matrix for display of antimicrobial substances produced by the epithelial cells. Some mucins act as decoy molecules, which can be shed from the epithelial cells and bind to the adhesin proteins that pathogenic bacteria use to attach to host cell membranes. In addition to the secreted mucus, the apical surface of gastrointestinal epithelial cells is coated with membrane-bound mucin proteins, including MUC1, MUC3A/b, MUC12, MUC13, MUC17. These membrane-bound mucins combine with various glycolipids to form a dense macromolecular layer at the epithelial cell surface called the glycocalyx, which ranges from 30 to 500 nm in thickness in different locations in the gut. The glycocalyx, like the secreted mucus, serves as a physical barrier to prevent microbial contact.

A remarkable property of the mucous barrier of the intestine is its rapid turnover and responsiveness to various environmental and immune signals, which

allows rapid increases in mucosal barrier function. Mucins are constitutively produced both by the surface epithelial cells in the gastrointestinal tracts and by submucosal glands and are replaced by newly synthesized molecules every 6 to 12 hours. Several different environmental and immune stimuli can induce dramatic increases in mucin production. These stimuli include cytokines (IL-1, IL-4, IL-6, IL-9, IL-13, tumor necrosis factor [TNF], and type I interferons), neutrophil products (such as elastase), and microbial adhesive proteins. These stimuli not only increase mucin gene expression but also alter the glycosylation of the mucins because of induced changes in the expression of glycosyltransferase enzymes. The changes in quantity and glycosylation of mucins are thought to increase barrier function against pathogens.

Defensins produced by intestinal epithelial cells provide innate immune protection against luminal bacteria, and defects in their production are associated with bacterial invasion and inflammatory bowel disease. Defensins are peptides produced by various cell types in the body that exert lethal toxic effects on microbes by inserting into and causing loss of integrity of their outer phospholipid membranes (see Chapter 4). In the small bowel, the major defensins are the α-defensins, including human defensin 5 (HD5) and HD6, produced constitutively as inactive precursor proteins by Paneth cells located at the base of crypts between microvilli. Active HD5 and HD6 peptides are generated by proteolytic cleavage mediated by trypsin, also produced by Paneth cells. In the colon, β-defensins are produced by absorptive epithelial cells in the intestinal crypts, some constitutively and others in response to IL-1 or invasive bacteria. In addition, neutrophil granules are rich in α-defensins, which likely contributes to their antimicrobial functions in the setting of infections of the bowel wall. Several studies have identified defects in defensin production by epithelial cells in affected regions of bowel in Crohn's disease, a chronic inflammatory disease that can involve the entire gastrointestinal tract. Because there is a significant inheritable risk for development of Crohn's disease, it is possible that a genetically determined defect in production of defensins may be a predisposing factor for the disease, and reduced expression of defensin genes has been associated with a subset of Crohn's disease.

Toll-like receptors (TLRs) and cytoplasmic Nod-like receptors (NLRs) expressed by intestinal epithelial cells promote immune responses to invasive pathogens but are also regulated to limit inflammatory responses to commensal bacteria. In Chapter 4, we defined TLRs and NLRs as cellular receptors that recognize PAMPs produced by microbes and generate signals that promote inflammatory and antiviral responses by the cells. Most luminal bacteria of the gut are nonpathogenic if they are retained outside the epithelial barrier, yet they may express the same array of PAMPs that pathogenic bacteria express, such as lipopolysaccharide, peptidoglycans, CpG DNA, and flagellin. Because inflammatory responses that involve the intestinal epithelial cells can impair barrier function and can lead to bacterial invasion and pathologic inflammation, it is not surprising that stringent control mechanisms have evolved to limit TLR-induced proinflammatory responses to commensal bacteria. Intestinal epithelial cells express a wide range of TLRs, including TLRs 2, 4, 5, 6, 7, and 9, with different receptors expressed in different regions of the gut. Ligation of some TLRs results in the phosphorylation and reorganization of zona occludens 1 and increased strength of the tight junctions between epithelial cells, and TLR signaling also increases intestinal epithelial motility and proliferation. These functional responses to TLR signaling increase barrier function but not inflammation. TLR responses in the gut appear also to be regulated by levels of expression or compartmentalized expression in only certain sites (Fig. 13-2). For example, TLR5, which recognizes bacterial flagellins, is exclusively expressed on the basolateral surface of intestinal epithelial cells, where it will be accessible only to bacteria that have invaded through the barrier. Similarly, NLR family receptors for flagellins (e.g., NAIP and IPAF-1) are expressed in the cytoplasm of intestinal epithelial cells and will activate inflammatory responses only when pathogenic bacteria or their products gain access to the cytosol. There is also evidence that regulators of TLR signaling inside intestinal epithelial cells maintain a higher threshold for activation of inflammatory responses compared with epithelial cells and DCs in other tissues (see Fig. 13-2).

In healthy individuals, lamina propria DCs and macrophages in the gut inhibit inflammation and serve to maintain homeostasis. Overall, intestinal macrophages have a unique phenotype that enables them to phagocytose and kill microbes but at the same time to secrete anti-inflammatory cytokines, such as IL-10. This phenotype is apparently induced in the local mucosal environment by transforming growth factor-β (TGF-β). TLR4 expression on both macrophages and DCs in the lamina propria is lower than in other tissues, and inflammatory gene expression in these cells is often inhibited by microbial products. This may be an evolved mechanism to prevent damaging inflammation in response to commensal bacteria and bacterial products that may traverse the epithelial barrier.

Adaptive Immunity in the Gastrointestinal Tract

The adaptive immune system in the gastrointestinal tract has features that are distinct from adaptive immune functions in other organ systems.

- The major form of adaptive immunity in the gut is humoral immunity directed at microbes in the lumen, which prevents commensals and pathogens from colonizing and invading through the mucosal epithelial barrier. This function is mediated by dimeric IgA antibodies that are secreted into the lumen of the gut or, in the case of breast-feeding infants, IgA that is secreted into colostrum and mother's milk and ingested by the infant. Significant quantities of IgG and IgM antibodies are also present in the gut lumen and contribute to humoral immunity in this location.
- The dominant protective cell-mediated immune response consists of T_H17 effector cells.
- The adaptive immune system in the gut must continuously suppress potential immune responses to food antigens and commensal microbial antigens to prevent

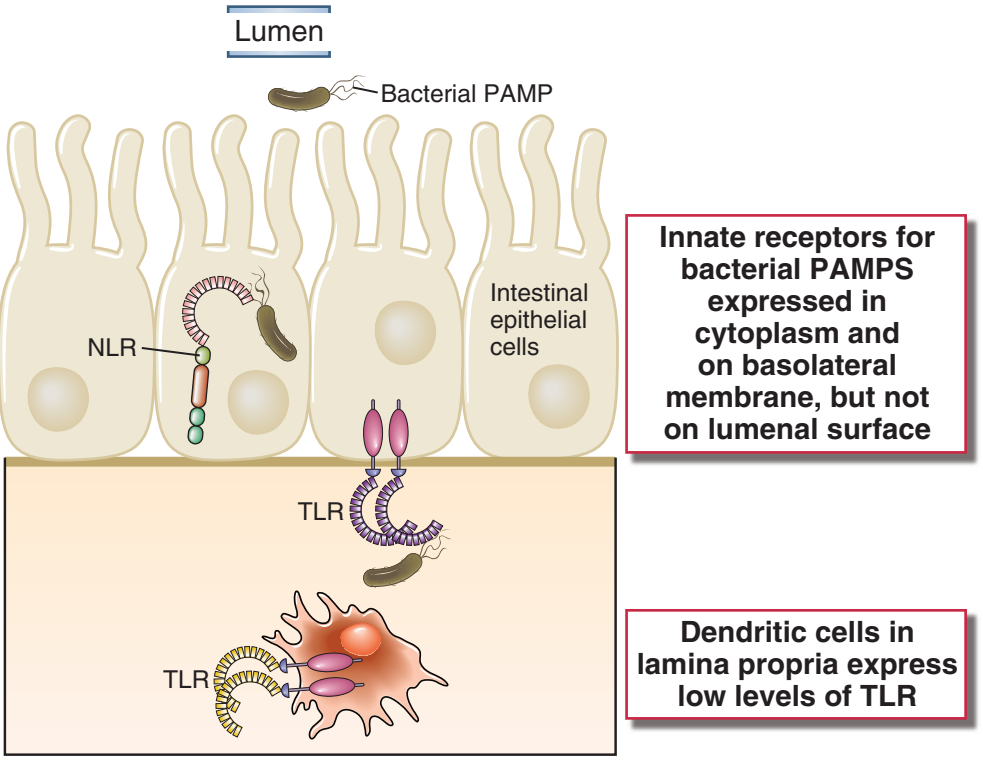


FIGURE 13–2 Mechanism of regulation of innate immune responses in the intestinal mucosa. Pattern recognition receptor expression and function in intestinal epithelial cells and lamina propria DCs minimize inflammatory responses to commensal bacteria in the lumen but promote responses to microbes that traverse the barrier and enter the lamina propria. *Top,* Pattern recognition receptors that recognize bacterial flagellin are compartmentalized in the cytosol (NLR) or basal membrane (TLR5) of intestinal epithelial cells but not on the apical/lumen membrane. *Bottom,*TLR4, which recognizes bacterial lipopolysaccharides, is expressed at low levels on intestinal epithelial cells and lamina propria DCs. TLR signaling does not induce inflammatory gene expression in lamina propria DCs because of more dominant effect of intracellular regulators of TLR signal transduction, such as TOLLIP and IRAK-M, compared with conventional DCs in other tissues.

inflammatory reactions that would compromise the mucosal barrier. Nowhere else in the body is there such an extensive commitment of the immune system to maintaining tolerance to foreign antigens. A major mechanism for controlling responses in the gut is the activation of regulatory T cells (Treg), and some subsets of Treg are more abundant in mucosa-associated lymphoid tissues (MALT) than in other lymphoid organs.

We will now discuss the special features of adaptive immunity in the gastrointestinal system, including anatomic organization, antigen sampling, lymphocyte homing and differentiation, and antibody delivery to the lumen.

The Functional Anatomy of the Adaptive Immune System in the Gastrointestinal Tract

In this section, we will discuss the anatomic organization of cells within the intestines and the relationship of this organization to how adaptive immune responses are initiated, carried out, and regulated. In general terms, the functional anatomy of the adaptive immune system in the gut has evolved to effectively deal with the conditions we emphasized earlier of abundant commensal microbes and rare pathogens just outside an epithelial barrier of enormous surface area.

Adaptive immune responses in the gut are initiated in discretely organized collections of lymphocytes and antigen-presenting cells closely associated with the mucosal epithelial lining of the bowel and in mesenteric lymph nodes (see Fig. 13-1). Naive lymphocytes are exposed to antigens in these sites and differentiate into effector cells. These gut-associated lymphoid tissues adjacent to the mucosal epithelium are sometimes referred to as GALT, which is the gastrointestinal version of MALT, although the terms are often used interchangeably. Up to 30% of the lymphocytes in the body are found in the GALT. The most prominent GALT structures are Peyer's patches, found mainly in the distal ileum, and smaller aggregates of lymphoid follicles or isolated follicles in the appendix and colon. Peyer's patches have the structure of lymphoid follicles, with germinal centers containing B lymphocytes, follicular helper T cells, follicular dendritic cells (FDCs), and macrophages. The germinal centers in the follicles are surrounded by IgM- and IgD-expressing naive follicular B cells. A region called the dome is located between the follicles and the overlying epithelium and contains B and T lymphocytes, DCs, and macrophages. Between the follicles are T cell–rich parafollicular areas, similar to lymph nodes, but overall, the ratio of B cells to T cells in GALT is about five times higher than in lymph nodes. Also in distinction from lymph nodes, GALT structures are not encapsulated, and there are routes of antigen delivery to these structures that are independent of lymphatics. Development of both aggregates of follicles, such as Peyer's patches, and isolated

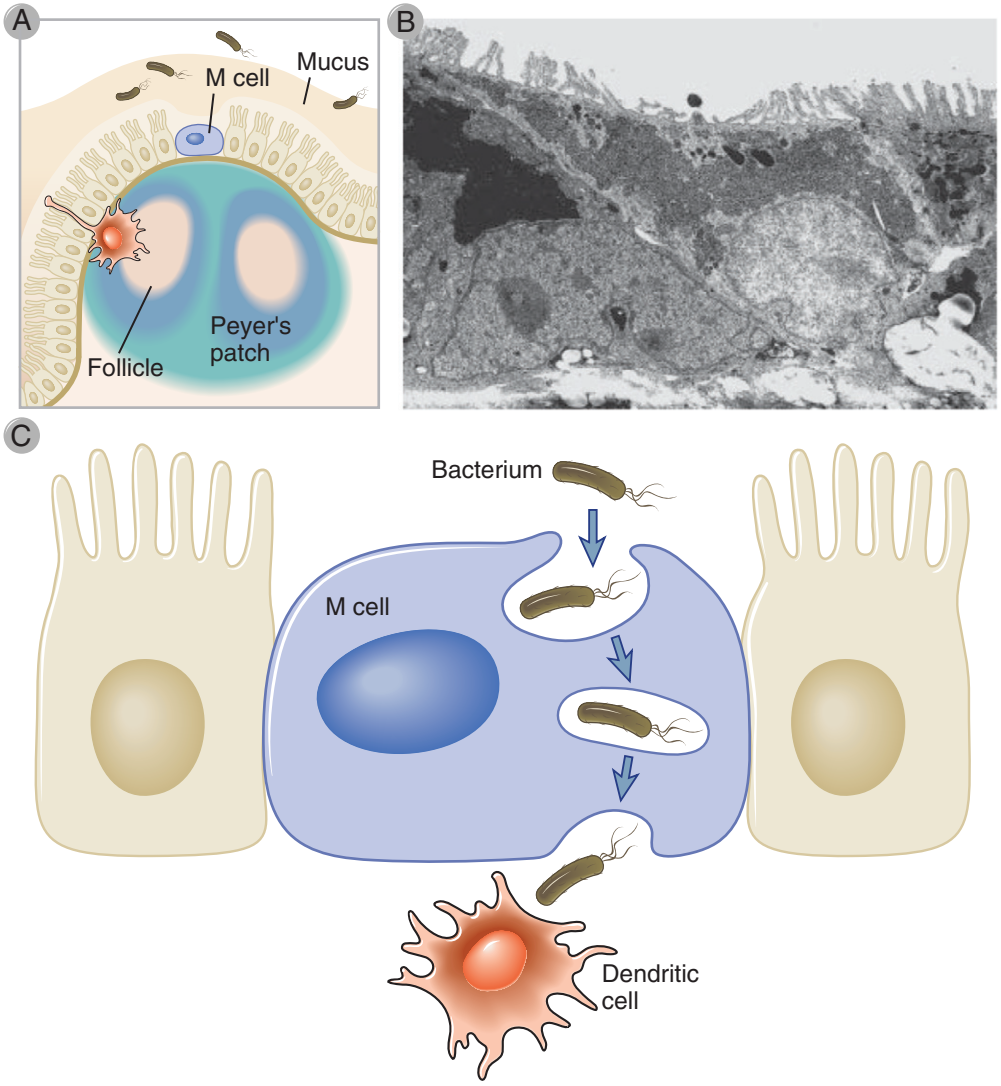


FIGURE 13–3 M cells in the small intestine. M cells are specialized intestinal epithelial cells found in the small bowel epithelium overlying Peyer's patches and lamina propria lymphoid follicles **(A)**. Unlike neighboring epithelial cells with tall microvillous borders and primary absorptive functions, M cells have shorter villi **(B)** and engage in transport of intact microbes or molecules across the mucosal barrier into gut-associated lymphoid tissues, where they are handed off to DCs **(C)**. *(Electron micrograph from Corr SC, CC Gahan, and C Hill. M-cells: origin, morphology and role in mucosal immunity and microbial pathogenesis. FEMS Immunology and Medical Microbiology 52:2-12, 2008.)*

follicles in the gut lamina propria requires lymphoid tissue inducer cells, which express the RORγT transcription factor and produce the cytokine lymphotoxin-β (LTβ).

A major pathway of antigen delivery from the lumen to the GALT is through specialized cells within the gut epithelium called microfold (M) cells (Fig. 13-3). M cells are located in regions of the gut epithelium called follicle-associated or dome epithelium that overlie the domes of Peyer's patches and other GALT structures. Although M cells and the more numerous epithelial cells with absorptive function likely arise from a common epithelial precursor, the M cells are distinguishable by a thin glycocalyx, their relatively short, irregular microvilli (referred to as microfolds), and large fenestrations in their membranes, all features that enhance the uptake of antigens from the gut lumen. The main function of M cells is transcellular transport of various substances from the lumen of the intestine across the epithelial barrier to underlying antigen-presenting cells. M cells take up luminal contents efficiently and in various ways, including phagocytosis in a manner similar to macrophages, and either clathrin-coated vesicular or fluid-phase endocytosis. These pathways enable uptake of whole bacteria, viruses, and soluble microbial products. Unlike macrophages or DCs, M cells do not engage in extensive processing of the substances they take up, but rather they move the particles and molecules through endocytic vesicles across the cytosol and deliver them by exocytosis at the basolateral membrane to DCs in the dome regions of underlying GALT structures. Although M cells play an important role in protective immunity to luminal microbes, some microbes have evolved to take advantage of M cells as a route of invasion through the mucosal barrier. The best described example of this is *Salmonella typhimurium*, similar to the human pathogen *S. typhi* that causes

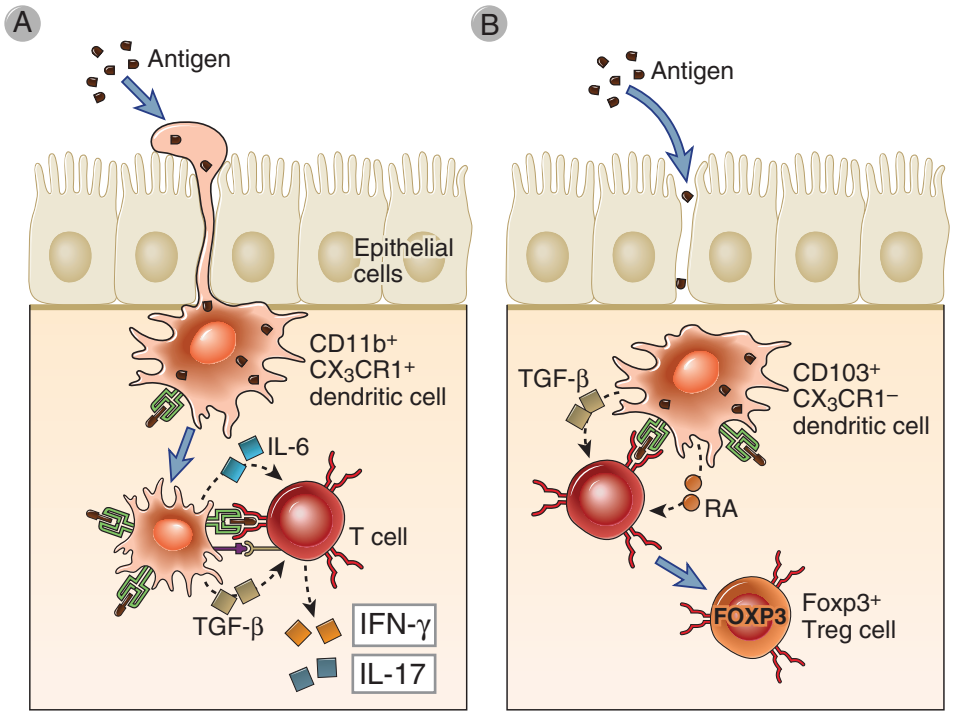


FIGURE 13–4 DCs in the intestinal mucosa. There are several different subsets of DCs constitutively present in the intestinal mucosa that are defined by cell surface molecules and function. Two such subsets are shown that are also present in other mucosal tissues. **A,** Antigen-sampling DCs extend dendritic processes between intestinal epithelial cells into the lumen to sample antigens and then migrate to mesenteric lymph nodes, where they initiate activation and differentiation of proinflammatory effector T cells. These DCs express the CD11b integrin chain and the CX_3CR1 chemokine receptor. **B,** Other DCs present in the lamina propria, which express the integrin CD103, present antigens to naive T cells and induce their differentiation of regulatory T cells, in part by secreting TGF-β and retinoic acid (RA). The regulatory function of these DCs depends on factors secreted by intestinal epithelial cells.

typhoid fever. M cells express specific lectins that allow these bacteria to specifically bind and be internalized. The bacteria are cytotoxic to the M cells, leading to gaps in the epithelium that promote invasion of more organisms. M cell lectins may also promote infection by certain enteric viruses.

Microbial antigens in the gut lumen can be sampled by lamina propria DCs that extend cytoplasmic processes between the intestinal epithelial cells and by Fc receptor–dependent uptake of IgG opsonized antigens by the epithelial cells (Fig. 13-4). Antigen-sampling DCs are numerous in certain regions of the intestine, especially the terminal ileum, where they extend dendrites through the junctions between adjacent epithelial cells, apparently without disrupting the tight junctions. These antigen-sampling DCs belong to a subset of mucosal DCs that promote effector T cell responses, which we will discuss later in the chapter. Unlike M cells, these DCs are capable of processing and presenting protein antigens to T cells within the GALT. Antigens in the lumen that have been opsonized by antibodies can be delivered to the GALT by Fc receptor–mediated pathways. There is evidence from mouse studies that IgG-opsonized antigens, such as bacterial flagellin, can be transported across the gut epithelium through the neonatal Fcγ receptor (FcRn, see Chapter 12) and passed on to DCs in the GALT, leading to T cell responses to the antigens.

Mesenteric lymph nodes collect lymph-borne antigens from the small and large bowel and are sites of differentiation of effector and regulatory lymphocytes that home back to the lamina propria. There are 100 to 150 of these lymph nodes located between the membranous layers of the mesentery. Mesenteric lymph nodes serve some of the same functions as GALT, including differentiation of B cells into dimeric IgA–secreting plasma cells and the development of effector T cells as well as regulatory T cells. The cells that differentiate in the mesenteric lymph nodes in response to bowel wall invasion by pathogens or commensals often home to the lamina propria. We will discuss imprinting of homing properties of lymphocytes activated in mesenteric lymph nodes later.

Lingual and palatine tonsils are nonencapsulated lymphoid structures located beneath stratified squamous epithelial mucosa in the base of the tongue and oropharynx, respectively, and are sites of immune responses to microbes in the oral cavity. These tonsils, together with nasopharyngeal tonsils, form a ring of lymphoid tissues called Waldeyer's ring. The bulk of the tonsillar tissue is composed of lymphoid follicles, usually with prominent germinal centers. There are multiple narrow and deep invaginations of the surface squamous epithelium, called crypts, that grow into the follicular tissue. Although these tonsils are often considered part of the GALT, they are distinct in that they are separated from the microbe-rich oral cavity by multiple layers of squamous epithelial cells rather than the single epithelial cell layer of the gut. The mechanism of antigen sampling from oral cavity microbes

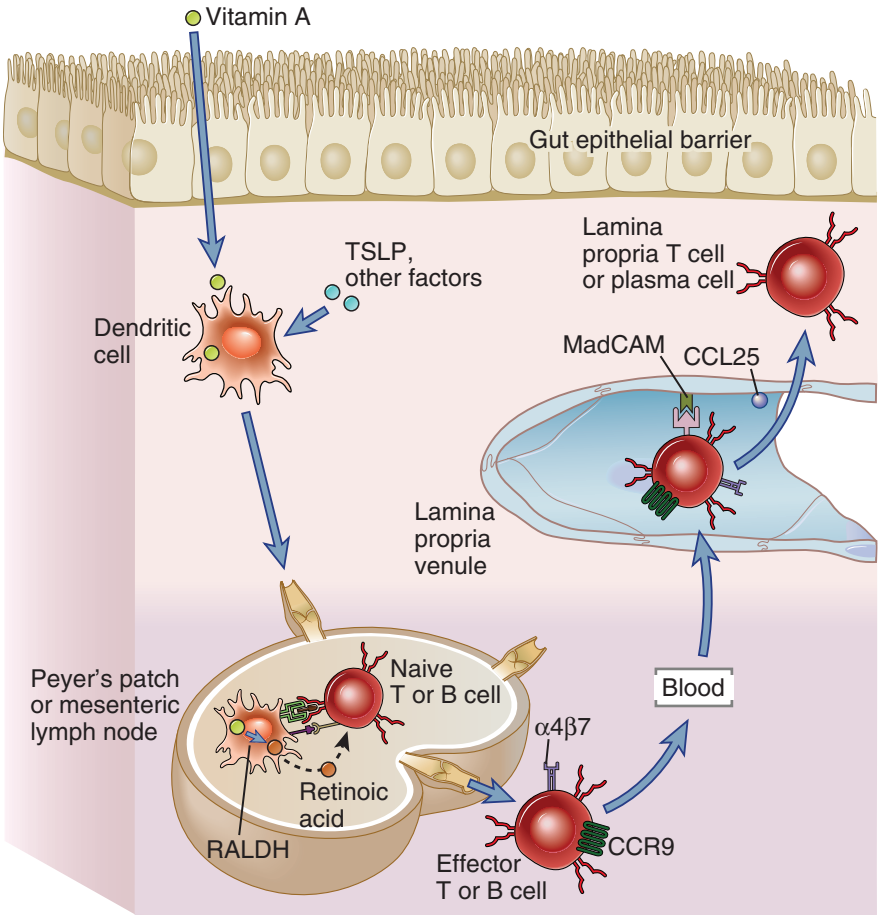


FIGURE 13–5 Homing properties of intestinal lymphocytes. The gut-homing properties of effector lymphocytes are imprinted in the lymphoid tissues where they have undergone differentiation from naive precursors. DCs in gut-associated lymphoid tissues, including Peyer's patches and mesenteric lymph nodes, are induced by thymic stromal lymphopoietin (TSLP) and other factors to express retinaldehyde dehydrogenase (RALDH), which converts dietary vitamin A into retinoic acid. When naive B or T cells are activated by antigen in GALT, they are exposed to retinoic acid produced by the DCs, and this induces the expression of the chemokine receptor CCR9 and the integrin $\alpha_4\beta_7$ on the plasma cells and effector T cells that arise from the naive lymphocytes. The effector lymphocytes enter the circulation and home back into the gut lamina propria because the chemokine CCL25 (the ligand for CCR9) and the adhesion molecule MadCAM (the ligand for $\alpha_4\beta_7$) are displayed on lamina propria venular endothelial cells.

is not well described; the crypts are possible sites where this may happen. Nonetheless, the lingual and palatine tonsils respond to infections of the epithelial mucosa by significant enlargement and vigorous, mainly IgA, antibody responses. Typical infections that are associated with tonsillar enlargement, usually in children, are caused by streptococci and the Epstein-Barr virus.

Effector lymphocytes that are generated in the GALT and mesenteric lymph nodes are imprinted with selective integrin- and chemokine receptor–dependent gut-homing properties, and they circulate from the blood back into the lamina propria of the gut (Fig. 13-5). Both IgA-secreting B cells and effector T cells acquire the gut-homing phenotype. Thus, the effector arm of the gastrointestinal immune system depends on a large number of antibody-secreting cells and T cells that recirculate back into the lamina propria and respond rapidly to pathogens. The major integrin on gut-homing B and T lymphocytes is $\alpha_4\beta_7$, which binds to the MadCAM-1 protein expressed on postcapillary venular endothelial cells in the gut lamina propria. Gut homing also requires the chemokine receptor CCR9 on the B and T lymphocytes and its chemokine ligand CCL25, which is produced by intestinal epithelial cells. The combined expression of MadCAM-1 and CCL25 is restricted to the gut. Homing of IgA-producing cells to the colon also requires CCR10 expression and the chemokine CCL28, but this is not a gut-specific pathway because CCL28 is expressed by epithelial cells in other mucosal tissues, such as the lung and genitourinary tract. Blocking monoclonal antibodies specific for the α_4 chain of $\alpha_4\beta_7$ have been used to treat patients with inflammatory bowel disease on the basis of the knowledge that effector T cells use this integrin to enter gut tissues in this disease. (We will discuss inflammatory bowel disease later in the chapter.)

The gut-homing phenotype of IgA-producing cells and effector T cells is imprinted by DCs and the action of retinoic acid during the process of T cell activation (see Fig. 13-5). In addition to promoting naive T cell differentiation into effector T cells and naive B cell differentiation into IgA antibody–secreting cells, discussed later in the chapter, DCs in GALT and mesenteric lymph nodes also provide signals that lead to the expression of $\alpha_4\beta_7$ and CCR9 on these effector cells. The induction of these homing molecules depends on secretion of retinoic acid by the DCs, although the mechanisms are not well understood. The selective induction of gut-homing cells in the gut lymphoid tissues is explained by the fact that gut lymphoid tissues are exposed to dietary vitamin A, and DCs in GALT and mesenteric lymph nodes express retinal dehydrogenases (RALDH), the enzyme needed for retinoic acid synthesis from vitamin A, whereas DCs in other tissues do not. In addition, intestinal epithelial cells also express RALDH and can synthesize retinoic acid. Consistent with these properties of the intestinal humoral immune system, it is known that oral vaccination not

only favors the expansion of IgA-producing B cells, compared with intradermal immunization, but that oral vaccines also induce higher levels of $\alpha_4\beta_7$ on B cells.

The lamina propria contains diffusely distributed effector lymphocytes, DCs, and macrophages and is the site of the effector phase of gastrointestinal adaptive immune responses. As discussed before, effector lymphocytes generated in Peyer's patches, other GALT structures, and mesenteric lymph nodes home back into the lamina propria. In this location, T cells can respond to invading pathogens, and B cells can secrete antibodies that are transported into the lumen and neutralize pathogens before they invade.

Humoral Immunity in the Gastrointestinal Tract

Humoral immunity in the gut is dominated by production of secretory IgA in the GALT and transport of the antibody across the mucosal epithelium into the lumen. Smaller but significant quantities of IgG and IgM are also secreted into the gut lumen. Within the lumen, IgA, IgG, and IgM antibodies bind to microbes and toxins and neutralize them by preventing their binding to receptors on host cells. This form of humoral immunity is sometimes called **secretory immunity** and has evolved to be particularly prominent in mammals. Antibody responses to antigens encountered by ingestion are typically dominated by IgA, and secretory immunity is the mechanism of protection induced by oral vaccines such as the polio vaccine. Several unique properties of the gut environment result in selective development of IgA-secreting cells that either stay in the gastrointestinal tract or, if they enter the circulation, home back to the lamina propria of the intestines. The result is that IgA-secreting cells efficiently accumulate next to the epithelium that will take up the secreted IgA and transport it into the lumen.

IgA is produced in larger amounts than any other antibody isotype. It is estimated that a normal 70-kg adult secretes about 2 g of IgA per day, which accounts for 60% to 70% of the total production of antibodies. This tremendous output of IgA is because of the large number of IgA-producing plasma cells in the GALT, which by some estimates amount to about 10^{10} cells per meter of bowel (Fig. 13-6). Because IgA synthesis occurs mainly in mucosal lymphoid tissue and transport into the mucosal lumen is efficient, this isotype constitutes less than one quarter of the antibody in plasma and is a minor component of systemic humoral immunity compared with IgG and IgM.

The dominance of IgA production by intestinal plasma cells is due in part to selective induction of IgA isotype switching in B cells in GALT and mesenteric lymph nodes. IgA class switching in the gut can occur by T-dependent and T-independent mechanisms (Fig. 13-7). In both cases, the molecules that drive IgA switching include both soluble cytokines and membrane proteins on other cell types that bind to signaling receptors on B cells (see Chapter 11). TGF-β is required for IgA isotype switching in the gut as well as in other mucosal compartments, and this cytokine is produced by intestinal epithelial cells and DCs in GALT. Furthermore, GALT DCs express the $\alpha_v\beta_8$ integrin, which is required for activation of TGF-β. Several molecules that promote IgA class switching are expressed by intestinal epithelial cells or GALT DCs in response to TLR signaling, and the commensal bacteria in the gut lumen produce the ligands that bind to the relevant TLRs. For example, T-independent IgA and IgG switching requires binding of the TNF family cytokine APRIL to the TACI receptor on B cells, and intestinal epithelial cells produce APRIL in response to TLR ligands made by commensal bacteria. Intestinal epithelial cells also produce thymic stromal lymphopoietin (TSLP) in response to TLR signals, and TSLP stimulates additional APRIL production by GALT DCs. TLR ligands made by commensal bacteria in the gut also increase expression of inducible nitric oxide synthase in DCs, leading to nitric oxide production. Nitric oxide is thought to promote both T-dependent and T-independent IgA class switching, in part because nitric oxide enhances TGF-β signaling in B cells and also synthesis of APRIL by GALT DCs. Finally, intestinal B cell IgA production is at least partly dependent on the vitamin A metabolite all-*trans* retinoic acid, which is made by intestinal epithelial cells and GALT DCs, although the mechanisms by which retinoic acid promotes IgA production are not known. Retinoic acid is also important in B cell homing to the gut, as we discussed earlier. There is an abundance of many of these molecules within the GALT and mesenteric lymph nodes compared with nonmucosal lymphoid tissues such as spleen and skin-draining lymph nodes, largely accounting for the propensity of B cells in the GALT to switch to IgA production.

The dominance of IgA production by intestinal plasma cells is enhanced by selective gut-homing properties of

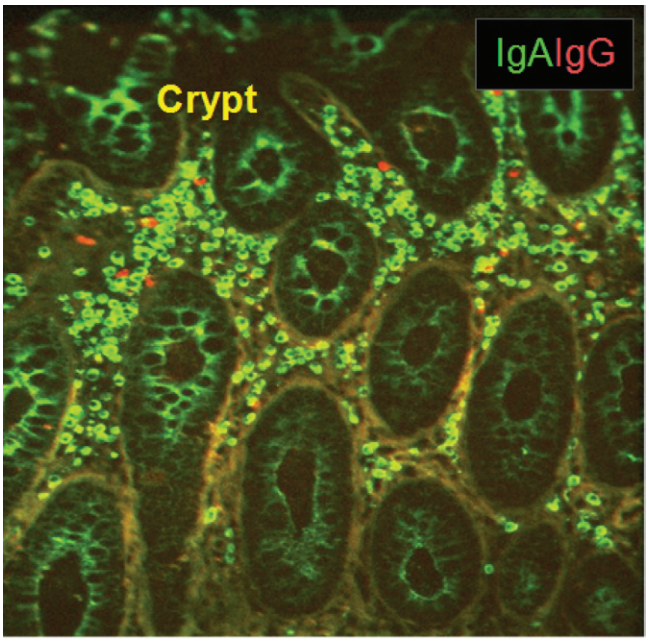


FIGURE 13–6 IgA-secreting plasma cells in the intestine. The abundance of IgA-producing plasma cells (green) in colon mucosa compared with IgG-secreting cells (red) is shown by immunofluorescence staining. IgA that is being secreted can be seen as green cytoplasm in the crypt epithelial cells. *(From Brandtzaeg P. The mucosal immune system and its integration with the mammary glands. The Journal of Pediatrics 156[Suppl 1]:S8-S16, 2010.)*

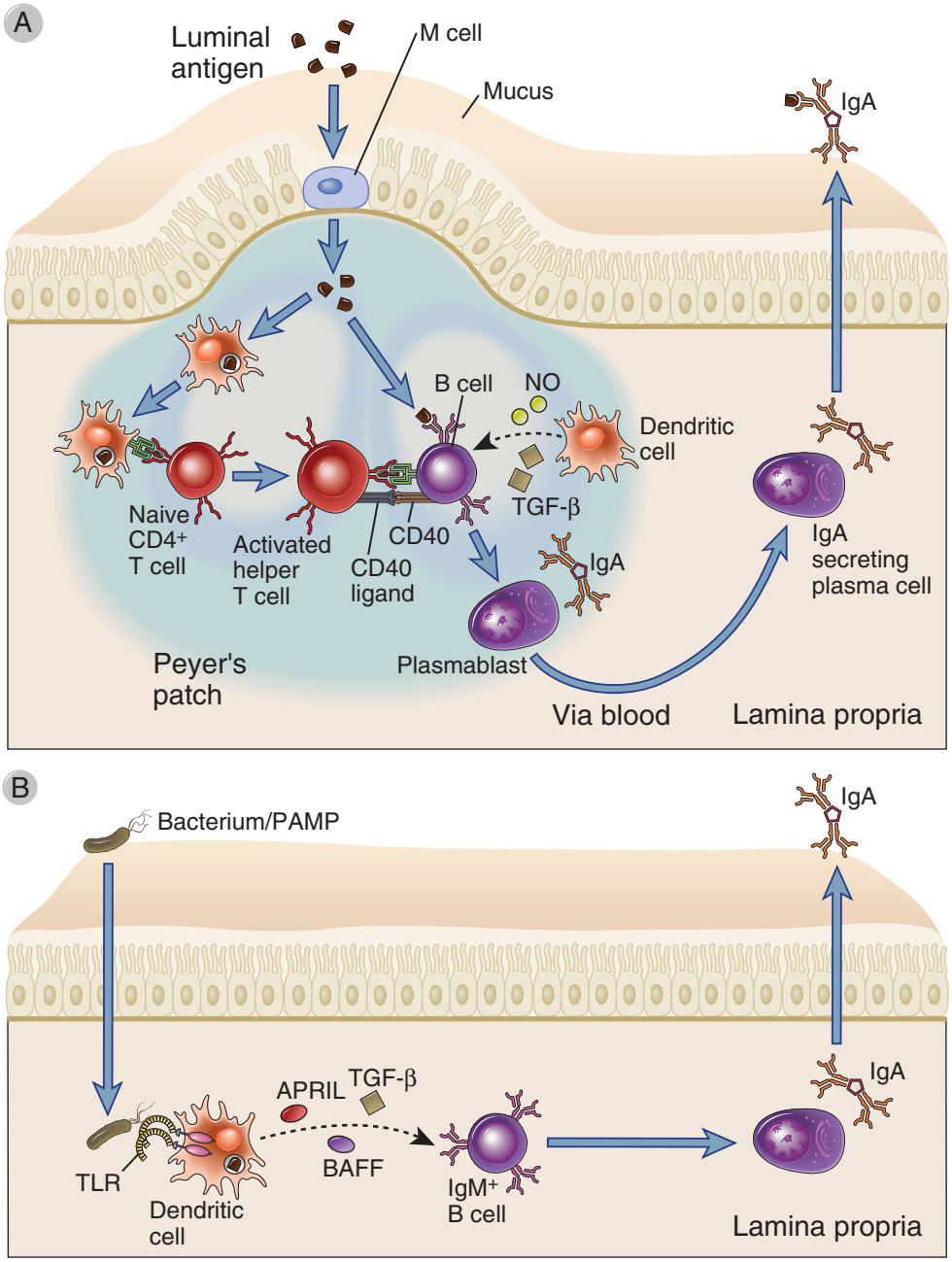


FIGURE 13–7 IgA class switching in the gut. IgA class switching in the gut occurs by both T-dependent and T-independent mechanisms. **A,** In T-dependent IgA class switching, DCs in the subepithelial dome of Peyer's patches capture bacterial antigens delivered by M cells and migrate to the interfollicular zone, where they present antigen to naive $CD4^+$ T cells. The activated T cells differentiate into helper T cells and engage in cognate interactions with antigen-presenting IgM^+IgD^+ B cells that have also taken up and processed the bacterial antigen. B cell class switching to IgA is stimulated through T cell CD40L binding to B cell CD40, together with the action of TGF-β. IgA class switching may be enhanced by NO production by DCs, which upregulates TGF-β receptor on B cells. This T cell–dependent pathway yields high-affinity IgA antibodies that preferentially target pathogens and toxins. **B,** T-independent IgA class switching involves dendritic cell activation of IgM^+IgD^+ B cells, including B-1 B cells. TLR ligand–activated DCs secrete factors that induce IgA class switch, including BAFF, APRIL, and TGF-β. The DCs also produce IL-6 and retinoic acid. This T cell–independent pathway yields relatively low-affinity IgA antibodies to intestinal bacteria. The molecular mechanisms of class switching are described in Chapter 11.

IgA-producing cells that arise in GALT and mesenteric lymph nodes (see Fig. 13-5). Some of the IgA that is transported across the intestinal epithelium may be produced by plasma cells that differentiated and remained within underlying GALT follicles. However, IgA-secreting plasma cells are widely dispersed in the lamina propria of the gastrointestinal tract, not just in lymphoid follicles. As we discussed earlier, activated B cells that undergo isotype switching into IgA-producing cells in the GALT and mesenteric lymph nodes may enter into the systemic circulation and then selectively home back to the intestinal lamina propria, where they may reside as plasma cells.

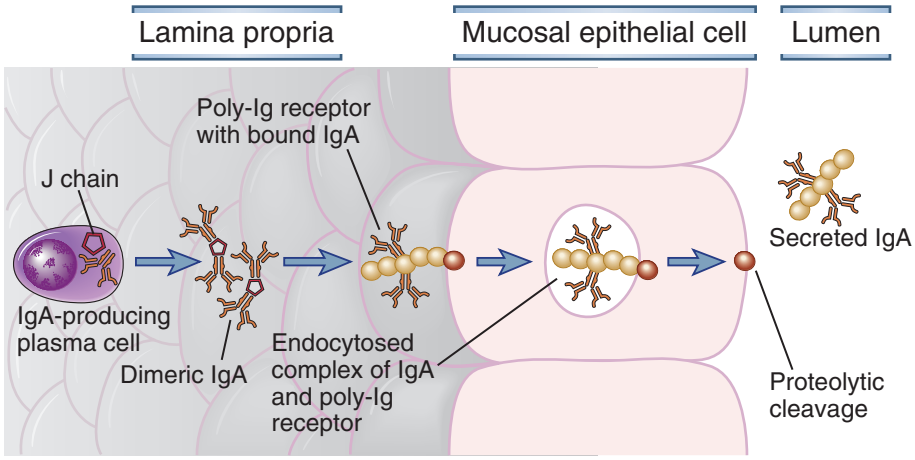


FIGURE 13–8 Transport of IgA across epithelial cells. IgA is produced by plasma cells in the lamina propria of mucosal tissue and binds to the poly-Ig receptor at the base of an epithelial cell. The complex is transported across the epithelial cell, and the bound IgA is released into the lumen by proteolytic cleavage. The process of transport across the cell, from the basolateral to the luminal surface in this case, is called transcytosis.

Secreted IgA is transported through epithelial cells into the intestinal lumen by an IgA/IgM-specific Fc receptor called the poly-Ig receptor (Fig. 13-8). The IgA produced by plasma cells in the lamina propria is in the form of a dimer that is held together by the coordinately produced J chain, which is covalently bound by disulfide bonds to the Fc regions of the α heavy chains of two IgA molecules. Mucosal plasma cells produce abundant J chain, even more than plasma cells in nonmucosal tissues, and serum IgA is usually a monomer lacking the J chain. From the lamina propria, the IgA must be transported across the epithelium into the lumen by a process known as transcytosis, and this function is mediated by the **poly-Ig receptor**. This receptor is synthesized by mucosal epithelial cells and expressed on their basal and lateral surfaces. It is an integral membrane glycoprotein with five extracellular domains homologous to Ig domains and is thus a member of the Ig superfamily. The J chain of secreted dimeric IgA and pentameric IgM contains a domain required for poly-Ig receptor binding, and therefore dimeric IgA (and multimeric IgM) binds to the poly-Ig receptor on mucosal epithelial cells (see Fig. 13-8). This complex is endocytosed into the epithelial cell and actively transported in vesicles to the luminal surface. Here the poly-Ig receptor is proteolytically cleaved, its transmembrane and cytoplasmic domains are left attached to the epithelial cell, and the extracellular domain of the receptor, which carries the IgA molecule, is released into the intestinal lumen. The soluble IgA–associated component of the receptor is called the secretory component. IgM produced by lamina propria plasma cells is also a polymer (pentamer) associated noncovalently with the J chain, and the poly-Ig receptor also transports IgM into intestinal secretions. This is why this receptor is called the poly-Ig receptor. It is believed that the bound secretory component protects polymeric IgA and IgM from proteolysis by enzymes present in the intestinal lumen and these antibodies are therefore able to serve their function of neutralizing microbes and toxins in the lumen. The poly-Ig receptor is also responsible for the secretion of IgA into bile, milk, sputum, saliva, and sweat.

IgG is present in intestinal secretions at levels equal to IgM but lower than IgA. In some mucosal secretions (i.e., in the rectum, genitourinary tract, and airways), IgG levels are high and often exceed IgA. The transport of IgG into mucosal secretions is due to another transcytosing receptor, the neonatal Fc receptor (FcRn), which we discussed in Chapter 12. In contrast to the poly-Ig receptor, which transports IgA unidirectionally (from the basal side to the apical/lumen side), FcRN can mediate bidirectional transport of IgG. Therefore, FcRn-mediated IgG transport likely contributes to humoral immunity against luminal intestinal pathogens and may also contribute to uptake of antibody-coated microbes and other antigens from the lumen into the GALT.

IgA produced in lymphoid tissues in the mammary gland is secreted into colostrum and mature breast milk through poly-Ig receptor–mediated transcytosis and mediates passive mucosal immunity in breast-fed children. The human lactating mammary gland contains a large number of IgA-secreting plasma cells, and the mammary gland epithelium can store large quantities of secretory IgA. The plasma cells in the breast originate from various mucosa-associated lymphoid tissues. They home to the breast because most IgA plasmablasts express CCR10, no matter which lymphoid tissues they were generated in, and the breast tissues express CCL28, the chemokine that binds CCR10. Therefore, during breast-feeding, a child ingests a significant quantity of maternal IgA, which provides broad polymicrobial protection in the infant's gut. Moderate amounts of IgG and IgM are also secreted into breast milk and contribute to the passive immunity of breast-fed children. Many epidemiologic studies have shown that breast-feeding significantly reduces the risk of diarrheal disease and sepsis, especially in developing countries, and this correlates with the presence of secretory IgA in breast milk specific for

enterotoxic species of bacteria including *Escherichia coli* and *Campylobacter*.

T Cell–Mediated Immunity in the Gastrointestinal Tract

T cells play important roles in protection against microbial pathogens in the gastrointestinal system and in regulating responses to food and commensal antigens. Furthermore, T cells contribute to inflammatory diseases in the gastrointestinal tract. As in other parts of the body, T cell immunity in the gut involves different subsets of T cells and is influenced in various ways by antigen-presenting DCs, which also belong to different subsets. In this section, we will discuss important features of T cell and DC functions in the intestines.

T cells are found within the gut epithelial layer, scattered throughout the lamina propria and submucosa, and within Peyer's patches and other organized collections of follicles. In humans, most of the intraepithelial T cells are $CD8^+$ cells. In mice, about 50% of intraepithelial lymphocytes express the γδ form of the TCR, similar to intraepidermal lymphocytes in the skin. In humans, only about 10% of intraepithelial lymphocytes are γδ cells, but this proportion is still higher than the proportions of γδ cells found among T cells in other tissues. Both the αβ and the γδ TCR-expressing intraepithelial lymphocytes show limited diversity of antigen receptors. These findings support the idea that mucosal intraepithelial lymphocytes have a limited range of specificity, distinct from that of most T cells, and this restricted repertoire may have evolved to recognize microbes that are commonly encountered at the epithelial surface. Lamina propria T cells are mostly $CD4^+$, and most have the phenotype of activated effector or memory T cells, the latter with an effector memory phenotype (see Chapter 9). Recall that these lamina propria effector and memory T cells are generated from naive precursors in the GALT and mesenteric lymph nodes, enter the circulation, and preferentially home back into the lamina propria (see Fig. 13-5). T cells within Peyer's patches and in other follicles adjacent to the intestinal epithelium include $CD4^+$ helper T cells and regulatory T cells.

DCs are abundant in the gastrointestinal immune system and can be broadly divided into two functional subgroups, which participate in stimulating protective effector T cell responses or inducing regulatory T cell responses that suppress immunity to ingested antigens and commensal organisms (see Fig. 13-4). These subsets, which are also present in mucosal tissues other than the gut, are sometimes called effector DCs and regulatory DCs. They can be distinguished by their differential expression of integrins and chemokine receptors: effector DCs are $CD11b^+CX_3CR1^+$, and regulatory DCs are $CD103^+CX_3CR1^-$. Effector DCs are the antigen-sampling DCs we discussed earlier in the chapter that project dendrites between epithelial cells and sample luminal contents. They can interact with naive T cells (and B cells) in the GALT, or they can migrate, through lymphatic drainage, into mesenteric lymph nodes, where they present processed protein antigens to naive T cells and induce the differentiation of these T cells into IFN-γ– or IL-17–producing effector cells. Regulatory DCs, which do not directly sample luminal contents, induce the differentiation of naive T cells into $FoxP3^+$ regulatory T cells. Regulatory DCs are thought to be conditioned by mucosal epithelial cells to secrete TGF-β and retinoic acid at the time of antigen presentation to naive T cells; these molecules favor Treg differentiation. DC heterogeneity in the gut as well as in other tissues is actually more complex than we have discussed, with 5 to 10 different subsets having been identified by patterns of expression of multiple surface molecules. The functional relevance of these subsets remains incompletely understood.

In the gastrointestinal tract, different subsets of effector $CD4^+$ T cells are induced by and protect against different microbial species. In Chapters 9 and 10, we introduced the concept that helper T cell subsets that secrete different cytokines are specialized for particular types of antimicrobial responses. This fundamental concept is highly relevant to the mucosal immune system. The commensal bacterial microflora of the gut lumen exerts profound influences on T cell phenotypes even during homeostasis.

- **T_H17 cells.** Studies in mice have shown that certain classes of bacteria, or in some cases individual species of bacteria, can shift the dominant pattern of T cell cytokine production. For example, the lamina propria of the small bowel in healthy mice is particularly rich in IL-17–producing cells, whereas the colon is not, and the presence of the T_H17 cells depends on colonization of the gut with a certain phylum of bacteria (segmented filamentous bacteria) in the postnatal period. This steady-state presence of T_H17 cells is required for protection against pathogenic species of bacteria (e.g., *Citrobacter rodentium*). Another example of bacterial microflora-induced changes in gut T cell phenotypes is the finding that colonization of the bowel with either polysaccharide A non-expressing or expressing strains of *Bacteroides fragilis* induces IL-17–producing T cells or IL-10–producing regulatory T cells, respectively. T_H17 cells appear to play a special role in maintaining mucosal epithelial barrier function because of the actions of the two signature cytokines they produce, IL-17 and IL-22. The receptors for both these cytokines are expressed on intestinal epithelial cells, and both induce the expression of proteins important for barrier function, such as mucins and β-defensins, which protect the epithelial cells against microbe-induced injury. The mechanisms underlying these microbe-induced changes in T cell responses are not well understood but likely involve microbe-induced signals in both intestinal epithelial cells and DCs. These signals change the phenotype and cytokine secretion profile of DCs, which in turn influence T cell subset differentiation when the DCs present antigen to microbial antigen-specific naive T cells.
- **T_H2 cells.** Intestinal helminthic infections induce strong T_H2 responses, which are effective in eliminating the worms because the T_H2 cytokines IL-4 and IL-13 cooperate in enhancing fluid and mucus secretions and inducing smooth muscle contraction and bowel motility. This type of host defense is called

barrier immunity because it is active at epithelial barriers (see Chapter 10).

Regulation of Immunity in the Gastrointestinal Tract by Regulatory T Cells and Cytokines

Regulatory T cells are abundant in GALT and prevent inflammatory reactions against intestinal commensal microbes. It is estimated that there are about twice as many FoxP3$^+$ Treg among CD4$^+$ cells in the lamina propria as in other peripheral lymphoid tissues. Many of these Treg are likely induced in the gut in response to antigens encountered locally and thus belong to the category of adaptive Treg (see Chapter 14). The factors that contribute to the generation of these Treg include CD103$^+$ DCs, local production of retinoic acid (which promotes FoxP3 expression), and local production of TGF-β (which also promotes FoxP3 expression and inhibits the generation of T_H1 and T_H2 cells). As we discuss in Chapter 14, Treg are thought to suppress immune responses by several mechanisms. Of these, the dominant mechanism in the gut seems to be production of the immunosuppressive cytokine IL-10, as discussed later.

Several cytokines, including TGF-β, IL-10, and IL-2, appear to play crucial roles in maintaining homeostasis in the gut immune system, and deficiencies in these cytokines or their receptors result in pathologic bowel inflammation. Much of our knowledge of cytokine-mediated regulation in the gut comes from studies with cytokine or cytokine receptor gene knockout mice. A major feature of the phenotype of mice with engineered deficiencies in TGF-β, IL-10, IL-10 receptor, IL-2, and IL-2 receptor is uncontrolled inflammation in the bowel. Mutations in the IL-10 and IL-10 receptor genes are also associated with severe inflammatory bowel disease in children, confirming the importance of IL-10 in preventing pathologic gut inflammation in humans. The uncontrolled inflammation observed in the gut in the absence of these cytokines or their receptors is most likely caused by innate and adaptive immune responses to commensal gut flora because the inflammation does not occur in mice raised in germ-free conditions. The cellular sources of the cytokines and the relevant receptor-expressing target cells that are critical for prevention of bowel inflammation are not completely understood. Mouse models in which cytokines, cytokine receptors, and cytokine receptor signaling are genetically ablated only in specific cell types have been used to address the question of which cell types are important. In the case of TGF-β– and IL-10–dependent regulation of gut inflammation, evidence indicates that Treg and macrophages are both important sources of these cytokines. For example, selective deletion of the *IL10* gene in FoxP3$^+$ cells rapidly leads to severe colitis but no other manifestations of inflammatory disease, consistent with the critical role of Treg-produced IL-10 in maintaining homeostasis in the gastrointestinal tract. The target cells that express receptors for and are regulated by TGF-β and IL-10 likely include DCs, effector T cells, innate effector cells such as macrophages, and epithelial cells. Inflammatory bowel disease in mice lacking IL-2 or its receptor is a consequence of the defects in the development and function of Treg, which require IL-2, (see Chapter 14).

Oral Tolerance and Oral Vaccines

Oral tolerance is systemic adaptive immune tolerance to antigens that are ingested or otherwise administered orally and is a potential way of treating diseases in which unwanted immune responses occur, such as autoimmunity. Oral tolerance has been most clearly demonstrated in experimental rodent models. Mice fed high doses of a protein antigen may subsequently have impaired humoral and T cell–mediated responses to the same antigen administered by other routes, such as through the skin. A similar phenomenon can be demonstrated when antigens are administered through the nasal passages into the respiratory mucosa, and the more general term *mucosal tolerance* is used to describe tolerance induced by either oral or nasal antigen administration. The physiologic role of oral tolerance is speculated to be the prevention of potentially harmful immune responses to food proteins and commensal bacteria. The underlying mechanisms of oral tolerance are not well understood but likely include the same mechanisms of peripheral tolerance discussed in Chapter 14, such as anergy, deletion, and Treg-mediated suppression. The propensity of the immune system in the gut to suppress local immune responses to antigens in the intestinal lumen could be manifested in other parts of the body because of circulation of Treg to other tissues and deletion or anergy of effector T cells in the gut, which are no longer available to respond to antigens at other sites. Attempts to treat autoimmune disease or allergies by oral or nasal administration of relevant self antigens or allergens have so far been unsuccessful.

Oral administration of antigen in the setting of concomitant stimulation of innate immunity can lead to productive adaptive immune responses, as in the use of oral viral vaccines to induce protective antibody responses to viruses. These vaccines are live attenuated viruses that may infect DCs in the intestine and stimulate strong innate responses that then promote T and B cell activation.

Diseases Related to Immune Responses in the Gut

Given the abundance of immune cells and their constant activity in the intestinal mucosa, it is not surprising that there are many intestinal diseases related to abnormal immune responses. These diseases are generally caused by unregulated responses to commensal organisms or to antigens in the food. We will now discuss selected examples of these diseases; they are more thoroughly described in medical textbooks.

Inflammatory bowel disease is a heterogeneous group of disorders characterized by chronic remitting inflammation in the small or large bowel, likely due to poorly regulated responses to commensal bacteria. The two main types of inflammatory bowel disease are **Crohn's disease,** which can affect the entire thickness of the bowel wall tissue in any part of the gastrointestinal tract but most frequently involves the terminal ileum, and **ulcerative colitis**, which is restricted to the colonic mucosa.

Symptoms include abdominal pain, vomiting, diarrhea, and weight loss. Treatments include various anti-inflammatory drugs, such as sulfasalazine, corticosteroids, TNF antagonists, and antimetabolites. Although the etiology of Crohn's disease and ulcerative colitis is poorly understood, several types of evidence suggest that these disorders are a result of defects in the regulation of immune responses to commensal organisms in the gut in a genetically susceptible host. A number of immunologic abnormalities may contribute to the development of inflammatory bowel disease.

- *Defects in innate immunity to gut commensals.* Earlier we discussed the possibility that inflammatory bowel disease results from either or both of two types of innate immune defects. First, there may be defective expression of molecules such as defensins, leading to increased commensal bacterial invasion through the intestinal epithelium. Second, there may be inadequate negative regulation of innate immune responses to commensal organisms. Polymorphisms in the gene encoding the NOD2 cytoplasmic innate immune sensor are associated with a subset of Crohn's disease and may lead to either of these two types of abnormalities in innate immunity.
- *Abnormal T_H17 and T_H1 responses.* Analysis of T cell responses in animal models and patients with inflammatory bowel disease indicates that there is an active T_H17 response in the affected parts of the bowel. Furthermore, studies of genes associated with risk for development of inflammatory bowel disease indicate that polymorphisms of the IL-23 receptor are associated with altered risk, which is also consistent with a role for T_H17 responses because IL-23 is required for T_H17 differentiation and maintenance. Crohn's disease is also characterized by granulomatous inflammation driven by IFN-γ–producing T_H1 cells (see Chapter 18). These findings are the basis for clinical trials in which inflammatory bowel disease patients are treated with a monoclonal antibody that binds a polypeptide (p40) shared by IL-23 and IL-12. IL-23 is required for T_H17-mediated immune responses, as mentioned before, and IL-12 is required for T_H1 responses.
- *Defective function of regulatory T cells.* It is possible that inflammatory bowel disease may be caused by inadequate Treg-mediated suppression of immune responses to commensal organisms. The evidence supporting this hypothesis comes from mouse models in which an absence of Treg leads to inflammatory bowel disease. In fact, one of the first experiments demonstrating the existence of Treg was the development of gastrointestinal inflammation in immunodeficient mice injected with naive $CD4^+CD25^-$ T cells, which we now know contain precursors of effector T cells but lack $CD4^+CD25^+$ Treg. Mice deficient in Treg because of deletion of IL-2 or IL-2R genes, as mentioned before, or knockout of the *FOXP3* gene also develop inflammatory bowel disease. In humans, *FOXP3* mutations result in a failure to develop Treg and cause the disease called immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX), which includes severe gut inflammation as well as autoimmunity in many other tissues. Although all these observations are consistent with a need for Treg to maintain intestinal homeostasis, as discussed earlier, it is not known if Treg defects underlie most cases of human inflammatory bowel disease.
- ***Polymorphisms of genes that are associated with macroautophagy and the unfolded protein response to endoplasmic reticulum stress are risk factors for inflammatory bowel disease.*** Experimental evidence suggests that the connection between inflammatory bowel disease and variants in the unfolded protein responses and autophagy genes relates to diminished Paneth cell secretion of antimicrobial enzymes and defensins. Macroautophagy is a process by which cells sequester cytoplasmic organelles within autophagosomes, which then fuse with lysosomes, promoting the destruction of the organelles. Genetic variations of autophagy genes (including *ATG16L1* and *LBKK2*) that are associated with Crohn's disease impair autophagy in Paneth cells, and, for unclear reasons, this reduces secretion of lysozyme and defensins into the intestinal lumen. Endoplasmic reticulum stress occurs when misfolded proteins accumulate in the endoplasmic reticulum. This leads to the activation of a XBP-1, that work together to block protein translation and increase expression of chaperones that promote proper protein folding. Paneth cells, like other secretory cells, depend on the unfolded protein response to maintain protein secretory function.

Celiac disease (gluten-sensitive enteropathy or nontropical sprue) is an inflammatory disease of the small bowel mucosa caused by immune responses against ingested gluten proteins present in wheat. Celiac disease is characterized by chronic inflammation in the small bowel mucosa, leading to atrophy of villi, malabsorption, and various nutritional deficiencies that lead to extraintestinal manifestations. The disease is treated by dietary restriction to gluten-free foods. Patients produce IgA and IgG antibodies specific for gluten as well as IgA and IgG autoantibodies specific for transglutaminase 2A, an enzyme that modifies the gluten protein gliadin. These autoantibodies are thought to arise when transglutaminase-specific B cells endocytose host transglutaminase covalently associated with gliadin and then present gliadin peptides to helper T cells, which then provide help for the anti-transglutaminase antibody response. Whether these antibodies contribute to disease development is not known, but they are a sensitive diagnostic marker for the disease. There is strong evidence that $CD4^+$ T cell responses to gliadin are involved in disease pathogenesis. T cells specific for gliadin peptides are found in celiac patients, and the inflammatory process in the bowel includes T cells and T cell cytokines. Gliadin peptides bind strongly to two class II MHC alleles found in most patients, namely, HLA-DQ2 and HLA-DQ8, and there is a high relative risk for development of the disease among people with these two alleles. We will further discuss the association of autoimmune diseases with MHC alleles in Chapter 14. In addition to $CD4^+$ T cell responses, $CD8^+$ cytotoxic T lymphocyte (CTL) killing of intestinal epithelial cells may also contribute to celiac

disease, although not by recognition of gliadin peptides. Instead, gliadin stimulates epithelial cells to secrete IL-15, which induces expression of the activating receptor NKG2D on CTLs, and gliadin induces the expression of the ligands for NKG2D (MICA, MICB) on intestinal epithelial cells. The net result is a lowering of the threshold for CTL killing of the epithelial cells, although the source of the actual peptides recognized by the CTLs is not clear.

Food allergies are caused by T_H2 responses to many different food proteins and cause acute inflammatory responses locally in the gut and systemically on ingestion of these proteins. Allergies result from T_H2-dependent IgE responses to environmental antigens (allergens), which are either proteins or chemicals that modify (haptenate) self proteins. In the case of food allergies, the environmental antigens are ingested, and this is another example of a failure of adaptive immune tolerance to food antigens. The anti-allergen antibodies bind to Fc receptors on mast cells, and subsequent exposure to the allergen will cause cross-linking of the Fc receptors, activation of the mast cells, and release of potent proinflammatory amine and lipid mediators and cytokines. There are abundant mast cells in the lamina propria of the bowel. Therefore, reingestion of a food allergen by a person who has previously mounted a T_H2 and IgE response to the allergen will trigger mast cell activation, with its pathologic consequences. Cytokines produced by T_H2 cells also directly stimulate peristalsis and may trigger symptoms of food allergies even without the participation of IgE. These reactions may cause gastrointestinal symptoms like nausea, vomiting, diarrhea, and abdominal pain, but the allergen can be absorbed into the blood and end up activating mast cells in many different tissues, producing systemic manifestations. We will discuss allergic reactions in more detail in Chapter 19.

Prolonged immune responses to gastrointestinal microbes can lead to tumors arising in the gastrointestinal tract. The best documented example of this is the so-called MALT lymphomas in the stomach of people with chronic *Helicobacter pylori* infection. These lymphomas are tumors arising from malignantly transformed follicular B cells in lymphoid follicles of the gastric lamina propria. It is believed that *H. pylori* sets up an inflammatory reaction that promotes the development and growth of tumors induced by B cell intrinsic oncogenic events. Remarkably, if gastric MALT lymphomas are diagnosed before they spread beyond the stomach wall, patients can be cured by antibiotic treatment of the *H. pylori* infection.

IMMUNITY IN OTHER MUCOSAL TISSUES

Like the gastrointestinal mucosa, the mucosae of the respiratory system, the genitourinary system, and the conjunctiva must maintain a barrier against invasion of diverse microbes in the environment and balance effective protective responses to invading microbes and suppression of responses to numerous commensal organisms. Many of the features we described for gastrointestinal immunity are shared by mucosal immunity in these different locations. These shared features include relatively impermeable mucus- and defensin-secreting epithelial barriers; localized collections of lymphoid tissues just beneath the epithelium; the constant sampling of antigens located outside the barriers by immune cells within the barrier; the constant integration of proinflammatory and regulatory signals generated by microbial products binding to epithelial and DC TLRs; the strong reliance on secretory IgA–mediated humoral immunity to prevent microbial invasion; and the presence of effector and regulatory DC populations that stimulate particular types of effector and regulatory T cell responses. In addition to these shared features, each different mucosal tissue has unique features that reflect the distinct functions and anatomy of the organs it is part of and the distinct range of environmental antigens and microbes that are present at each site. We will now discuss some of the major features of mucosal immunity in these organs, focusing mainly on the respiratory system.

Mucosal Immunity in the Respiratory System

The mucosa of the respiratory system lines the nasal passages, nasopharynx, trachea, and bronchial tree. Alveoli, the epithelium-lined sac-like termini of the bronchial airways, may also be considered part of the respiratory mucosa. Inhalation of air exposes the respiratory mucosa to a wide variety of foreign substances, including airborne infectious organisms, plant pollens, dust particles, and various other environmental antigens. The microbial flora of the airways is far less dense and less diverse than that in the gut, and the deep airways and alveoli are usually sterile. Nonetheless, similar mechanisms have evolved in the respiratory mucosal immune system to achieve a fine balance between immune activation to protect against pathogens and immune regulation to avoid unnecessary or excessive responses that might impair the physiologic functions. Failure of the immune system to control bronchopulmonary infections and excessive immune or inflammatory responses to infections are major causes of morbidity and mortality worldwide.

Innate Immunity in the Respiratory System

The pseudostratified, ciliated columnar epithelium that lines most of the respiratory mucosa, including nasal passages, nasopharynx, and bronchial tree, performs similar physical and chemical barrier functions as gut epithelium, by virtue of tight junctions between cells and secretion of mucus, defensins, and cathelicidins. The mucus in the airways traps foreign substances including microbes, and the cilia move the mucus and trapped microbes up and out of the lungs. The importance of mucus and cilia in innate immune protection in the lung is illustrated by the greatly increased frequency of serious bronchopulmonary infections in people with decreased cilia function, such as heavy smokers, or impaired mucus production, such as patients with cystic fibrosis.

Innate responses in the alveolus serve antimicrobial functions but are tightly controlled to prevent inflammation, which would impair gas exchange. The alveoli are normally sterile but are susceptible to infection spreading from bronchopneumonia, and alveolar lining cells can be

directly infected by viruses. Surfactant proteins A (SP-A) and D (SP-D), which are secreted into the alveolar spaces, are members of the collectin family (see Chapter 4) and bind to carbohydrate PAMPs on the surface of many pathogens. These surfactants are involved in viral neutralization and clearance of microbes from the airspaces, but they also suppress inflammatory and allergic responses in the lung. For example, SP-A inhibits TLR2 and TLR4 signaling and inflammatory cytokine expression in alveolar macrophages, and SP-A also binds to TLR4 and inhibits lipopolysaccharide binding. SP-A and SP-D reduce the phagocytic activity of alveolar macrophages.

Alveolar macrophages represent the majority of free cells within the alveolar spaces. These cells are functionally distinct from macrophages in most other tissues in that they maintain an anti-inflammatory phenotype. They express IL-10, nitric oxide, and TGF-β and are poorly phagocytic compared with resident macrophages in other tissues, such as spleen and liver. Alveolar macrophages inhibit T cell responses as well as the antigen presentation function of $CD103^+$ airway DCs.

Adaptive Immunity in the Respiratory System

Protective humoral immunity in the airways is dominated by secretory IgA, as in other mucosal tissues, although the amount of IgA secreted is much less than in the gastrointestinal tract. Secretory IgG plays an important role in the upper airway. The anatomic sites of naive B cell activation, differentiation, and IgA isotype class switching may vary but include tonsils and adenoids in the nasopharynx and lymph nodes in the mediastinum and adjacent to bronchi in the lungs. There are relatively few aggregated or isolated lymphoid follicles in the lamina propria in the lower airways compared with the gut and likely less initiation of humoral immune responses in these locations. The homing of IgA-secreting plasma cells back into the airway tissue in proximity to respiratory mucosal epithelium depends on the chemokine CCL28 secreted by respiratory epithelium and its receptor CCR10 on the plasma cells. IgA and IgG are transported into the airway lumen by the same poly-Ig receptor and FcRn mechanism of transcellular transport as in the gut. IgE responses to airway antigens occur frequently and are involved in allergic diseases of the respiratory system, including hay fever and asthma. IgE performs its inflammatory effector functions when bound to mast cells, which are abundant in the airways.

T cell responses in the lung are initiated by DC sampling of airway antigens and presentation of these antigens to naive T cells in peribronchial and mediastinal lymph nodes. A network of DCs is present in the mucosa of the airways, and these DCs can be subdivided into subsets based on surface markers and location. The $CD103^+CD11b^-$ DCs extend dendrites between the bronchial epithelial cells into the airway lumen. These DCs sample airway antigens, migrate to draining lymph nodes, present the processed antigens to naive T cells, and have a propensity to drive differentiation of these T cells to the T_H2 subset. The T_H2 cells home back into the bronchial mucosa, where they may be reactivated by allergens presented by DCs in lamina propria. This pathway is considered central to the development of allergic asthma (see Chapter 19). Other DCs are found in the lamina propria beneath the epithelial cells, and these are mainly $CD103^-CD11b^+$.

Mucosal Immunity in the Genitourinary System

Innate immune defense against microbial invasion and infection in the genitourinary mucosa relies mainly on the epithelial lining, as in other mucosal barriers. Stratified squamous epithelium lines the vaginal mucosa and terminal male urethra, and a single layer of mucus-secreting columnar epithelium lines the upper female genital tract. The vaginal epithelium contains Langerhans cells, and a variety of DCs and macrophages have been described beneath the epithelium in vagina, endocervix, and urethra. There are also resident B and T cells in the genital mucosa. Differences in the phenotype of the DCs and macrophages in the female genital mucosa from those in the gastrointestinal tract may underlie the greater susceptibility of the latter to HIV infection. There is little regional specialization of the adaptive immune system in the genitourinary mucosa, which lacks prominent mucosa-associated lymphoid tissues. Unlike other mucosa, in which IgA is the dominant antibody isotype, most of the antibodies in genital secretions are IgGs, about half of which are produced by plasma cells in genital tract mucosa; the rest are from the circulation.

THE CUTANEOUS IMMUNE SYSTEM

The skin includes two main layers, the outer epidermis composed mainly of epithelial cells and, separated by a thin basement membrane, the underlying dermis composed of connective tissue and specialized adnexal structures such as hair follicles and sweat glands. Within both of these layers, a variety of different cell types and their products, composing the cutaneous immune system (Fig. 13-9), provide physical barrier and active immune defense functions against microbes. The skin of an adult is about 2 m^2 in area and is the second largest barrier of the body against environmental microbes and other foreign materials. Nonetheless, given its outermost location, the skin is normally colonized by many microbes and is frequently breached by trauma and burns. Therefore, the skin is a common portal of entry of a wide variety of microbes and other foreign substances and is the site of many immune responses.

Innate and Adaptive Immune Reponses in the Skin

The epidermis provides a physical barrier to microbial invasion. The epidermis consists of multiple layers of stratified squamous epithelium, made up almost entirely of specialized epithelial cells called keratinocytes. The basal layer of keratinocytes, anchored onto the basement membrane, are continuously proliferating, and their maturing progeny cells are displaced upward and differentiate to form several different layers. In the top

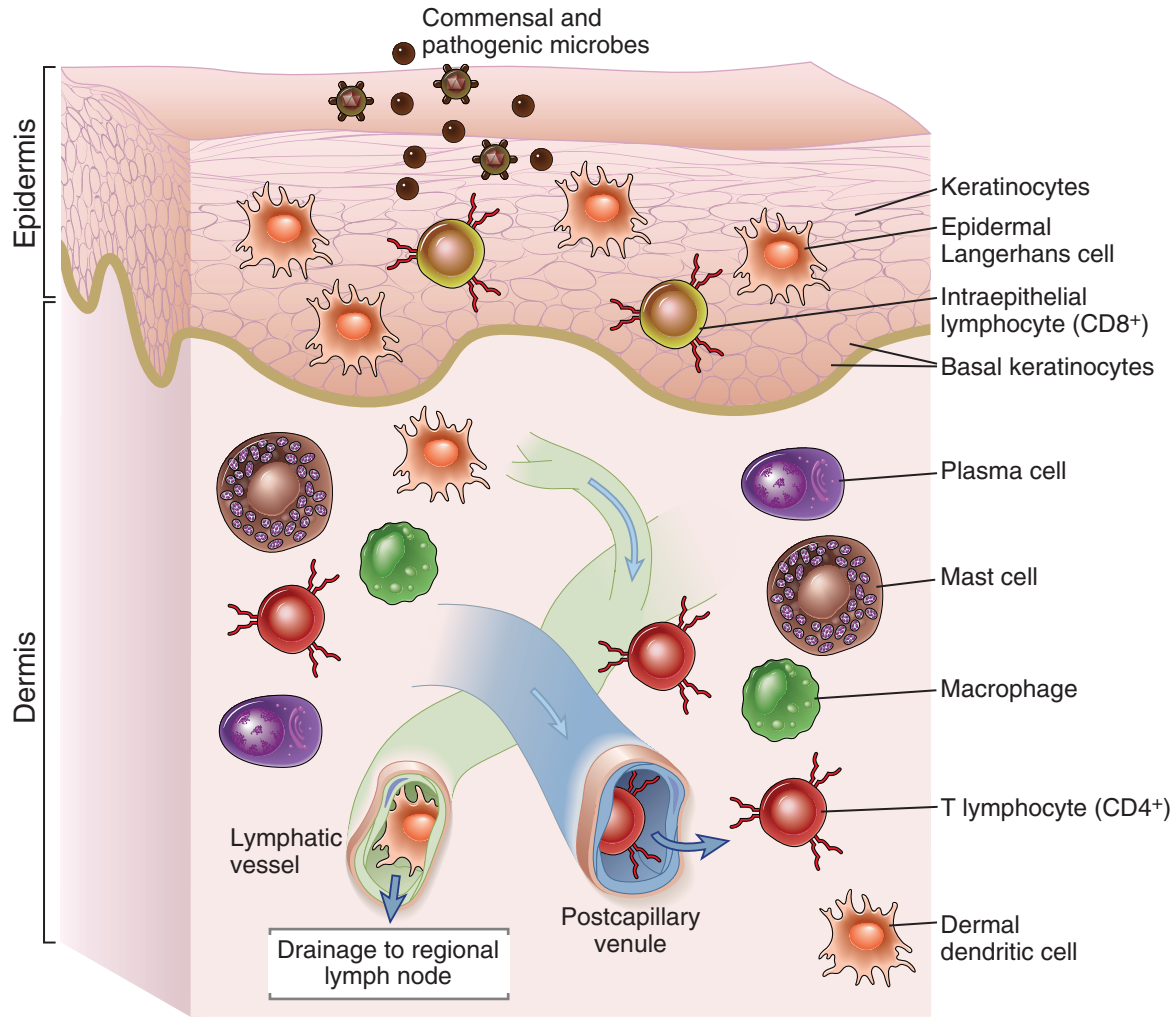


FIGURE 13–9 Cellular components of the cutaneous immune system. The major components of the cutaneous immune system shown in this schematic diagram include keratinocytes, Langerhans cells, and intraepidermal lymphocytes, all located in the epidermis, and T lymphocytes, DCs, and macrophages, located in the dermis.

layer, called the stratum corneum, the cells undergo programmed death, thereby forming a keratin- and lipid-rich permeability barrier that is important for protection against microbes as well as harmful physical and chemical agents.

In addition to their role in forming a physical barrier, keratinocytes respond to pathogens and injury by producing antimicrobial peptides, which kill microbes, and various cytokines, which promote and regulate immune responses. The antimicrobial peptides that keratinocytes produce include defensins, S100, and cathelicidins (see Chapter 4). The cytokines made by keratinocytes include TNF, IL-1, IL-6, and IL-18, which promote inflammation; GM-CSF, which induces differentiation and activation of DCs in the epidermis, discussed later; and IL-10, which controls immune responses. Keratinocytes produce the chemokines CCL17, CCL20, and CCL27, which participate in recruitment of lymphocytes expressing CCR4, CCR6, and CCR27. The induced expression of defensins, cytokines, and chemokines by keratinocytes depends on innate immune receptors including TLRs and NLRs. Keratinocytes express most of the TLRs and NLRP3, which forms the IL-1–processing inflammasome (see Chapter 4). Keratinocytes in normal skin constitutively synthesize pro–IL-1 and pro–IL-18. Stimuli such as UV irradiation activate the inflammasome to process these pro-cytokines to the active forms, which explains the inflammatory response to sunburn. When signal transduction pathways linked to inflammatory responses, such as the NF-κB and STAT3 pathways, are genetically activated only in keratinocytes, mice develop inflammatory skin diseases, showing the potential of keratinocytes to act as central players of cutaneous immune responses.

Several DC populations are normally present in the skin and contribute both to innate immune responses and to initiation of T cell responses to microbial and environmental antigens that enter the body through the skin. In the epidermis, the most abundant DCs are the Langerhans cells, which express a C-type lectin receptor called langerin (CD207) and have numerous Birbeck granules in the cytoplasm (see Fig. 6-4, Chapter 6). The dendrites of Langerhans cells form a dense meshwork between the

keratinocytes of the epidermis. In the dermis, there are relatively sparse langerin-expressing $CD103^+$ DCs, which are a distinct lineage from Langerhans cells, and langerin-negative DCs, such as plasmacytoid DCs. Each of these dendritic cell populations express innate pattern recognition receptors for PAMPs expressed on microbes and damage-associated molecular patterns (DAMPs) expressed on injured cells, and the DCs respond by secreting inflammatory cytokines.

Skin DCs take up foreign proteins, transport them to draining lymph nodes, and present processed peptides from these proteins to T cells or pass the protein antigens to other lymph node–resident DCs. When Langerhans cells encounter microbes, they are activated by engagement of Toll-like receptors (see Chapter 6). The cells lose their adhesiveness for the epidermis, enter lymphatic vessels, begin to express the CCR7 chemokine receptor, and migrate to the T cell zones of draining lymph nodes in response to chemokines produced in that location. The Langerhans cells also mature into efficient antigen-presenting cells. What remains unclear is the relative contribution of the different skin DC subsets to the initiation of T cell responses. Mouse models have been developed in which langerin-expressing DCs can be selectively eliminated, and under the proper conditions, the mice lack Langerhans cells but have dermal DCs. Using these models, investigators have shown that some T cell responses to chemically modified self proteins, a model for contact hypersensitivity, occur in the absence of Langerhans cells. Furthermore, T cell responses to certain viruses, including herpes viruses, depend on dermal $langerin^+$ $CD103^+$ DCs but not Langerhans cells. Langerhans cells do appear to be required for T_H2 responses that cause atopic dermatitis (contact hypersensitivity and atopic dermatitis will be discussed later). The role of the different skin DC populations could vary with the antigen dose and type and likely will differ between mice and humans.

Normal human skin contains many T cells, 95% of which have a memory phenotype. Human skin contains about 1 million T cells/cm^2, which is about 2×10^{10} total T cells in the skin. About 98% of these T cells are present in the dermis, and 2% are intraepidermal lymphocytes. Dermal T lymphocytes (both $CD4^+$ and $CD8^+$ cells) are predominantly in a perivascular location and usually express phenotypic markers typical of activated or memory cells. It is not clear whether these cells reside permanently within the dermis or are merely in transit between blood and lymphatic capillaries as part of memory T cell recirculation. $CD4^+$ T cells of each major subset, T_H1, T_H2, T_H17, and Treg, are found in the skin. T_H1 and T_H17 cells are important for microbial defense, against intracellular and extracellular microbes, respectively, as in other tissues. The two signature T_H17 cytokines, IL-17 and IL-22, are known to induce expression of defensins by keratinocytes. Intraepidermal T cells, most of which are $CD8^+$ cells, may express a more restricted set of antigen receptors than do T lymphocytes in most extracutaneous tissues. In mice (and some other species), many intraepidermal lymphocytes are T cells that express the $\gamma\delta$ T cell antigen receptor.

T cells in the skin express homing molecules that direct their migration out of dermal microvessels (Fig. 13-10). Migration of effector or memory T cells into the skin depends on T cell expression of cutaneous lymphocyte antigen (CLA), which is an E-selectin–binding carbohydrate moiety displayed on various glycoproteins on the endothelial cell plasma membrane. In addition, T cell expression of CCR4 and CCR10, which bind the chemokines CCL17 and CCL27, respectively, is also required for T cell trafficking to skin. The skin-homing properties of T cells are imprinted during activation in skin-draining lymph nodes, by a process analogous to imprinting of gut-homing properties of T cells in mesenteric lymph nodes, discussed earlier in the chapter. When naive T cells recognize antigens presented by DCs in skin-draining lymph nodes, they receive signals from the DCs that not only induce proliferation and differentiation into effector cells but also induce expression of the skin-homing molecules CLA, CCR4, and CCR10. Interestingly, sunlight and vitamin D appear to play an important role in T cell migration to the skin, analogous to the role of vitamin A and its metabolite retinoic acid in lymphocyte migration to the gut. UVB rays in sunlight act on 7-dehydrocholesterol made in the basal layer of the epidermis, converting it to previtamin D_3. Dermal DCs express vitamin D_3 hydroxylases that convert previtamin D_3 to the active form, $1,25(OH)_2D_3$. When the DCs present antigen to T cells, the active $1,25(OH)_2D_3$ is released, enters the T cells, translocates to the nucleus, and activates transcription of CCR10. $1,25(OH)_2D_3$ produced in dermal DCs may be transported in free form or within migrating DCs to skin-draining lymph nodes. Within the node, $1,25(OH)_2D_3$ induces CCR10 expression on naive T cells activated by antigen-presenting DCs. IL-12 made by the DCs participates in induction of CLA. CCR4 is also upregulated, and the gut-homing integrin $\alpha_4\beta_7$ is downregulated, both by unknown signals, during T cell activation in skin-draining lymph nodes. Thus, naive T cells activated in skin-draining lymph nodes will differentiate into effector T cells that preferentially home back into the skin. $1,25(OH)_2D_3$ may also act locally within the dermis on effector and memory T cells to upregulate CCR10 and promote migration of the T cells into the epidermis because the CCR10 ligand CCL27 is made by keratinocytes.

Diseases Related to Immune Responses in the Skin

There are many different inflammatory diseases that are caused by dysregulated or inappropriately targeted immune responses in the skin. We will now discuss two selected examples of these diseases. In addition to these inflammatory diseases, there are several malignant lymphomas that primarily affect the skin. Most of these are derived from skin-homing T cells.

Psoriasis, a chronic inflammatory disorder of the skin characterized by red scaly plaques, is caused by dysregulated innate and T cell–mediated immune responses triggered by various environmental stimuli. DCs are central to the pathogenesis, and reduction in numbers of skin DCs by treatment with the ultraviolet A (UVA) absorbing chemical psoralen plus UVA light (PUVA therapy) reduces disease manifestations. There is evidence that psoriasis is

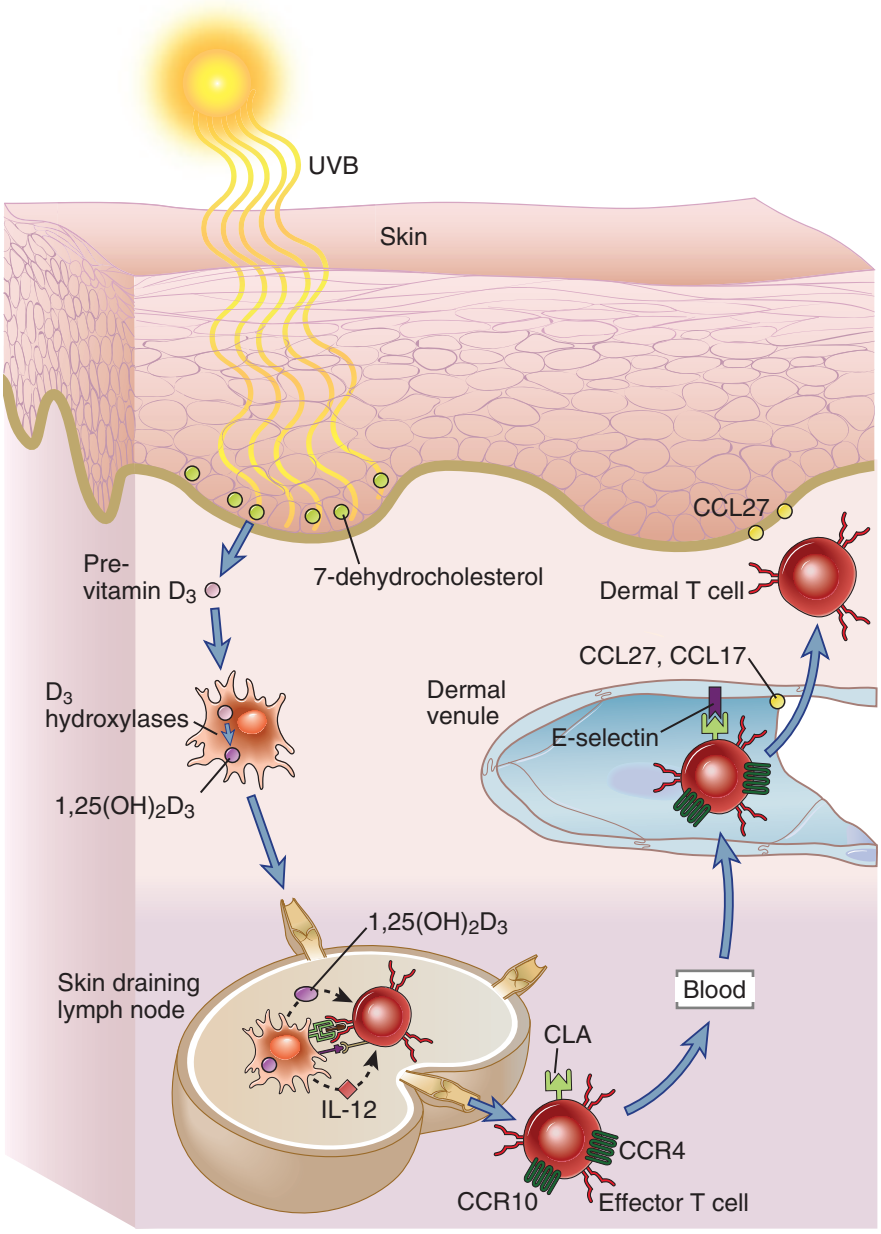


FIGURE 13–10 Homing properties of skin lymphocytes. The skin-homing properties of effector lymphocytes are imprinted in skin-draining lymph nodes where they have undergone differentiation from naive precursors. The vitamin D precursor 7-dehydrocholesterol made in the basal layer of the epidermis is converted into $1,25(OH)_2D_3$ (active vitamin D) by sequential actions of ultraviolet rays in sunlight (UVB) and hydroxylases in dermal dendritic cells. During naive T cell activation by dendritic cells in skin-draining lymph nodes, $1,25(OH)_2D_3$ induces expression of CCR10, IL-12 induces expression of the E-selectin ligand cutaneous lymphocyte antigen (CLA), and other signals induce CCR4 expression. The differentiated effector T cells enter the circulation, and the homing molecules they now express direct migration out of dermal venules into the skin because of the expression of E-selectin on the endothelium of the venules and production of CCL27 and CCL17 (the ligands of CCR10 and CCR4) in the skin.

initiated when trauma or infection induces production of the cathelicidin LL-37 by keratinocytes, which forms complexes with host DNA and then activates plasmacytoid DCs in the skin through TLR9. Activated plasmacytoid DCs produce abundant IFN-α, and psoriatic skin has a strong type I interferon signature (i.e., expression of many interferon-inducible genes). One of the effects of IFN-α is activation of other DCs that are induced to migrate to lymph nodes, activate helper T cells of unknown antigen specificity, and induce their differentiation into skin-homing effector cells. These T cells circulate to the dermis and further promote an inflammatory cascade and persistent keratinocyte proliferation. Both T_H1 and T_H17 cells have been implicated in this phase of the disease. Various therapies have been approved or are in clinical trials targeting T cell migration and cytokines that drive T_H1 and T_H17 differentiation (IL-12, IL-23) or are produced by these T cells (IL-17, IL-22, TNF). A central unanswered question about this disease is the identity of the antigens recognized by the T cells.

Atopic dermatitis is a chronic inflammatory disease of the skin characterized by itchy rashes, in which IgE specific for environmental antigens and cells expressing high-affinity Fc receptors for IgE (FcεRI) play a central role. Antigen-mediated cross-linking of FcεRI on mast cells is important in many allergic diseases, as we will discuss in detail in Chapter 19, and mast cells are implicated in the pathogenesis of atopic dermatitis. In addition, FcεRI is expressed on skin DCs, including Langerhans cells and plasmacytoid DCs, and DC activation also plays a central role in atopic dermatitis, as in psoriasis. However, in atopic dermatitis, the response of DCs to TLR signaling is believed to be influenced by FcεRI signaling and by the cytokine thymic stromal lymphopoietin (TSLP), made by

keratinocytes, leading to an initial T_H2 response. Underlying the inflammatory cascade of atopic dermatitis may be genetically determined sensitivity to environmental antigens, perhaps because of impaired epithelial barrier function.

IMMUNE PRIVILEGED TISSUES

Immune responses and associated inflammation in certain parts of the body, including brain, eye, testes, placenta, and fetus, carry a high risk of lethal organ dysfunction or reproductive failure. These tissues, which have evolved to be protected, to a variable degree, from immune responses, are called **immune privileged sites**. The Nobel laureate immunologist Sir Peter Medawar coined the term *immune privilege* in the 1940s to describe the lack of immune responses to tissue transplanted into the brain or the anterior chamber of the eye of experimental animals. Foreign antigens that would evoke an immune response in most tissues are often tolerated in these immune privileged sites. The mechanisms underlying immune privilege vary between these tissues and are not fully understood. Some of the mechanisms are similar to mechanisms of regulation in gut and skin discussed earlier in this chapter and mechanisms of self tolerance discussed in Chapter 14. In this section of the chapter, we will discuss some of the distinguishing features of immune privilege in different tissues.

Immune Privilege in the Eye, Brain, and Testis

The Eye

Vision, which is essential to survival to most mammals, can be easily impaired by inflammation within the eye. Evolved mechanisms that minimize the likelihood of immune responses and inflammation in the eye have been most thoroughly described in the anterior chamber, a fluid-filled space between the transparent cornea in front and the iris and lens behind. Inflammation in this chamber could lead to opacification of the transparent cornea and lens, with loss of sight. At least some of the properties of immune privilege studied in the anterior chamber also apply to other ocular sites, such as the vitreous cavity and the subretinal space. Anatomic features of the anterior chamber that contribute to immune privilege include the tight junctions and resistance to leakiness of blood vessels in the tissues adjacent to the anterior chamber (the so-called blood-eye barrier), the avascular nature of the cornea, and the absence of lymphatics draining the anterior chamber, which limits access of the adaptive immune system to antigens in the eye. There are several soluble factors with immunosuppressive/anti-inflammatory properties in the aqueous humor that fills the anterior chamber, including neuropeptides (α-melanocyte–stimulating hormone, vasointestinal peptide, somatostatin), TGF-β, and indolamine 2,3-dioxygenase. Cells lining the anterior chamber, including the epithelium of the iris and the endothelium, constitutively express Fas ligand and PD-L1, which can induce death or inactivation of T cells, respectively.

Anterior chamber–associated immune deviation is a phenomenon in which introduction of foreign protein antigen into the anterior of the eye actively induces systemic tolerance to that antigen. This phenomenon presumably reduces the chance that adaptive immune responses will be mounted to foreign antigens that may be located in the eye. The tolerance is detectable as a diminished inflammatory T cell or antibody response to the same antigen when it is introduced at extraocular sites compared with the response in individuals who were not given intraocular antigen. Anterior chamber–associated immune deviation may be mediated by Treg. Studies in mice show that the antigen introduced in the anterior chamber is transported by macrophages or DCs, through the blood, to the spleen, and presented by splenic B cells to naive T cells, inducing the generation of regulatory T cells specific for the antigen.

In contrast to induced tolerance to foreign antigens introduced into the anterior chamber, self antigens in the eye are isolated from the immune system, and systemic tolerance to these antigens is not induced. This lack of tolerance becomes a problem only when eye trauma exposes the eye antigens to the immune system. A striking example of this is sympathetic ophthalmia, in which trauma to one eye causes release of eye antigens leading to autoimmune disease in both the injured eye and the uninjured eye. Presumably, although self antigens in the normal eye are inaccessible to the extraocular immune system to induce tolerance, activated immune effector cells and antibodies that are generated in the periphery when one eye is injured have access to and cause injury to the normal eye.

The Brain

Inflammation in the brain can lead to functional derangement and death of neurons, with disastrous consequences. Anatomic features of the brain that impair initiation of adaptive immunity to antigens include an absence of conventional lymphatic drainage and a scarcity of DCs. Delivery of immune cells and inflammatory mediators into the brain is impaired by the nature of the tight junctions between brain microvascular endothelial cells (the so-called blood-brain barrier). Some of the mechanisms operative in the eye may also apply to the brain, including the action of neuropeptides. The brain is rich in resident macrophages, called microglia, which become activated in response to tissue damage or infections in the brain. The threshold for their activation, however, may be higher than that of macrophages in other tissues. One putative mechanism for maintaining this high threshold is inhibitory signaling by the CD200 receptor, which is expressed by microglia. The ligand for this receptor, CD200, is highly expressed in the brain on neurons and other cell types.

Contrary to previously common assumptions based on classic experiments, there is evidence that immune surveillance against microbes does occur in the central nervous system. For example, the frequency of some opportunistic infections within the brain increases significantly in immunosuppressed patients. Patients treated with certain monoclonal antibodies that block lymphocyte and monocyte adhesion to endothelial cells have a

significantly increased although still small risk for activation of latent JC virus, leading to a uniformly fatal central nervous system disease called progressive multifocal leukoencephalopathy. This finding suggests that T cell or monocyte trafficking into the brain is necessary to keep latent viruses in check and argues that the brain is not a stringently immune privileged site.

The Testis

Immune privilege in the testis serves to limit inflammation that may impair male fertility. Many self antigens in the adult testis are first expressed at the time of puberty, well after the development of a competent immune system, which may include testis antigen–specific precursor T and B cells. Therefore, immune privilege in the testis may also serve to prevent autoimmunity. The testis, like the eye and brain, has a blood-tissue barrier with specialized endothelial tight junctions that limit delivery of cells and molecules to the sites of spermatogenesis. The hormonal milieu of the testis, which is rich in androgens, has an anti-inflammatory influence on macrophages. TGF-β is produced by Leydig, Sertoli, and peritubular cells and likely contributes to local immune suppression.

Immune Privilege of the Mammalian Fetus

The mammalian fetus expresses paternally inherited genes that are allogeneic to the mother, but fetuses are not normally rejected by the mother. In essence, the fetus is a naturally occurring allograft, but one that is protected from graft rejection. It is clear that the mother is exposed to fetal antigens during pregnancy because maternal antibodies against paternal MHC molecules are easily detectable. Obviously, there has been very strong selective pressure that has led to the evolution of mechanisms that protect the fetus from the maternal immune system, yet these mechanisms remain poorly understood. Probably several different special molecular and barrier features of the placenta and local immunosuppression contribute.

Several experimental observations indicate that the anatomic location of the fetus is a critical factor in the absence of rejection. For example, pregnant animals are able to recognize and reject allografts syngeneic to the fetus placed at extrauterine sites without compromising fetal survival. Wholly allogeneic fetal blastocysts that lack maternal genes can successfully develop in a pregnant or pseudopregnant mother. Thus, neither specific maternal nor paternal genes are necessary for survival of the fetus. Hyperimmunization of the mother with cells bearing paternal antigens does not compromise placental and fetal growth.

The failure to reject the fetus has focused attention on the region of physical contact between the mother and fetus. The fetal tissues of the placenta that most intimately contact the mother are composed of either vascular trophoblast, which is exposed to maternal blood for purposes of mediating nutrient exchange, or implantation site trophoblast, which diffusely infiltrates the uterine lining (decidua) for purposes of anchoring the placenta to the mother.

One simple explanation for fetal survival is that trophoblast cells fail to express paternal MHC molecules. Class II molecules have not been detected on trophoblast. In mice, cells of implantation trophoblast, but not of vascular trophoblast, do express paternal class I MHC molecules. In humans, the situation may be more complex in that trophoblast cells express only a nonpolymorphic class IB molecule called HLA-G. This molecule may be involved in protecting trophoblast cells from maternal NK cell–mediated lysis. A specialized subset of NK cells called uterine NK cells are the major type of lymphocyte present at implantation sites, and IFN-γ production by these cells is essential for decidual development. The way in which uterine NK cells are stimulated and their role in maternal responses to fetal alloantigens are not known. Even if trophoblast cells do express classical MHC molecules, they may lack costimulator molecules and fail to act as antigen-presenting cells.

The uterine decidua may be a site where immune responses are functionally inhibited. In support of the idea is the observation that mouse decidua is highly susceptible to infection by *Listeria monocytogenes* and cannot support a delayed-type hypersensitivity response. The basis of immunologic privilege is clearly not a simple anatomic barrier because maternal blood is in extensive contact with trophoblast. Rather, the barrier is likely to be created by functional inhibition. Cultured decidual cells directly inhibit macrophage and T cell functions, perhaps by producing inhibitory cytokines, such as TGF-β. Some of these inhibitory decidual cells may be resident regulatory T cells, although the evidence for this is limited. Some experiments have led to the suggestion that T_H2 cytokines are produced at the maternal-fetal interface and are responsible for local suppression of T_H1 responses to fetal antigens. However, this idea is not supported by the finding that IL-4 and IL-10 knockout mice have normal pregnancies. There is expansion of systemic and decidual Treg in mothers during pregnancy, and the fetus contains abundant regulatory T cells.

Immune responses to the fetus may be regulated by local concentrations of tryptophan and its metabolites in the decidua. The enzyme indolamine 2,3-dioxygenase (IDO) catabolizes tryptophan, and the IDO-inhibiting drug 1-methyl-tryptophan induces abortions in mice in a T cell–dependent manner. These observations led to the hypothesis that T cell responses to the fetus are normally blocked because decidual tryptophan levels are kept low or the levels of toxic metabolites are high.

Several other mechanisms may also dampen maternal immune response of the fetus, including FasL expression by fetal trophoblast cells that promote apoptosis of activated Fas-expressing maternal lymphocytes, generation of tolerogenic DCs in response to galectin-1 expressed in the decidua, and impaired DC migration from the uterus to lymph nodes.

Trophoblast and decidua may also be resistant to complement-mediated damage activation. In mice, these tissues express a C3 and C4 inhibitor called Crry. Crry-deficient embryos die before birth and show evidence of complement activation on trophoblast cells. Thus, this inhibitor may block maternal alloantibody- and complement-mediated damage. However, Crry or equivalent molecules have not been found in humans.

SUMMARY

- Regional immune systems are specialized collections of innate and adaptive immune cells and tissues at particular anatomic locations, which perform protective and regulatory functions that are unique to those sites. The main regional immune systems are in the gastrointestinal tract and skin, which together protect against microbial invasion across an enormous surface area of exposure to the environment.
- The gastrointestinal immune system must cope with the presence of trillions of commensal bacteria in the gut lumen by preventing their invasion and tolerating their presence in the lumen, while also identifying and responding to numerically rare pathogenic organisms.
- Innate immunity in the gastrointestinal system is mediated in part by the mucosal epithelial lining cells, which impede microbial invasion by tight intercellular junctions, secretion of mucus which prevents microbial attachment to the lining cells, and the production of antimicrobial molecules, such as defensins. Innate immune effector cells in the lamina propria include macrophages, DCs, and mast cells. Intraepithelial lymphocytes, including γδ T cells, also provide innate defense against commonly a encountered microbes at the intestinal epithelial barrier. Specialized anatomic features of adaptive immunity in the intestinal tract include collections of lymphoid tissues just below the epithelial lining that comprise the gut-associated lymphoid tissues (GALT), including oropharyngeal tonsils, Peyer's patches in the ileum, and similar collections in the colon. M cells in the epithelial lining sample lumen antigens and transport them to antigen-presenting cells in the GALT. Lamina propria DCs extend processes through intestinal epithelial lining cells to sample lumenal antigens. There are also diffuse effector lymphocytes in the lamina propria of the gut and in mesenteric lymph nodes, where many of the adaptive immune responses in the bowel wall are initiated.
- Effector B and T lymphocytes that differentiate from naive T cells in the GALT or mesenteric lymph nodes enter the circulation, and selectively migrate back to the intestinal lamina propria. This tissue-specific homing is due to signals the naive T cells receive from DCs in the GALT and mesenteric nodes, including retinoic acid derived from dietary Vitamin A, which induce expression of chemokine receptors and adhesion molecules on the differentiated effector cells that favor homing back to the gut.
- Humoral immunity in the gastrointestinal tract is dominated by IgA secretion into the lumen, where the antibodies neutralize potentially invading pathogens. B cells in the GALT and mesenteric lymph nodes differentiate into IgA-secreting plasma cells under the influence of TGFβ, BAFF, and other cytokines, through both T-dependent and T-independent mechanisms, and the plasma cells migrate to the lamina propria beneath the epithelial barrier and secrete IgA. Dimerized IgA is transported across the epithelium by the poly-Ig-receptor and released into the lumen. IgA is also secreted into breast milk, and mediates passive immunity in the gut of breastfeeding infants.
- T_H17 cells play a dominant role in T cell–mediated immunity in the intestinal tract, in part because IL-17 and IL-22, which they secrete, enhance epithelial barrier function. T_H2 cells are important in defense against intestinal parasites. Changes in bacterial flora can influence the balance between different helper T cell subset responses, both in the gut and systemically throughout the adaptive immune system.
- Immune reposes to commensal organisms and food antigens in the lumen of the intestinal tract are minimized by a variety of mechanisms, including selective expression of pattern recognition receptors in the cytoplasm and basolateral surfaces of the epithelial lining cells, and sampling of luminal microbial antigen by DCs which are specialized to induce Treg that suppress adaptive immune responses. Several cytokines are essential to maintain immune homeostasis is the bowel wall, including TGFβ, IL-10, and IL-2. Systemic tolerance to some antigens can be induced by feeding the antigens to mice, a phenomenon called oral tolerance.
- Several intestinal diseases are related to abnormal immune responses, including inflammatory bowel diseases (Crohn's disease and ulcerative colitis), in which innate and adaptive immune responses to normal gut flora are not adequately regulated, and celiac disease, in which humoral and cell-mediated responses to dietary wheat glutens occur.
- Mucosal immunity in the respiratory system defends against airborne pathogens, and is the cause of allergic airway diseases, such as asthma. Innate immunity in the bronchial tree depends on the ciliated epithelial lining, which secretes mucus and defensins and moves the mucus with entrapped microbes out of the lungs. Surfactant proteins and alveolar macrophages provide both antimicrobial and anti-inflammatory functions. As in the intestines, regulatory mechanisms, including Treg and immunosuppressive cytokines are important for prevention harmful responses to nonpathogenic organisms or other inhaled antigens inhaled antigens.
- The cutaneous immune system defends against microbial invasion through the skin, and suppresses responses against numerous commensal organisms. The outer multilayered keratinized squamous epithelial layer, called the epidermis, performs innate immune defense functions, providing a physical barrier to microbial invasion. Keratinocytes secrete defensins as well as inflammatory cytokines in response to various PAMPs and DAMPs. The dermis contains a mixed

population of mast cells, macrophages, and DCs that respond to microbes and injury and mediate inflammatory responses.

- DCs are abundant in the skin and mediate innate immune responses. These DCs also transport microbial and environmental antigens that enter through the skin to draining lymph nodes where they initiate T cells responses. Langerhans cells are the main DC type in the epidermis, and there are several different dermal DC subsets. Skin-derived DCs provide signals, including Vitamin D, to the naive T cells they activate in draining lymph, which induce chemokines and adhesion molecules that favor homing of the effector T cells back to the skin.
- A significant fraction of the body's T cells are present in the skin. Most of these T cells are $CD4^+$ or $CD8^+$ effectors or memory T cells in the dermis. T_H1, T_H2, and T_H17 cells are important for defense against different types of skin-invading pathogens, while T_H1 and T_H17 cells contribute to inflammatory dermatoses such as psoriasis.
- Immune privileged sites, which are tissues where immune responses are not readily initiated, include the brain, anterior chamber of the eye, and testis. The mechanisms of immune privilege include the tight junctions of endothelial cells in blood vessels at these sites, local production of immunosuppressive cytokines, and expression of cell surface molecules that inactivate or kill lymphocytes.
- Mammals have evolved mechanisms that prevent maternal immunologic rejection of the developing fetus, which invariably expresses paternal antigens that are allogeneic to the mother. These mechanisms appear to act locally at the placental maternal-fetal interface, since systemic maternal tolerance to paternal alloantigens does not occur. Possible mechanisms include lack of MHC expression on fetal trophoblasts, expression of immunosuppressive cytokines, the actions of Treg, and local indolamine 2,3-dioxygenase mediated depletion of tryptophan needed for lymphocyte growth.

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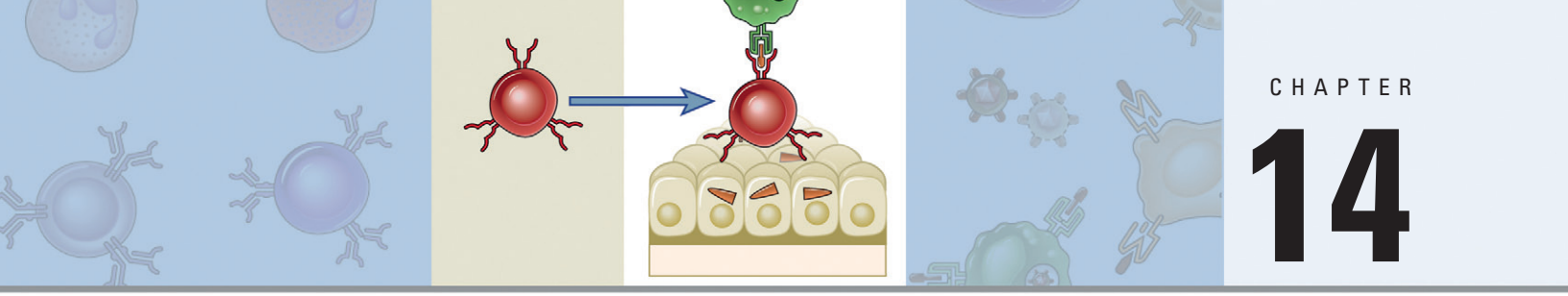
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CHAPTER

14

Immunologic Tolerance and Autoimmunity

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Immunologic tolerance is defined as unresponsiveness to an antigen that is induced by previous exposure to that antigen. When specific lymphocytes encounter antigens, the lymphocytes may be activated, leading to immune responses, or the cells may be inactivated or eliminated, leading to tolerance. Different forms of the same antigen may induce an immune response or tolerance. Antigens that induce tolerance are called **tolerogens**, or tolerogenic antigens, to distinguish them from **immunogens**, which generate immunity. A single antigen may be an immunogen or a tolerogen, depending on the conditions in which it is displayed to specific lymphocytes (e.g., in the presence or absence, respectively, of inflammation and innate immune responses). Tolerance to self antigens, also called **self-tolerance**, is a fundamental property of the normal immune system, and failure of self-tolerance results in immune reactions against self (autologous) antigens. Such reactions are called **autoimmunity**, and the diseases they cause are called **autoimmune diseases**. Elucidating the mechanisms of self-tolerance is the key to understanding the pathogenesis of autoimmunity.

In this chapter, we discuss immunologic tolerance mainly in the context of self-tolerance and how self-tolerance may fail, resulting in autoimmunity. We also mention the relevance of tolerance to unresponsiveness to foreign antigens and the potential of tolerance induction as a therapeutic strategy for immunologic diseases and to prevent the rejection of cell and organ transplants. Because of the importance of self-tolerance for the health of individuals and the therapeutic promise of tolerance, there has been great interest in understanding this phenomenon and learning how to apply it to humans.

GENERAL FEATURES OF IMMUNOLOGIC TOLERANCE

There are several characteristics of tolerance in T and B lymphocyte populations. It is important to appreciate the general principles before we discuss the specific mechanisms of tolerance in these lymphocytes.

- ***Normal individuals are tolerant of their own (self) antigens because the lymphocytes that recognize self antigens are killed or inactivated or the specificity of these lymphocytes is changed.*** All individuals inherit essentially the same antigen receptor gene segments, and these recombine and are expressed in lymphocytes as they arise from stem cells. The specificities of the receptors encoded by the recombined genes are random, and are not influenced by what is foreign or self for each individual (see Chapter 8). It is not surprising that during this process of generating a large and diverse repertoire, some developing T and B cells in every individual may express receptors capable of recognizing normal molecules in that individual (i.e., self antigens). Therefore, there is a risk for lymphocytes to react against that individual's cells and tissues, causing disease. The mechanisms of immunologic tolerance are designed to prevent such reactions.

The importance of self-tolerance for the health of individuals was appreciated from the early days of immunology. In Chapter 1, we introduced the concept of self-nonself discrimination, which is the ability of the immune system to recognize and respond to foreign antigens but not to self antigens. Macfarlane Burnet added to his clonal selection hypothesis the corollary that lymphocytes specific for self antigens are eliminated to prevent immune reactions against one's own tissues. As we shall see later in this chapter, self-tolerance is maintained by several different mechanisms that prevent the maturation and activation of potentially harmful self-reactive lymphocytes.

- ***Tolerance results from the recognition of antigens by specific lymphocytes.*** In other words, tolerance, in its strict definition, is antigen specific. This contrasts with therapeutic immunosuppression and inherited or acquired immunodeficiencies, which affect lymphocytes of many specificities. The key advances that allowed immunologists to study tolerance were induction of this phenomenon in animals by exposure to defined antigens under various conditions and analysis of the functions of the lymphocytes that had encountered tolerogenic antigens. The results that definitively established tolerance as an immunologically specific phenomenon that could be induced experimentally came from studies of graft rejection in inbred mice done by Peter Medawar and colleagues in the 1950s. An adult mouse of strain A will reject a skin graft from an allogeneic mouse of strain B that differs from strain A at the major histocompatibility complex (MHC). If the strain A mouse is injected with white blood cells of strain B during neonatal life (the cells serving as a source of strain B antigens), the injected cells will not be rejected (because the neonate is immunodeficient), and small numbers will survive indefinitely in the recipient. The persistence of allogeneic lymphoid cells in a host is called hematopoietic microchimerism. This strain A recipient will accept a graft from strain B even after it becomes an adult. However, the strain A recipient will reject skin grafts from all mouse strains whose MHC is different from that of strain B. Thus, tolerance to the graft is immunologically specific. Such experiments led to the concept that exposure of developing lymphocytes to foreign antigens induces tolerance to these antigens. Microchimerism is now being studied as a possible approach for preventing graft rejection in humans (see Chapter 16).
- ***Self-tolerance may be induced in immature self-reactive lymphocytes in the generative lymphoid organs (central tolerance) or in mature lymphocytes in peripheral sites (peripheral tolerance)*** (Fig. 14-1). Central tolerance ensures that the repertoire of mature lymphocytes becomes incapable of responding to self antigens that are expressed in the generative lymphoid organs (the thymus for T cells and the bone marrow for B lymphocytes, also called central lymphoid organs). However, central tolerance is not perfect, and it cannot account for unresponsiveness to antigens that are expressed only in peripheral tissues. Tolerance to such tissue-specific self antigens is maintained by peripheral mechanisms. Additional mechanisms of peripheral tolerance work in peripheral tissues to prevent activation of self-reactive lymphocytes that may have escaped central tolerance.
- ***Central tolerance occurs during the maturation of lymphocytes in the central (generative) lymphoid organs, where all developing lymphocytes pass through a stage at which encounter with antigen may lead to cell death or replacement of a self-reactive antigen receptor with a new one.*** The generative lymphoid organs contain mostly self antigens and not foreign antigens because foreign (e.g., microbial) antigens that enter from the external environment are typically captured and taken to peripheral lymphoid organs, such as the lymph nodes, spleen, and mucosal lymphoid tissues, and are not transported to the thymus or bone marrow. The antigens normally present in the thymus and bone marrow include ubiquitous, or widely disseminated, self antigens including those bought in by the blood. In addition, some peripheral tissue-specific antigens are expressed in specialized cells in the thymus. Therefore, in the generative lymphoid organs, the immature lymphocytes that specifically recognize antigens are typically cells specific for self, and not foreign, antigens. Strong recognition of self antigens by immature lymphocytes has several possible outcomes: the cells may die by apoptosis (called clonal deletion or negative selection because this process selects clones of antigen-specific cells for elimination); many immature B cells do not die but change their receptors (called receptor editing) and thus no longer recognize the self antigen that triggered this process; and some $CD4^+$ T cells differentiate into regulatory T cells, which migrate to the periphery and prevent responses to the self antigens (see Fig. 14-1).
- ***Peripheral tolerance occurs when, as a consequence of recognizing self antigens, mature lymphocytes become incapable of responding to that antigen, or are induced to die by apoptosis, or mature T cells are actively suppressed by regulatory T cells.*** Peripheral tolerance is most important for maintaining unresponsiveness to self antigens that are expressed in peripheral tissues and not in the generative lymphoid organs and for tolerance to self antigens that are expressed only in adult life, after mature lymphocytes have been generated. Peripheral mechanisms may also serve as a back-up for the central mechanisms, which may not eliminate all self-reactive lymphocytes.
- ***Whether lymphocytes that recognize antigens become activated or tolerant is determined by the properties of the antigens, the state of maturation of the antigen-specific lymphocytes, and the types of stimuli received when these lymphocytes encounter self antigens.*** As we shall see in this chapter, these factors affect in different ways the fates of lymphocytes that encounter their cognate antigens.
- ***Some self antigens may be ignored by the immune system.*** The importance of this phenomenon of "ignorance" for the maintenance of self-tolerance is not established. Some antigens may be anatomically sequestered from the immune system and thus cannot

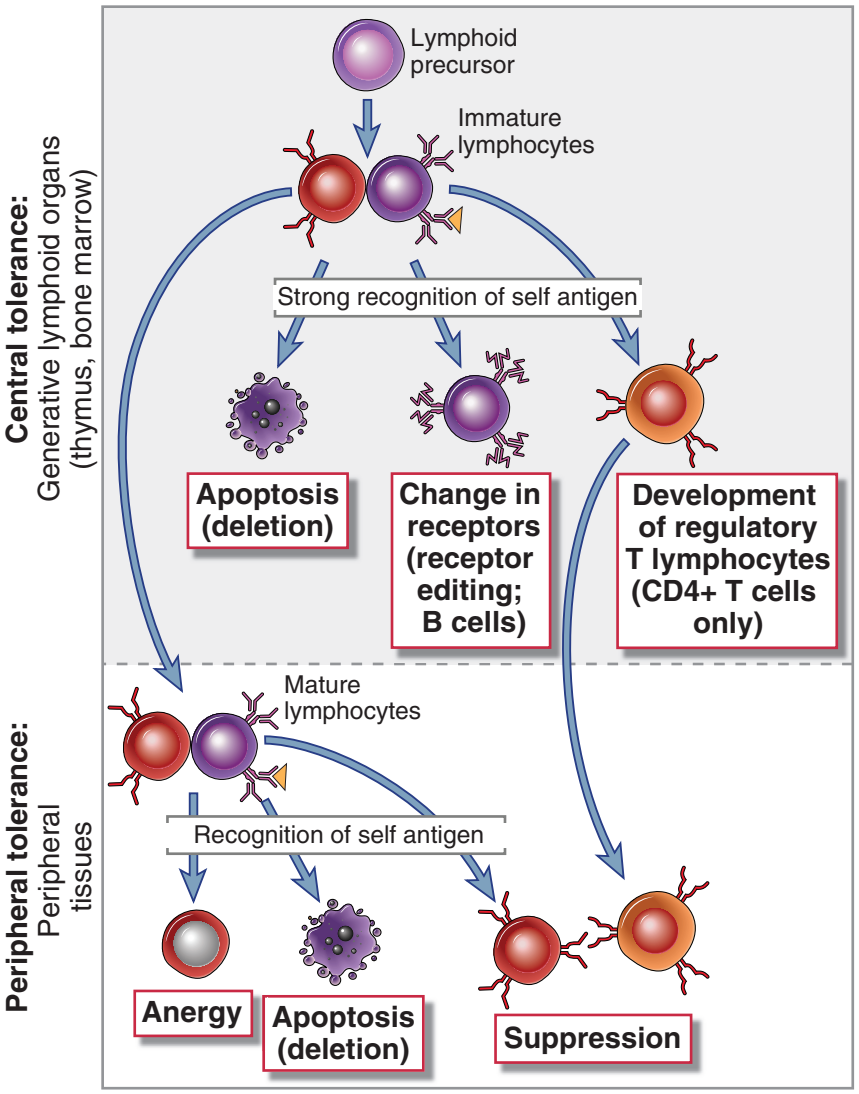


FIGURE 14–1 Central and peripheral tolerance to self antigens. Immature lymphocytes specific for self antigens may encounter these antigens in the generative lymphoid organs and are deleted, change their specificity (B cells only), or (in the case of $CD4^+$ T cells) develop into regulatory lymphocytes (central tolerance). Some self-reactive lymphocytes may mature and enter peripheral tissues and may be inactivated or deleted by encounter with self antigens in these tissues or are suppressed by the regulatory T cells (peripheral tolerance). (Note that T cells recognize antigens presented by antigen-presenting cells, which are not shown.)

engage antigen receptors. In experimental models, some self antigens are recognized by lymphocytes but, for unknown reasons, fail to elicit any response and are functionally ignored.

- ***Foreign antigens in the absence of costimulatory signals may inhibit immune responses by inducing tolerance in specific lymphocytes.*** Many of the mechanisms of tolerance to foreign antigens are similar to those of self-tolerance in mature lymphocytes. Effective immunization methods are designed to enhance the immunogenicity of antigens by administering them in ways that promote lymphocyte activation and prevent tolerance induction. Some microbes and tumors may also evade immune attack by inducing unresponsiveness in specific lymphocytes.
- ***The induction of immunologic tolerance may be exploited as a therapeutic approach for preventing harmful immune responses.*** A great deal of effort is being devoted to the development of strategies for inducing tolerance to treat autoimmune and allergic diseases and to prevent the rejection of organ transplants. Tolerance induction may also be useful for preventing immune reactions to the products of newly expressed genes in gene therapy protocols, for preventing reactions to injected proteins in patients with deficiencies of these proteins (e.g., hemophiliacs treated with factor VIII), and for promoting acceptance of stem cell transplants.

We do not know which self antigens induce central or peripheral tolerance (or are ignored). It is technically difficult to identify rare cells that may be specific for natural self antigens because reagents for detecting antigen-specific lymphocytes are not widely used and few self antigens are defined for which such reagents could even be produced. Experimental approaches, especially the creation of genetically modified mice, have provided valuable models for analysis of self-tolerance, and many of our current concepts are based on studies with such models. Furthermore, by identifying genes that may be associated with autoimmunity in mice and humans, it has been possible to deduce some of the critical

mechanisms of self-tolerance. In the sections that follow, we will discuss central and peripheral tolerance first in T cells and then in B lymphocytes, but many aspects of the processes are common to both lineages.

T LYMPHOCYTE TOLERANCE

Tolerance in $CD4^+$ helper T lymphocytes is an effective way of preventing immune responses to protein antigens because helper T cells are necessary inducers of both cell-mediated and humoral immune responses to proteins. This realization has been the impetus for a large amount of work on the mechanisms of tolerance in $CD4^+$ T cells. Immunologists have also developed experimental models for studying tolerance in $CD4^+$ T cells that have proved to be quite informative. Also, many of the therapeutic strategies that are being developed to induce tolerance to transplants and autoantigens are targeted to these T cells. Therefore, much of the following discussion, especially of peripheral tolerance, focuses on $CD4^+$ T cells. Less is known about peripheral tolerance in $CD8^+$ T cells, and this is summarized at the end of the section.

Central Tolerance in T Cells

During their maturation in the thymus, many immature T cells that recognize antigens with high avidity are deleted and some of the surviving cells in the $CD4^+$ lineage develop into regulatory T cells (Fig. 14-2). The process of deletion, or negative selection, of T lymphocytes was described in Chapter 8, when the maturation of T cells in the thymus was discussed. This process affects both class I and class II MHC–restricted T cells and is therefore important for tolerance in both $CD8^+$ and $CD4^+$ lymphocyte populations. Negative selection of thymocytes is responsible for the fact that the repertoire of mature T cells that leave the thymus and populate peripheral lymphoid tissues is unresponsive to the self antigens that are present in the thymus. The two main factors that determine if a particular self antigen will induce negative selection of self-reactive thymocytes are the presence of that antigen in the thymus, either by local expression or delivery by the blood, and the affinity of the thymocyte T cell receptors (TCRs) that recognize the antigen. Thus, the important questions that are relevant to negative selection are what self antigens are present in the thymus and how are immature T cells that recognize these antigens killed.

Self proteins are processed and presented in association with MHC molecules on thymic antigen-presenting cells (APCs). The antigens that are present in the thymus include many circulating and cell-associated proteins that are widely distributed in tissues. The thymus also has an unusual mechanism for expressing protein antigens that are typically present only in certain peripheral tissues, so that immature T cells specific for these antigens can be deleted from the developing T cell repertoire. Some of these peripheral tissue antigens are expressed in thymic medullary epithelial cells under the control of the **autoimmune regulator (AIRE)** protein. Mutations in the *AIRE* gene are the cause of a multiorgan autoimmune disease called the autoimmune polyendocrine syndrome (APS). This group of diseases is characterized by antibody- and lymphocyte-mediated injury to multiple endocrine organs, including the parathyroids, adrenals, and pancreatic islets. A mouse model of APS has been developed by knockout of the *AIRE* gene, and it recapitulates many of the features of the human disease. Studies with mice have shown that several proteins that are produced in peripheral organs (such as pancreatic insulin) are also normally expressed at low levels in thymic medullary epithelial cells, and immature T cells that recognize these antigens are deleted in the thymus. In the absence of functional AIRE (as in the patients and knockout mice) these antigens are not displayed in the thymus, and T cells specific for the antigens escape deletion, mature, and enter the periphery, where they attack the target tissues in which the antigens are expressed independent of AIRE. The AIRE protein may function as a transcription factor to promote the expression of selected tissue antigens in the thymus. It is a component of a multiprotein

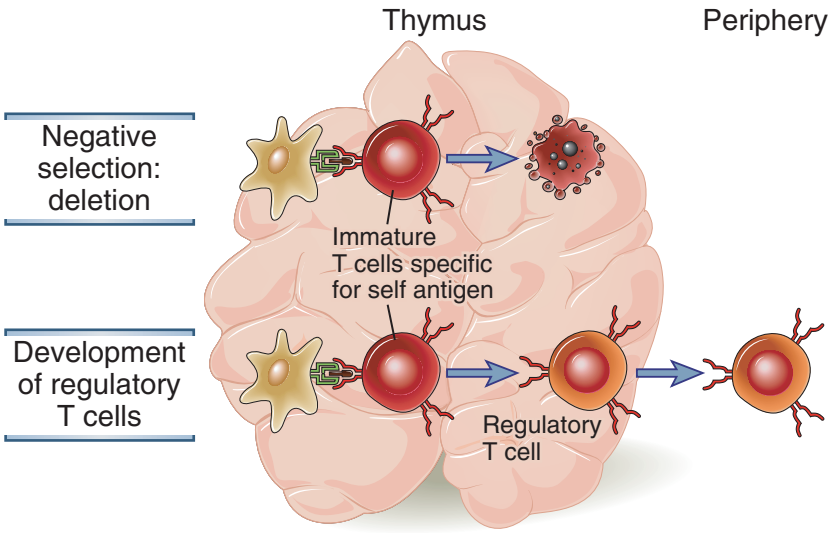


FIGURE 14–2 Central T cell tolerance. Recognition of self antigens by immature T cells in the thymus may lead to death of the cells (negative selection, or deletion) or the development of regulatory T cells that enter peripheral tissues.

complex that is involved in transcriptional elongation and chromatin unwinding and remodeling. AIRE also contributes to pre-mRNA processing and induces the accumulation of processed spliced mRNAs (as opposed to unspliced mRNAs) of genes encoding peripheral tissue antigens. There is also evidence for AIRE-independent mechanisms of deletion in the thymus.

Many immature thymocytes with high-affinity receptors for self antigens that encounter these antigens in the thymus die by apoptosis. Negative selection occurs in double-positive T cells in the thymic cortex or newly generated single-positive cells in the medulla. In these locations, immature thymocytes with high-affinity receptors for self antigens encounter these antigens and die by apoptosis. T cell receptor (TCR) signaling in immature T cells leads to the activation of a protein called Bim, which triggers the mitochondrial pathway of apoptosis. The mechanisms of apoptosis are described later in the chapter, when we discuss deletion as a mechanism of peripheral T cell tolerance. Clearly, immature and mature lymphocytes interpret antigen receptor signals differently—the former die and the latter are activated. The biochemical basis of this difference is not known.

Some self-reactive CD4$^+$ T cells that see self antigens in the thymus are not deleted but instead differentiate into regulatory T cells specific for these antigens (see Fig. 14-2). The regulatory cells leave the thymus and inhibit responses against self tissues in the periphery. Interestingly, deficiency of the AIRE protein, which interferes with deletion of T cells reactive with some antigens in the thymus, does not appear to prevent the development of thymic regulatory T cells specific for the same self antigens. This observation suggests that the requirements for T cell deletion and regulatory T cell development in the thymus are different, but what determines the choice between cell death and development of regulatory T cells is not known. The characteristics and functions of regulatory T cells are described later in the context of peripheral tolerance because these cells suppress immune responses in the periphery.

Although the importance of central T cell tolerance has been clearly established in animal models, and the autoimmune polyendocrine syndrome suggests that it has a fundamental role for tolerance to some peripheral tissue antigens, it is still not known if a failure of central tolerance contributes to common human autoimmune diseases.

Peripheral T Cell Tolerance

Peripheral tolerance is the mechanism by which mature T cells that recognize self antigens in peripheral tissues are rendered incapable of subsequently responding to these antigens. Peripheral tolerance mechanisms may be responsible for T cell tolerance to tissue-specific self antigens, especially those that are not abundant in the thymus. The same mechanisms may induce unresponsiveness to tolerogenic forms of foreign antigens. The mechanisms of peripheral tolerance are anergy (functional unresponsiveness), suppression, and deletion (cell death) (Fig. 14-3). We do not know if tolerance to different self antigens is maintained by one or another mechanism or if all these mechanisms function cooperatively to prevent dangerous autoimmunity.

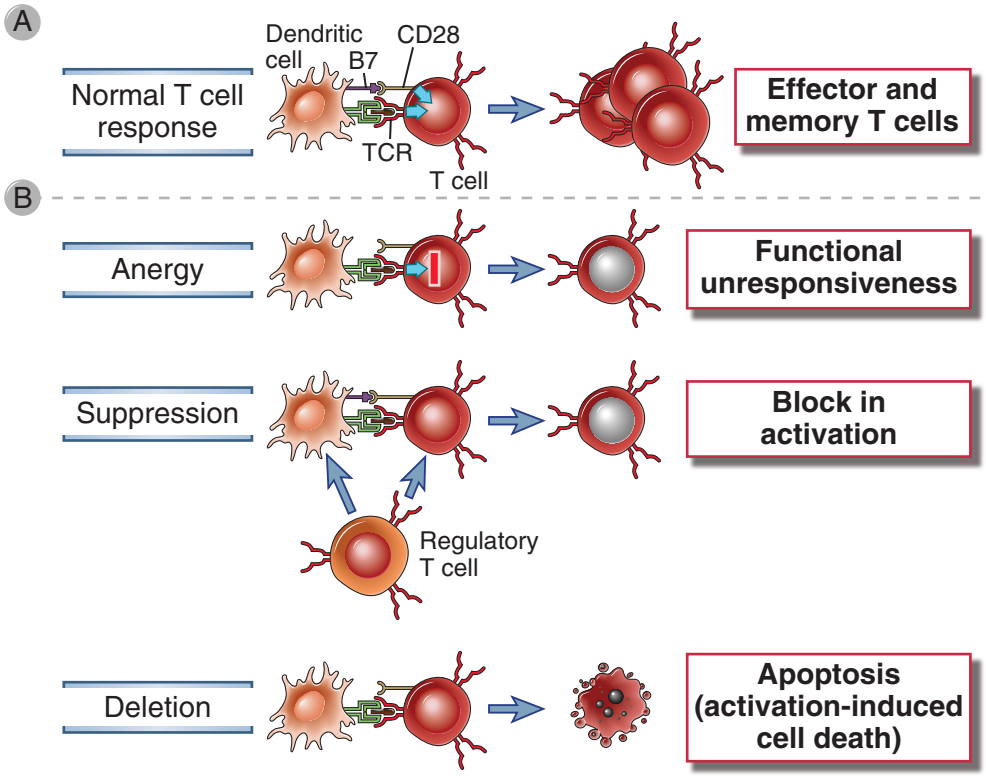


FIGURE 14–3 Mechanisms of peripheral T cell tolerance. The signals involved in a normal immune response **(A)** and the three major mechanisms of peripheral T cell tolerance **(B)** are illustrated.

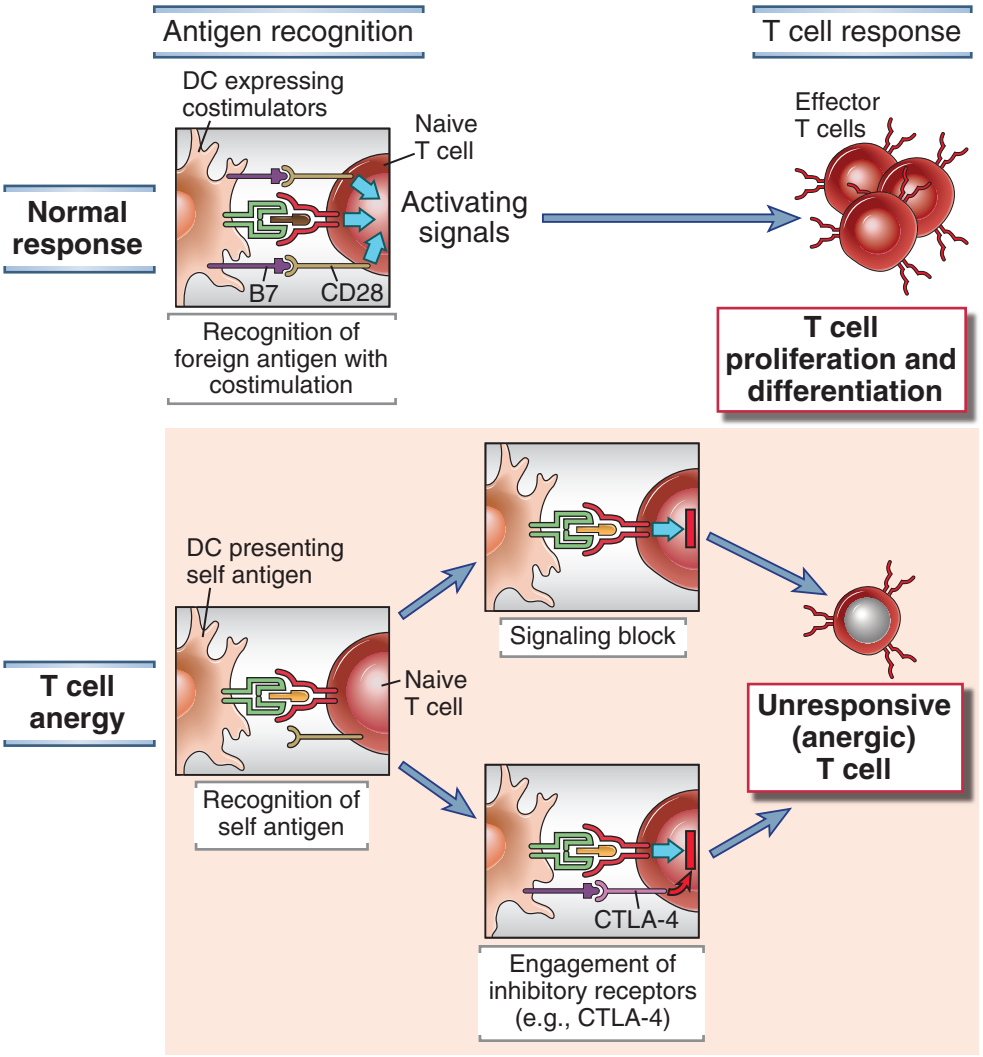


FIGURE 14–4 Mechanisms of T cell anergy. T cell responses are induced when the cells recognize an antigen presented by a professional antigen-presenting cell (APC) and activating receptors on the T cells (such as CD28) recognize costimulators on the APCs (such as B7). If the T cell recognizes a self antigen without costimulation, the T cell becomes unresponsive to the antigen because of a block in signaling from the TCR complex or engagement of inhibitory receptors (such as CTLA-4). The signaling block may be the result of recruitment of phosphatases to the TCR complex or the activation of ubiquitin ligases that degrade signaling proteins. The T cell remains viable but is unable to respond to the self antigen. DC, dendritic cell.

Anergy (Functional Unresponsiveness)

Exposure of mature CD4$^+$ T cells to an antigen in the absence of costimulation or innate immunity may make the cells incapable of responding to that antigen. In this process, the self-reactive cells do not die but become unresponsive to the antigen. We previously introduced the concept that full activation of T cells requires the recognition of antigen by the TCR (which provides signal 1) and recognition of costimulators, mainly B7-1 and B7-2, by CD28 (signal 2) (see Chapter 9). Prolonged signal 1 (i.e., antigen recognition) alone may lead to anergy. It is likely that self antigens are displayed to specific T cells in the absence of innate immunity and strong costimulation. Antigen-induced anergy has been demonstrated in a variety of experimental models, including studies with T cell clones exposed to antigens in vitro (which were the basis for the original definition of anergy), experiments in which antigens are administered to mice without adjuvants, and studies with transgenic mice in which particular protein antigens are expressed throughout life and are recognized by T cells in the absence of the inflammation and innate immune responses that normally accompany exposure to microbes. In many of these situations, the T cells that recognize the antigens become functionally unresponsive and survive for days or weeks in a quiescent state.

Anergy results from biochemical alterations that reduce the ability of lymphocytes to respond to signals from their antigen receptors (Fig. 14-4). It is believed that several biochemical pathways cooperate to maintain this unresponsive state.

- ***Anergic cells show a block in TCR-induced signal transduction.*** The mechanisms of this signaling block are not fully known. In different experimental models,

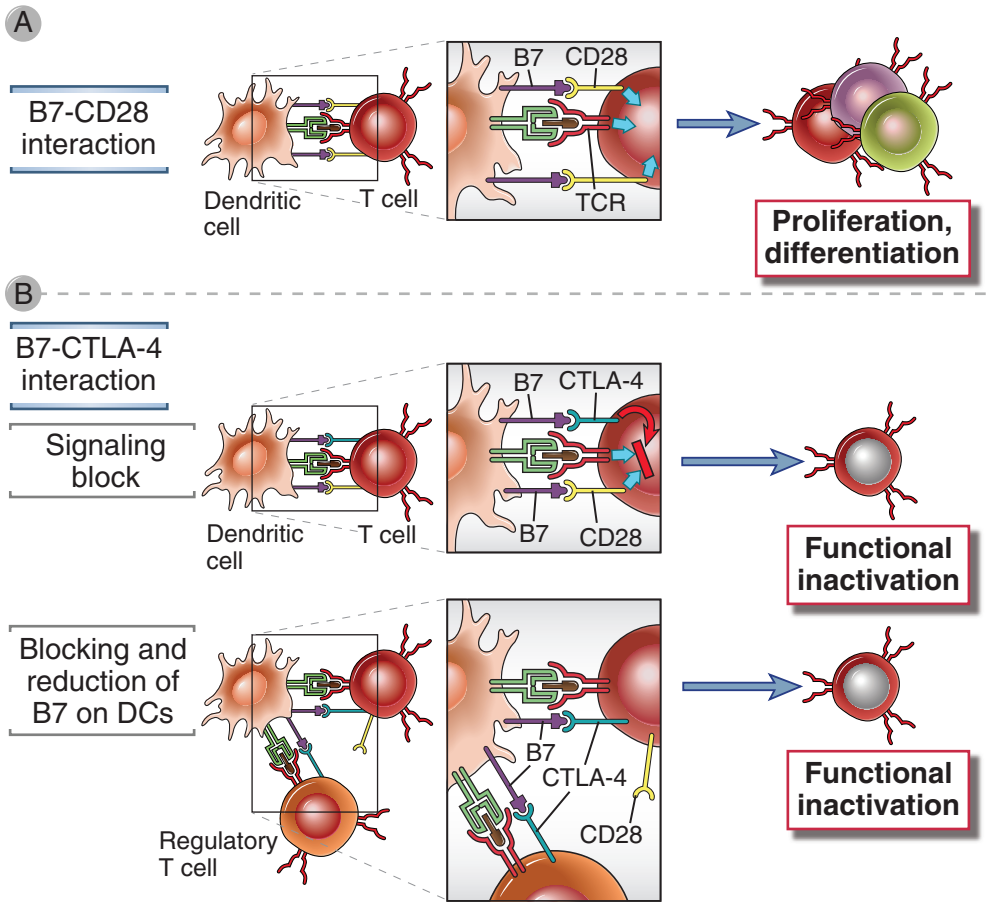


FIGURE 14–5 Mechanisms of action of CTLA-4. A, The top panel shows the activation of T cells by antigen recognition and costimulation through CD28. **B,** The bottom panel shows the two postulated mechanisms of action of CTLA-4: delivery of inhibitory signals that block TCR- and CD28-mediated signals, and engagement of B7 molecules on APCs so they are inaccessible to CD28. Note that regulatory T cells (described later in the chapter) may also use CTLA-4 to block B7 and thus inhibit immune responses. There is some evidence that in addition to blocking B7, CTLA-4 may remove these molecules from the APC surface and internalize them (not shown).

it is attributable to decreased TCR expression (perhaps because of increased degradation; see later) and recruitment to the TCR complex of inhibitory molecules such as tyrosine phosphatases.

- ***Self antigen recognition may activate cellular ubiquitin ligases, which ubiquitinate TCR-associated proteins and target them for proteolytic degradation in proteasomes or lysosomes.*** The net result is loss of these signaling molecules and defective T cell activation. One ubiquitin ligase that is important in T cells is called Cbl-b. Mice in which Cbl-b is knocked out show spontaneous T cell proliferation and manifestations of autoimmunity, suggesting that this enzyme is involved in maintaining T cell unresponsiveness to self antigens. It is not known why self antigen recognition, which occurs typically without strong costimulation, activates these ubiquitin ligases, whereas foreign antigens that are recognized with costimulation do so much less or not at all.
- ***When T cells recognize self antigens, they may engage inhibitory receptors of the CD28 family, whose function is to terminate T cell responses.*** In Chapter 9, we introduced the general concept that the outcome of antigen recognition by T cells, particularly $CD4^+$ cells, is determined by a balance between engagement of activating and inhibitory receptors. Although many inhibitory receptors have been described, the two whose physiologic role in self-tolerance is best established are CTLA-4 and PD-1 (see Fig. 9-5, Chapter 9).

CTLA-4, like the activating receptor CD28, binds to B7 molecules. CTLA-4 has a higher affinity than CD28 for B7 molecules and thus prevents B7 costimulators on APCs from engaging CD28; it may also remove B7 molecules from the surface of APCs (Fig. 14-5). In addition, CTLA-4 delivers inhibitory signals that negate the signals triggered by the TCR. In fact, the cytoplasmic tail of CTLA-4 has a potentially inhibitory motif that may counteract ITAM-dependent signals from the TCR and CD28. As we shall discuss later, CTLA-4 is also a mediator of the inhibitory function of regulatory T cells. The importance of CTLA-4 in tolerance induction is illustrated by the finding that knockout mice lacking CTLA-4 develop uncontrolled lymphocyte activation with massively enlarged lymph nodes and spleen and fatal multiorgan lymphocytic infiltrates suggestive of systemic autoimmunity. In other words, elimination of this one control mechanism results in a severe T cell–mediated disease,

presumably because of defects in both T cell anergy and suppression by regulatory T cells. Blocking of CTLA-4 with antibodies also enhances autoimmune diseases in animal models, such as encephalomyelitis induced by immunization with myelin antigens and diabetes induced by T cells reactive with antigens in the β cells of pancreatic islets. In clinical trials of anti–CTLA-4 antibody to boost immune responses to cancers, some of the treated patients develop manifestations of autoimmunity with inflammation in various organs. Polymorphisms in the *CTLA4* gene are associated with several autoimmune diseases in humans, including type 1 diabetes and Graves' disease. All these findings indicate that CTLA-4 functions continuously to keep self-reactive T cells in check.

Another inhibitory receptor of the CD28 family is **PD-1** (programmed cell death 1, so called because it was originally thought to be involved in programmed cell death but now known to not have a role in T cell apoptosis). PD-1 recognizes two ligands, called PD-L1 and PD-L2; PD-L1 is expressed on APCs and many other tissue cells and PD-L2 mainly on APCs. Engagement of PD-1 by either ligand leads to inactivation of the T cells. Mice in which PD-1 is knocked out develop autoimmune diseases, including lupus-like kidney disease and arthritis in different inbred strains. The autoimmune disorders in PD-1 knockout mice are less severe than in CTLA-4 knockouts. It has been postulated that CTLA-4 functions mainly to control initial T cell activation in lymphoid organs whereas PD-1 is more important for limiting responses of differentiated effector cells in peripheral tissues.

How the balance of activating and inhibitory receptor signaling is normally regulated is not understood. As we mentioned in Chapter 9, one possible explanation for the engagement of CTLA-4 vs. CD28 by B7 molecules is that APCs that are presenting self antigens normally express low levels of B7-1 and B7-2, which is sufficient to engage the high-affinity inhibitory receptor CTLA-4. In contrast, microbes activate the APCs to increase the expression of B7 costimulators, and CD28, which has a lower affinity for B7 molecules than does CTLA-4, is engaged at these higher levels of B7 expression. This might explain why self antigen recognition tilts the balance toward CTLA-4, whereas microbial infections induce relatively more CD28 signals.

Dendritic cells that are resident in lymphoid organs and nonlymphoid tissues may present self antigens to T lymphocytes and maintain tolerance. Tissue dendritic cells are normally in a resting (immature) state and express few or no costimulators. Such APCs may be constantly presenting self antigens without activating signals, and T cells that recognize these antigens become anergic. There is also some evidence that resting dendritic cells tend to promote the development of regulatory T lymphocytes instead of effector and memory lymphocytes. By contrast, dendritic cells that are activated by microbes are the principal APCs for initiation of T cell responses (see Chapter 6). As we shall discuss later, local infections and inflammation may activate resident dendritic cells, leading to increased expression of costimulators, breakdown of tolerance, and autoimmune reactions against tissue antigens. The characteristics of dendritic cells that make them tolerogenic are not defined but presumably include low expression of costimulators. There is great interest in manipulating the properties of dendritic cells as a way of enhancing or inhibiting immune responses for therapeutic purposes.

Suppression of Self-Reactive Lymphocytes by Regulatory T Cells

The concept that some lymphocytes could control the responses of other lymphocytes was proposed many years ago and was soon followed by experimental demonstrations of populations of T lymphocytes that suppressed immune responses. These initial findings led to enormous interest in the topic, and "suppressor T cells" became one of the dominant themes of immunology in the 1970s. However, this field of research has had a somewhat checkered history, mainly because initial attempts to define populations of suppressor cells and their mechanisms of action were largely unsuccessful. More than 20 years later, the idea had an impressive rebirth, with the application of better approaches to define, purify, and analyze populations of T lymphocytes that inhibit immune responses. These cells are called regulatory T lymphocytes; their properties and functions are described next.

Regulatory T lymphocytes are a subset of $CD4^+$ T cells whose function is to suppress immune responses and maintain self-tolerance (Fig. 14-6). The majority of these $CD4^+$ regulatory T lymphocytes express high levels of the interleukin-2 (IL-2) receptor α chain (CD25) but not other markers of T cell activation. A transcription factor called FoxP3, a member of the forkhead family of transcription factors, is critical for the development and function of the majority of regulatory T cells. Mice with mutations in the *foxp3* gene, and mice in which this gene has been knocked out, develop a multisystem autoimmune disease associated with an absence of $CD25^+$ regulatory T cells. A rare autoimmune disease in humans called IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome) is also associated with deficiency of regulatory T cells and is now known to be caused by mutations in the *FOXP3* gene. These results establish the importance of regulatory T cells for maintaining self-tolerance. The recent surge of interest in regulatory T cells is because of an increasing appreciation of their physiologic roles, as well as the possibility that defects in these cells may result in various autoimmune diseases and, conversely, that regulatory T cells can be used to treat inflammatory diseases.

Phenotypic Markers and Heterogeneity of Regulatory T Cells

Regulatory T cells are phenotypically distinct from other lymphocyte populations (Table 14-1). Although numerous T cell populations have been described as possessing suppressive activity, the cell type whose regulatory role is best established is $CD4^+$ $FoxP3^+$ $CD25^{high}$. Both FoxP3 and CD25 are essential for the generation, maintenance, and function of these cells. These cells typically express low levels of receptors for IL-7 (CD127), and as predicted

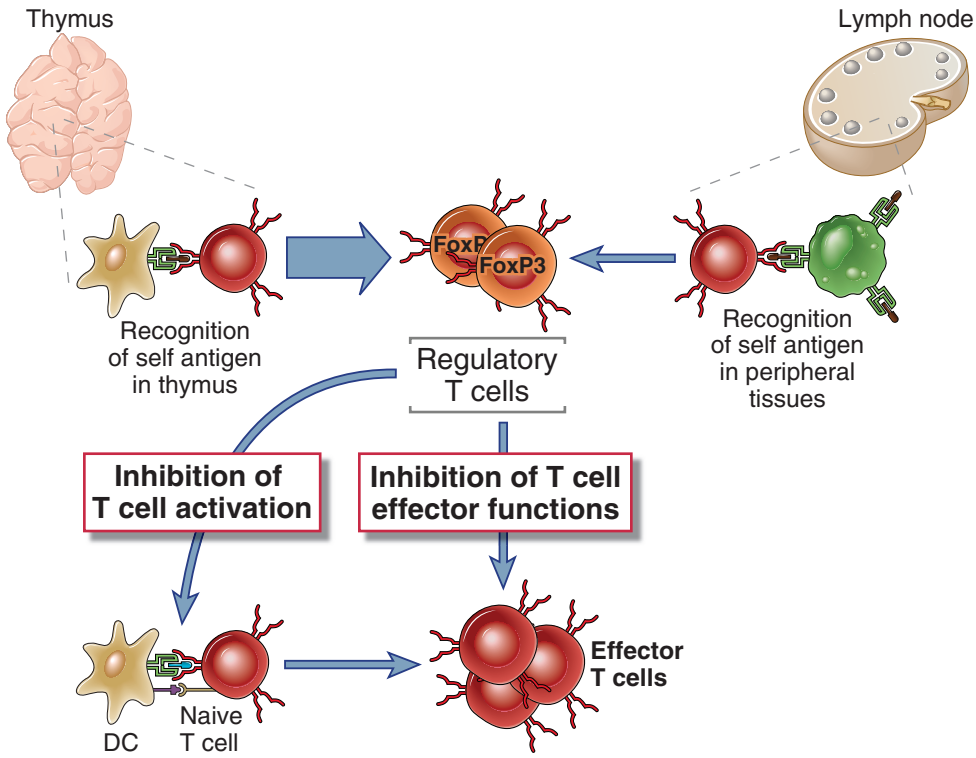


FIGURE 14–6 Regulatory T cells. Regulatory T cells are generated by self antigen recognition in the thymus (sometimes called natural regulatory cells) and (probably to a lesser extent) by antigen recognition in peripheral lymphoid organs (called inducible or adaptive regulatory cells). The development and survival of these regulatory T cells require IL-2 and the transcription factor FoxP3. In peripheral tissues, regulatory T cells suppress the activation and effector functions of other, self-reactive and potentially pathogenic lymphocytes.

TABLE 14–1 Phenotypic Characteristics of Regulatory T Lymphocytes

	Regulatory T Cells	Naive T Cells	Effector and Memory T Cells
Surface markers	CD25 high CTLA-4 GITR CD127 (IL-7R α chain) low	CD25 CD127 (IL-7Rα) high	CD25 high or medium CD127 (IL-7Rα) low on effector cells, high on memory cells
Cytokines produced on activation	TGF-β, IL-10	IL-2	Different subsets of effector and memory cells produce IFN-γ, IL-4 and IL-5, IL-17, others CXCR3, others
Chemokine receptors	CCR6	CCR7	CXCR3, others
Growth factor requirement	IL-2	IL-7	Effector cells: IL-2, IL-4 Memory cells: IL-7
Major transcription factors expressed	FoxP3, STAT5	KLF-2, absence of transcription factors specific for effector cells	In different effector cell subsets: T-bet, GATA-3, RORγt, and various STATs Some memory cells: BLIMP-1

from this pattern of receptor expression, they use IL-2 but not IL-7 as their growth and survival factor. Intriguingly, memory T cells have the opposite receptor expression and growth factor dependence; they are typically $CD127^{high}$ and $CD25^{low}$ and rely on IL-7 for their maintenance. $FoxP3^{+}$ regulatory T cells also typically express high levels of CTLA-4, which is required for their function (discussed later).

Generation and Maintenance of Regulatory T Cells

Regulatory T cells are generated mainly by self antigen recognition in the thymus and by recognition of self and foreign antigens in peripheral lymphoid organs. In the thymus, development of regulatory T cells is one of the fates of T cells committed to the CD4 lineage that recognize self antigens; these thymus-derived cells are sometimes called natural regulatory T cells. In peripheral

lymphoid organs, antigen recognition in the absence of strong innate immune responses favors the generation of regulatory cells from naive $CD4^+$ T lymphocytes, although regulatory T cells can also develop after inflammatory reactions. These peripherally generated regulatory cells have been called adaptive or inducible because they may be induced to develop from naive $CD4^+$ T cells as an adaptation of the immune system in response to certain types of antigen exposure. Predictably, thymically derived regulatory cells are specific for self antigens because these are the antigens mainly encountered in the thymus. Peripherally generated regulatory cells may be specific for self or foreign antigens. Also, it is not clear if both thymic-derived and peripheral regulatory T cells contribute to the maintenance of self-tolerance or if one of the populations is more important than the other for prevention of autoimmunity.

The generation and survival of regulatory T cells are dependent on the cytokines TGF-β and IL-2. Culture of naive T cells with activating anti-TCR antibodies together with TGF-β and IL-2 can induce the development of regulatory cells in vitro; these are sometimes also called induced regulatory T cells. In mice, elimination of TGF-β or blocking of TGF-β signals in T cells leads to a systemic inflammatory disease mainly because of a deficiency of functional regulatory T cells. TGF-β stimulates expression of FoxP3, the transcription factor that drives differentiation of T cells toward the regulatory lineage. Similarly, mice in which the gene for IL-2 or for the α or β chain of the IL-2 receptor is knocked out develop autoimmunity, manifested by inflammatory bowel disease, autoimmune hemolytic anemia, and multiple autoantibodies (including anti-erythrocyte and anti-DNA). These mice lack a full complement of $CD25^+$ $FoxP3^+$ regulatory T cells, and their disease can be corrected by restoring these cells (by providing bone marrow cells from normal animals that can generate $FoxP3^+$ cells). IL-2 promotes differentiation of T cells into the regulatory subset and is also required for the survival and maintenance of this cell population. IL-2 activates the transcription factor STAT5, which may enhance expression of FoxP3 as well as other genes known to be involved in the function of regulatory T cells.

Particular populations or subsets of dendritic cells may be especially important for stimulating the development of regulatory T cells in peripheral tissues. There is some evidence that dendritic cells exposed to retinoic acid, the vitamin A analogue, are inducers of regulatory T cells, especially in mucosal lymphoid tissues (see Chapter 13).

Mechanisms of Action of Regulatory T Cells

Regulatory T cells appear to suppress immune responses at multiple steps—at the induction of T cell activation in lymphoid organs as well as the effector phase of these responses in tissues. Although several mechanisms of suppression have been described, the two that are supported by the most data involve inhibitory cytokines and a contact-mediated effect on APCs.

- ***Regulatory T cells produce IL-10 and TGF-β*** both of which inhibit immune responses. The biology of these cytokines is described in more detail below.
- ***Regulatory T cells inhibit the ability of APCs to stimulate T cells.*** One proposed mechanism of this action is dependent on CTLA-4, which is expressed by $FoxP3^+$ regulatory cells and appears to be required for their function. It may be that CTLA-4 on regulatory cells binds to B7 molecules on APCs and either blocks these molecules or removes them by internalizing them, resulting in reduced availability of B7 and an inability to provide adequate costimulation for immune responses (see Fig. 14-5).

Other mechanisms of suppression by regulatory T cells that have been reported include consumption of IL-2, thus starving responding lymphocytes of this essential growth factor, and killing of responding T cells.

It is not established if all regulatory cells work by all these mechanisms or if there are subpopulations that use different mechanisms to control immune responses. In fact, there is some evidence in humans that two different populations of regulatory T cells can be distinguished by the expression of FoxP3 or production of IL-10 (see later), but this separation may not be absolute.

Inhibitory Cytokines Produced by Regulatory T Cells

TGF-β and IL-10 are involved in both the generation and the functions of regulatory T cells. These cytokines are produced by and act on many other cell types besides regulatory cells. Here we describe the properties and actions of these cytokines.

Transforming Growth Factor-β

TGF-β was discovered as a tumor product that promoted the survival of tumor cells in vitro. It is actually a family of closely related molecules encoded by distinct genes, commonly designated TGF-β1, TGF-β2, and TGF-β3. Cells of the immune system synthesize mainly TGF-β1. TGF-β1 is a homodimeric protein that is synthesized and secreted by $CD4^+$ regulatory T cells, activated macrophages, and many other cell types. It is synthesized as an inactive precursor that is proteolytically cleaved in the Golgi complex and forms a homodimer. This mature TGF-β1 homodimer is secreted in a latent form in association with other polypeptides, which must be removed extracellularly by enzymatic digestion before the cytokine can bind to receptors and exert biologic effects. The TGF-β1 receptor consists of two different proteins, TGF-βRI and TGF-βRII, both of which phosphorylate transcription factors called SMADs. On cytokine binding, a serine/threonine kinase domain of TGF-βRI phosphorylates SMAD2 and SMAD3, which in complex with SMAD4 translocate to the nucleus, bind to promoters of target genes, and regulate their transcription.

TGF-β has many important and quite diverse roles in the immune system.

- ***TGF-β inhibits the proliferation and effector functions of T cells and the activation of macrophages.*** TGF-β inhibits classical macrophage activation but is one of the mediators secreted by alternatively activated macrophages (see Chapter 10). TGF-β also suppresses the activation of other cells, such as neutrophils

and endothelial cells. By these inhibitory actions, TGF-β functions to control immune and inflammatory responses. Mice in which the gene encoding TGF-β1 is knocked out or in which signaling encoding TGF-β is blocked develop uncontrolled inflammatory lesions and lymphoproliferation.

- ***TGF-β regulates the differentiation of functionally distinct subsets of T cells.*** As described before, the development of peripheral $FoxP3^+$ regulatory T cells depends on TGF-β. However, in combination with cytokines produced during innate immune responses, such as IL-1 and IL-6, TGF-β promotes the development of the T_H17 subset of $CD4^+$ T cells by virtue of its ability to induce the transcription factor RORγt (see Chapter 9). The ability of TGF-β to suppress immune and inflammatory responses, in part by generating regulatory T cells, and also to promote the development of proinflammatory T_H17 cells in the presence of other cytokines is an interesting example of how a single cytokine can have diverse and sometimes opposing actions, depending on the context in which it is produced. TGF-β can also inhibit development of T_H1 and T_H2 subsets.
- ***TGF-β stimulates production of IgA antibodies by inducing B cells to switch to this isotype.*** IgA is the antibody isotype required for mucosal immunity (see Chapter 13).
- ***TGF-β promotes tissue repair after local immune and inflammatory reactions subside.*** This function is mediated mainly by the ability of TGF-β to stimulate collagen synthesis and matrix-modifying enzyme production by macrophages and fibroblasts and by promotion of angiogenesis. This cytokine may play a pathologic role in diseases in which fibrosis is an important component, such as pulmonary fibrosis and systemic sclerosis. In repair and fibrotic reactions, alternatively activated macrophages may be a major source of TGF-β.

Interleukin-10

IL-10 is an inhibitor of activated macrophages and dendritic cells and is thus involved in the control of innate immune reactions and cell-mediated immunity. It is a member of a family of heterodimeric cytokines, each chain of which contains a six-helix bundle domain that intercalates with that of the other chain. Other members of the family include IL-19, IL-20, IL-22, IL-24, IL-26, and IL-27. The IL-10 receptor belongs to the type II cytokine receptor family (similar to the receptor for interferons) and consists of two chains, which associate with JAK1 and TYK2 Janus family kinases and activate STAT3. IL-10 is produced by many immune cell populations, including activated macrophages and dendritic cells, regulatory T cells, and T_H1 and T_H2 cells. Because it is both produced by and inhibits macrophage and dendritic cell functions, it is an excellent example of a negative feedback regulator. IL-10 is also produced by some nonimmune cell types (e.g., keratinocytes).

The biologic effects of IL-10 result from its ability to inhibit many of the functions of activated macrophages and dendritic cells.

- ***IL-10 inhibits the production of IL-12 by activated dendritic cells and macrophages.*** Because IL-12 is a critical stimulus for IFN-γ secretion, which plays an important role in innate and adaptive cell-mediated immune reactions against intracellular microbes, IL-10 functions to suppress all such reactions. In fact, IL-10 was first identified as a protein that inhibited IFN-γ production.
- ***IL-10 inhibits the expression of costimulators and class II MHC molecules on dendritic cells and macrophages.*** Because of these actions, IL-10 serves to inhibit T cell activation and terminate cell-mediated immune reactions.

A rare inherited autoimmune disease has been described in which mutations in the IL-10 receptor cause a severe colitis that develops early in life, before the age of 1 year. Knockout mice lacking IL-10 also develop colitis, probably as a result of uncontrolled activation of macrophages reacting to enteric microbes. It is believed that this cytokine is especially important for controlling inflammatory reactions in mucosal tissues, particularly in the gastrointestinal tract (see Chapter 13).

The Epstein-Barr virus contains a gene homologous to human IL-10, and viral IL-10 has the same activities as the natural cytokine. This raises the intriguing possibility that acquisition of the IL-10–like gene during the evolution of the virus has given it the ability to inhibit host immunity and thus a survival advantage in the infected host.

Roles of Regulatory T Cells in Self-Tolerance and Autoimmunity

The elucidation of the genetic basis of the disease IPEX and the similar disease in mice caused by mutations in the *FoxP3* gene, described before, is convincing proof of the importance of regulatory T cells in maintaining self-tolerance and homeostasis in the immune system. Numerous attempts are being made to identify defects in the development or function of regulatory T cells in more common autoimmune diseases, such as inflammatory bowel disease, type 1 diabetes, and multiple sclerosis, in humans. It appears likely that defects in the generation or function of regulatory T cells or resistance of effector cells to suppression contribute significantly to the pathogenesis of many autoimmune diseases. There is also potential for generating regulatory cells and using them to control pathologic immune responses, and many attempts are ongoing to develop such therapies, particularly to treat transplant rejection (see Chapter 16).

Deletion of T Cells by Apoptotic Cell Death

T lymphocytes that recognize self antigens without inflammation or that are repeatedly stimulated by antigens die by apoptosis. There are two major pathways of apoptosis in various cell types (Fig. 14-7), both of which have been implicated in deletion of T cells by self antigens.

- The **mitochondrial** (or **intrinsic**) **pathway** is regulated by the Bcl-2 family of proteins, named after the founding member, Bcl-2, which was discovered as an oncogene in a B cell lymphoma and shown to inhibit apoptosis. Some members of this family are

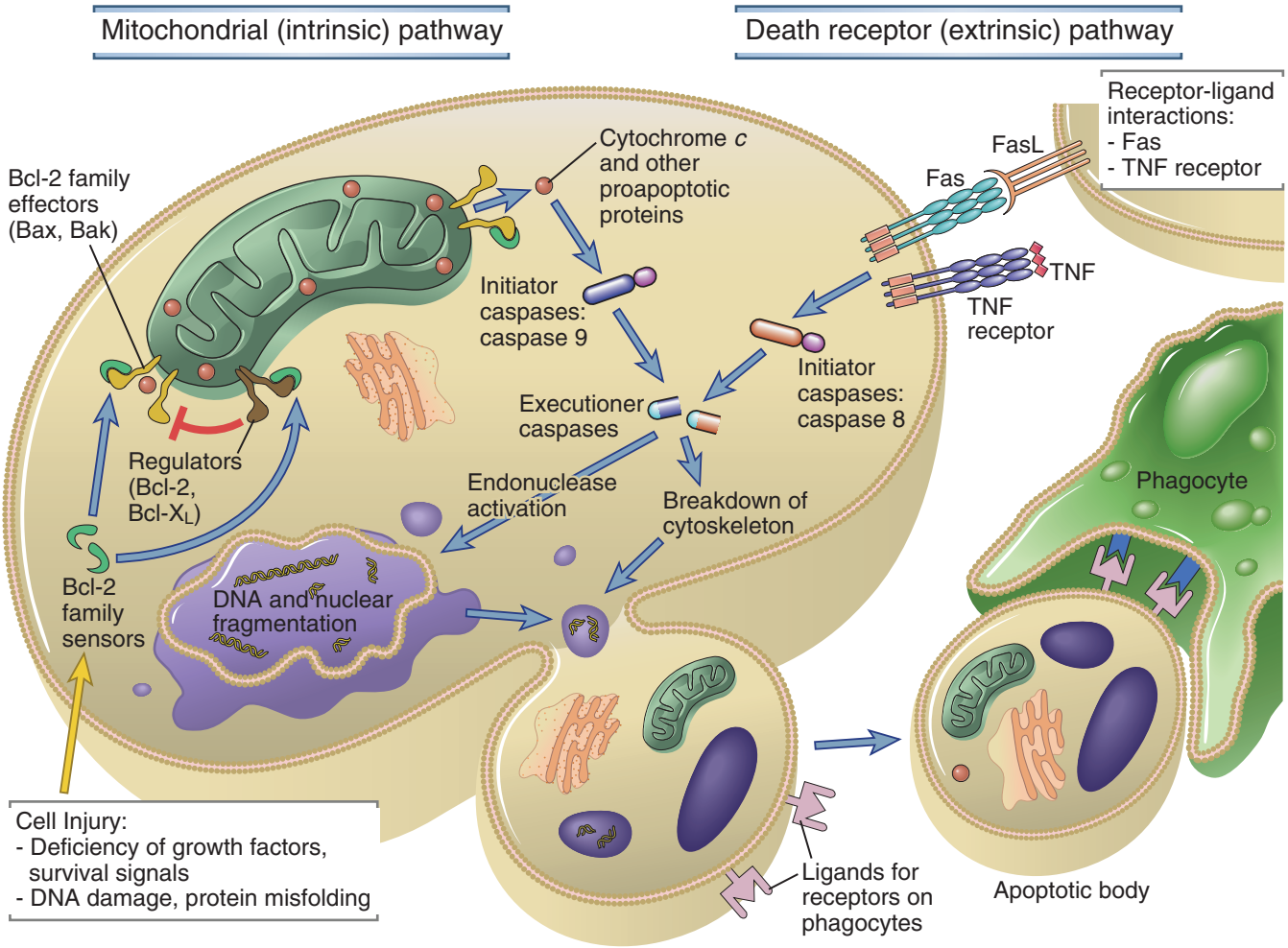


FIGURE 14–7 Pathways of apoptosis. Apoptosis is induced by the mitochondrial and death receptor pathways, described in the text, which culminate in fragmentation of the dead cell and phagocytosis of apoptotic bodies.

proapoptotic and others are antiapoptotic. The pathway is initiated when cytoplasmic proteins of the Bcl-2 family that belong to the "BH3-only" subfamily (so called because they contain one domain that is homologous to the third conserved domain of Bcl-2) are induced or activated as a result of cell signaling, growth factor deprivation, noxious stimuli, or DNA damage. BH3-only proteins can be considered to be "sensors" of cell stress that can bind to and influence death effectors and regulators. The most important of these sensors in lymphocytes is a protein called Bim. Activated Bim binds to two proapoptotic effector proteins of the Bcl-2 family called Bax and Bak, which oligomerize and insert into the outer mitochondrial membrane, leading to enhanced mitochondrial permeability. Growth factors and other survival signals induce the expression of antiapoptotic members of the Bcl-2 family, such as Bcl-2 and Bcl-X_L, which function as regulators of apoptosis by inhibiting Bax and Bak and thus maintaining intact mitochondria. BH3-only proteins also antagonize Bcl-2 and Bcl-X_L. When cells are deprived of survival signals, the mitochondria become leaky because of the actions of the BH3 proteins and Bax and Bak and the relative deficiency of proteins such as Bcl-2 and Bcl-X_L. The result is that many mitochondrial components, including cytochrome *c*, leak out into the cytoplasm. These proteins activate cytoplasmic enzymes called caspases, initially caspase-9, which in turn cleaves and activates a series of other caspases that lead to nuclear DNA fragmentation and other changes that culminate in apoptotic death.

- In the **death receptor** (or **extrinsic**) **pathway**, cell surface receptors homologous to tumor necrosis factor (TNF) receptors are engaged by their ligands, which are homologous to the cytokine TNF. The receptors oligomerize and activate cytoplasmic adaptor proteins, which assemble and cleave caspase-8. The active caspase-8 then cleaves a series of other caspases, again resulting in apoptosis. In many cell types, caspase-8 cleaves and activates a BH3-only protein that induces mitochondrial apoptosis. The mitochondrial pathway may therefore serve to amplify death receptor signaling.

Cells undergoing apoptosis develop membrane blebs, and fragments of the nucleus and cytoplasm break off in membrane-bound structures called apoptotic bodies. There are also biochemical changes in the plasma

membrane, including the exposure of lipids such as phosphatidylserine, which is normally on the inner face of the plasma membrane. These alterations are recognized by receptors on phagocytes, and apoptotic cells are rapidly engulfed and eliminated, without ever having elicited a host inflammatory response.

The best evidence for the involvement of the two apoptotic pathways in the elimination of mature self-reactive lymphocytes is that genetic ablation of both in mice results in systemic autoimmunity. These two death pathways may function in different ways to maintain self-tolerance. Cell death that occurs as a consequence of exposure of mature T cells to antigen is sometimes called **activation-induced cell death**.

- ***T cells that recognize self antigens in the absence of costimulation may activate Bim, resulting in apoptosis by the mitochondrial pathway.*** In normal immune responses, the responding lymphocytes receive signals from the TCR, costimulators, and growth factors. These signals stimulate the expression of antiapoptotic proteins of the Bcl-2 family (Bcl-2, Bcl-X_L) and thus prevent apoptosis and promote cell survival, the necessary prelude to subsequent proliferation. When T cells avidly recognize self antigens, they may directly activate Bim, which triggers death by the mitochondrial pathway, as described before. At the same time, because of the relative lack of costimulation and growth factors, the antiapoptotic members of the Bcl-2 family, Bcl-2 and Bcl-X_L, are expressed at low levels and the actions of Bim, Bax, and Bak are thus not counteracted. The Bim-dependent mitochondrial pathway of apoptosis is also involved in negative selection of self-reactive T cells in the thymus (described before) and in the contraction phase (decline) of immune responses after the initiating antigen has been eliminated (see Chapter 9).
- ***Repeated stimulation of T cells results in the coexpression of death receptors and their ligands, and engagement of the death receptors triggers apoptotic death.*** In $CD4^+$ T cells, the most important death receptor is **Fas (CD95)**, and its ligand is Fas ligand (FasL). Fas is a member of the TNF receptor family, and FasL is homologous to TNF. When T cells are repeatedly activated, FasL is expressed on the cell surface, and it binds to surface Fas on the same or adjacent T cells. This activates a cascade of caspases, which ultimately cause the apoptotic death of the cells. The same pathway of apoptosis may be involved in the elimination of self-reactive B lymphocytes (discussed later). Mice carrying homozygous mutations of the genes encoding Fas or Fas ligand provided the first clear evidence that failure of apoptotic cell death results in autoimmunity. These mice develop a systemic autoimmune disease with multiple autoantibodies and nephritis, resembling human systemic lupus erythematosus (see Chapter 18). The *lpr* (for lymphoproliferation) mouse strain produces low levels of Fas protein, and the *gld* (for generalized lymphoproliferative disease) strain produces FasL with a point mutation that interferes with its signaling function. The cause of autoimmunity is believed to be accumulation of autoreactive B and helper T cells because of the failure of elimination by apoptosis in the periphery. Children with a phenotypically similar disease have been identified and shown to carry mutations in the gene encoding Fas or in genes encoding proteins in the Fas-mediated death pathway that result in a failure of activation-induced cell death. This disease is called the **autoimmune lymphoproliferative syndrome (ALPS)**.

Peripheral Tolerance in $CD8^+$ T Lymphocytes

Much of our knowledge of peripheral T cell tolerance is limited to $CD4^+$ T cells, and much less is known about the mechanisms of tolerance in mature $CD8^+$ T cells. It is likely that if $CD8^+$ T cells recognize class I MHC–associated peptides without costimulation, innate immunity, or T cell help, the $CD8^+$ cells become anergic. In this situation, the $CD8^+$ T cells would encounter signal 1 (antigen) without second signals, and the mechanism of anergy would be essentially the same as for $CD4^+$ T lymphocytes. The role of CTLA-4 and other inhibitory receptors in inducing anergy in $CD8^+$ T cells is not established. $CD25^+$ regulatory T cells can directly inhibit the activation of $CD8^+$ T cells or suppress $CD4^+$ helper cells that are required for full $CD8^+$ responses (see Chapter 9). $CD8^+$ T cells that are exposed to high concentrations of self antigens may also undergo apoptotic cell death.

Factors That Determine the Tolerogenicity of Self Antigens

Studies with a variety of experimental models have shown that many features of protein antigens determine whether these antigens will induce T cell activation or tolerance (Table 14-2). Self antigens have several properties that make them tolerogenic. Some self antigens are present in the thymus, and these antigens may induce

TABLE 14–2 Factors That Determine the Immunogenicity and Tolerogenicity of Protein Antigens

Factor	Features That Favor Stimulation of Immune Responses	Features That Favor Tolerance
Persistence	Short-lived (eliminated by immune response)	Prolonged
Portal of entry; location	Subcutaneous, intradermal; absence from generative organs	Intravenous, mucosal; presence in generative organs
Presence of adjuvants	Antigens with adjuvants: stimulate helper T cells	Antigens without adjuvants: nonimmunogenic or tolerogenic
Properties of antigen-presenting cells	High levels of costimulators	Low levels of costimulators and cytokines

negative selection or the development of regulatory T cells. In the periphery, self antigens, which are usually expressed for long times or throughout life, are capable of engaging antigen receptors for prolonged periods and are normally displayed to lymphocytes without inflammation or innate immunity. Under these conditions, APCs express few or no costimulators, and antigen recognition may either elicit no response (ignorance) or induce anergy, cell death, or regulatory T cells. A general concept that has emerged is that T cell receptor activation in the absence of innate immunity and inflammation tends to trigger one or more of the mechanisms of peripheral tolerance, whereas innate immunity, costimulation, and cytokines tilt the balance toward T cell proliferation and differentiation into effector and memory cells. Our understanding of the mechanisms that link the signals that a T cell receives at the time of antigen recognition with the fate of that T cell remains incomplete. These concepts are based largely on experimental models in which antigens are administered to mice or are produced by transgenes expressed in mice. One of the continuing challenges in this field is to define the mechanisms by which various normally expressed self antigens induce tolerance, especially in humans.

B LYMPHOCYTE TOLERANCE

Tolerance in B lymphocytes is necessary for maintaining unresponsiveness to thymus-independent self antigens, such as polysaccharides and lipids. B cell tolerance also plays a role in preventing antibody responses to protein antigens. Experimental studies have revealed multiple mechanisms by which encounter with self antigens may abort B cell maturation and activation.

Central Tolerance in B Cells

Immature B lymphocytes that recognize self antigens in the bone marrow with high affinity either change their specificity or are deleted. The mechanisms of central B cell tolerance have been best described in experimental models (Fig. 14-8).

- ***Receptor editing.*** If immature B cells recognize self antigens that are present at high concentration in the bone marrow and especially if the antigen is displayed in multivalent form (e.g., on cell surfaces), many antigen receptors on each B cell are cross-linked, thus delivering strong signals to the cells. One consequence of such signaling is that the B cells reactivate their *RAG1* and *RAG2* genes and initiate a new round of VJ recombination in the immunoglobulin (Ig) κ light chain gene locus. A V_κ segment upstream of the already rearranged $V_\kappa J_\kappa$ unit is joined to a downstream J_κ. As a result, the previously rearranged $V_\kappa J_\kappa$ exon in the self-reactive immature B cell is deleted and a new Ig light chain is expressed, thus creating a B cell receptor with a new specificity. This process is called **receptor editing** (see Chapter 8) and is an important

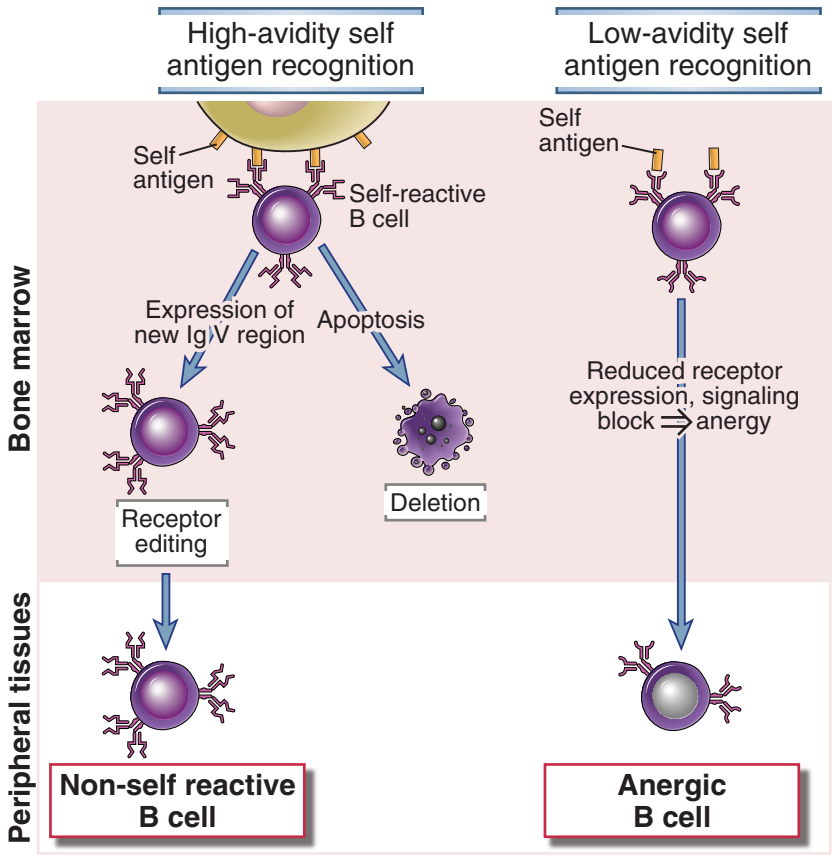


FIGURE 14–8 Central tolerance in B cells. Immature B cells that recognize self antigens in the bone marrow with high avidity (e.g., multivalent arrays of antigens on cells) die by apoptosis or change the specificity of their antigen receptors (receptor editing). Weak recognition of self antigens in the bone marrow may lead to anergy (functional inactivation) of the B cells.

mechanism for eliminating self-reactivity from the mature B cell repertoire. If the edited light chain rearrangement is nonproductive, rearrangement may proceed at the κ locus on the other chromosome, and if that is nonproductive, rearrangements at the λ light chain loci may follow. A B cell expressing a λ light chain is frequently a cell that has undergone receptor editing.

- ***Deletion.*** If editing fails, the immature B cells may be deleted (i.e., they die by apoptosis). The mechanisms of deletion are not well defined.
- ***Anergy.*** If developing B cells recognize self antigens weakly (e.g., if the antigen is soluble and does not cross-link many antigen receptors or if the B cell receptors recognize the antigen with low affinity), the cells become functionally unresponsive (anergic) and exit the bone marrow in this unresponsive state. Anergy is due to downregulation of antigen receptor expression as well as a block in antigen receptor signaling.

Peripheral B Cell Tolerance

Mature B lymphocytes that recognize self antigens in peripheral tissues in the absence of specific helper T cells may be rendered functionally unresponsive or die by apoptosis (Fig. 14-9). Signals from helper T cells may be absent if these T cells are deleted or anergic or if the self antigens are nonprotein antigens. Since self antigens do not elicit innate immune responses, B cells will also not encounter any of the cytokines or other signals that are induced during such responses. Thus, as in T cells, antigen recognition without additional stimuli results in tolerance. Peripheral tolerance mechanisms also eliminate autoreactive B cell clones that may be generated as an unintended consequence of somatic mutation in germinal centers.

- ***Anergy and deletion.*** Some self-reactive B cells that are repeatedly stimulated by self antigens become unresponsive to further activation. These cells require high levels of the growth factor BAFF/BLys for survival (see Chapter 11) and cannot compete efficiently with less BAFF–dependent normal naive B cells for survival in lymphoid follicles. As a result, these B cells that have encountered self antigens have a shortened life span and are eliminated more rapidly than cells that have not recognized self antigens. B cells that bind with high avidity to self antigens in the periphery may also undergo apoptotic death by the mitochondrial pathway independent of growth factor dependence.

  The high rate of somatic mutation of Ig genes that occurs in germinal centers has the risk of generating self-reactive B cells (see Chapter 11). These B cells may be actively eliminated by the interaction of FasL on helper T cells with Fas on the activated B cells. The same interaction was described before as a mechanism for the death of self-reactive T cells. Failure of this pathway of peripheral B cell tolerance may contribute to the autoimmunity that is caused by mutations in the *Fas* and *FasL* genes in mice, and in patients with the autoimmune lymphoproliferative syndrome, referred to earlier.
- ***Signaling by inhibitory receptors.*** B cells that recognize self antigens with low affinity may be prevented from responding by the engagement of various inhibitory receptors. The function of these inhibitory receptors is to set a threshold for B cell activation, which allows responses to foreign antigens with T cell help or innate immunity but does not allow responses to self antigens. This mechanism of peripheral tolerance was revealed by studies showing that mice with defects in the SHP-1 tyrosine phosphatase or the CD22 inhibitory receptor develop autoimmunity. ITIM motifs in

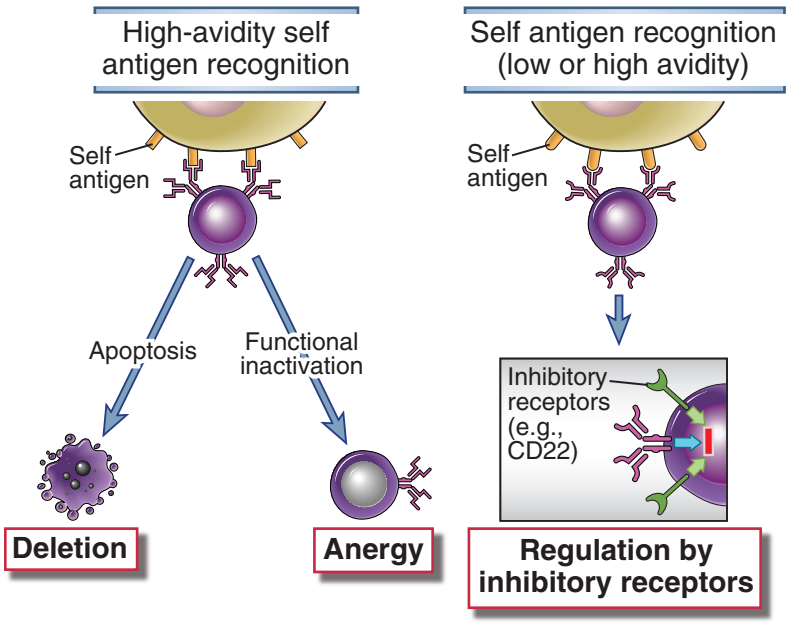


FIGURE 14–9 Peripheral tolerance in B cells. B cells that encounter self antigens in peripheral tissues become anergic or die by apoptosis. In some situations, recognition of self antigens may trigger inhibitory receptors that prevent B cell activation.

the cytoplasmic tail of CD22 are phosphorylated by Lyn, and this inhibitory receptor then recruits SHP-1, thus attenuating B cell receptor signaling. However, it is not known when inhibitory receptors such as CD22 are engaged and what ligands they recognize.

Much has been learned about the mechanisms of tolerance in T and B lymphocytes, largely from the use of animal models such as genetically modified mice. Application of this knowledge to understanding the mechanisms of tolerance to different self antigens in normal individuals and to defining why tolerance fails, giving rise to autoimmune diseases, is an area of active investigation.

TOLERANCE INDUCED BY FOREIGN PROTEIN ANTIGENS

Foreign antigens may be administered in ways that preferentially induce tolerance rather than immune responses. Understanding how to induce tolerance by antigen administration is the key to developing antigen-specific tolerance as a treatment strategy for immunologic diseases. In general, protein antigens administered subcutaneously or intradermally with adjuvants favor immunity, whereas high doses of antigens administered systemically without adjuvants tend to induce tolerance. The likely reason for this is that adjuvants stimulate innate immune responses and the expression of costimulators on APCs, and in the absence of these second signals, T cells that recognize the antigen may become anergic or die or may differentiate into regulatory cells. Many other features of antigens, and how they are administered, may influence the balance between immunity and tolerance (see Table 14-2).

The oral administration of a protein antigen often leads to suppression of systemic humoral and cell-mediated immune responses to immunization with the same antigen. This phenomenon, called **oral tolerance**, was discussed in Chapter 13.

Some systemic infections (e.g., with viruses) may initiate an immune response, but the response is impaired before the virus is cleared, resulting in a state of persistent infection. In this situation, virus-specific T cell clones are present but do not respond normally and are unable to eradicate the infection. This phenomenon has been called **clonal exhaustion**, implying that the antigen-specific lymphocyte clones make an initial response but then become anergic, or "exhausted." There is some evidence that clonal exhaustion is due to upregulation of inhibitory receptors such as PD-1 on virus-specific $CD8^+$ T cells. This phenomenon has been seen in patients infected with the human immunodeficiency virus (HIV) and in animal models of chronic viral infection. How some microbes upregulate expression of inhibitory molecules in T cells is not known. Clonal exhaustion may favor viral persistence and is thus a mechanism of immune evasion used by some pathogens. Understanding of this process may open new avenues for therapeutic interventions in some chronic viral diseases, such as treatment with PD-1–blocking antibodies.

PATHOGENESIS OF AUTOIMMUNITY

The possibility that an individual's immune system may react against autologous antigens and cause tissue injury was appreciated by immunologists from the time that the specificity of the immune system for foreign antigens was recognized. In the early 1900s, Paul Ehrlich coined the rather melodramatic phrase "horror autotoxicus" for harmful ("toxic") immune reactions against self. Autoimmunity is an important cause of disease in humans and is estimated to affect 2% to 5% of the U.S. population. The term *autoimmunity* is often erroneously used for any disease in which immune reactions accompany tissue injury, even though it may be difficult or impossible to establish a role for immune responses against self antigens in causing these disorders. Because inflammation is a prominent component of these disorders, they are sometimes grouped under *immune-mediated inflammatory diseases,* which does not imply that the pathologic response is directed against self antigens (see Chapter 18).

The fundamental questions about autoimmunity are how self-tolerance fails and how self-reactive lymphocytes are activated. Answers to these questions are needed to understand the etiology and pathogenesis of autoimmune diseases, which is a major challenge in immunology. Our understanding of autoimmunity has improved greatly during the past two decades, mainly because of the development of informative animal models of these diseases, the identification of genes that may predispose to autoimmunity, and improved methods for analyzing immune responses in humans. Several important general concepts have emerged from studies of autoimmunity.

- ***Autoimmunity results from a failure of the mechanisms of self-tolerance in T or B cells, which may lead to an imbalance between lymphocyte activation and control mechanisms.*** The potential for autoimmunity exists in all individuals because some of the randomly generated specificities of clones of developing lymphocytes may be for self antigens, and many self antigens are readily accessible to lymphocytes. As discussed before, tolerance to self antigens is normally maintained by selection processes that prevent the maturation of some self antigen–specific lymphocytes and by mechanisms that inactivate or delete self-reactive lymphocytes that do mature. Loss of self-tolerance may result if self-reactive lymphocytes are not deleted or inactivated during or after their maturation and if APCs are activated so that self antigens are presented to the immune system in an immunogenic manner. Some of the general mechanisms that are associated with autoimmune reactions are the following:
  - Defects in deletion (negative selection) of T or B cells or receptor editing in B cells during the maturation of these cells in the generative lymphoid organs
  - Defective numbers and functions of regulatory T lymphocytes
  - Defective apoptosis of mature self-reactive lymphocytes

- Inadequate function of inhibitory receptors
- Activation of APCs, which overcomes regulatory mechanisms and results in excessive T cell activation

In our earlier discussion of the mechanisms of self-tolerance, we have referred to many of these abnormalities to illustrate how self-tolerance may fail, resulting in autoimmunity. We will return to these immunological aberrations as the basis of autoimmunity in the discussion that follows and in Chapter 18, when we consider selected diseases.

Much recent attention has focused on the role of T cells in autoimmunity, for two main reasons. First, helper T cells are the key regulators of all immune responses to proteins, and most self antigens implicated in autoimmune diseases are proteins. Second, several autoimmune diseases are genetically linked to the MHC (the HLA complex in humans), and the function of MHC molecules is to present peptide antigens to T cells. Failure of self-tolerance in T lymphocytes may result in autoimmune diseases in which tissue damage is caused by cell-mediated immune reactions. Helper T cell abnormalities may also lead to autoantibody production because helper T cells are necessary for the production of high-affinity antibodies against protein antigens.

- ***The major factors that contribute to the development of autoimmunity are genetic susceptibility and environmental triggers, such as infections and local tissue injury.*** Susceptibility genes may disrupt self-tolerance mechanisms, and infection or necrosis in tissues promotes the influx of autoreactive lymphocytes and activation of these cells, resulting in tissue injury (Fig. 14-10). Infections and tissue injury may also alter the way in which self antigens are displayed to the immune system, leading to failure of self-tolerance and activation of self-reactive lymphocytes. The roles of these factors in the development of autoimmunity are discussed later.
- ***Autoimmune diseases may be either systemic or organ specific, depending on the distribution of the autoantigens that are recognized.*** For instance, the formation of circulating immune complexes

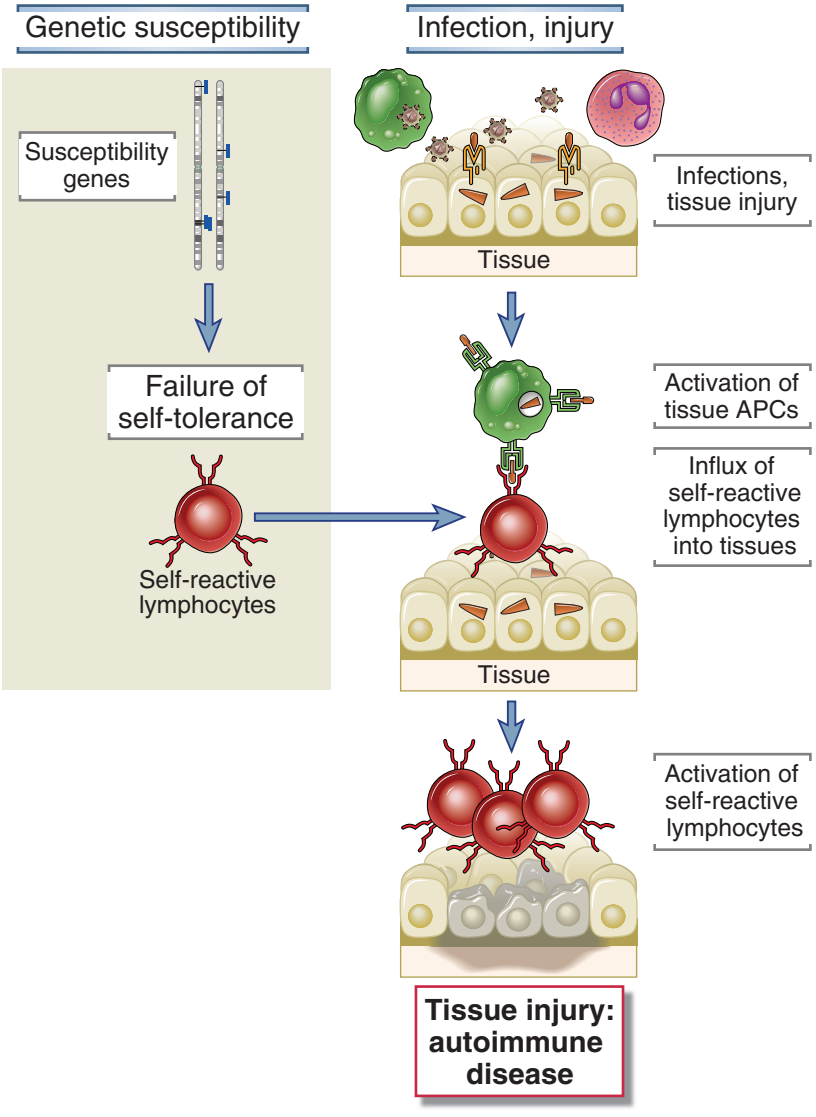


FIGURE 14–10 Postulated mechanisms of autoimmunity. In this proposed model of an organ-specific T cell–mediated autoimmune disease, various genetic loci may confer susceptibility to autoimmunity, in part by influencing the maintenance of self-tolerance. Environmental triggers, such as infections and other inflammatory stimuli, promote the influx of lymphocytes into tissues and the activation of self-reactive T cells, resulting in tissue injury.

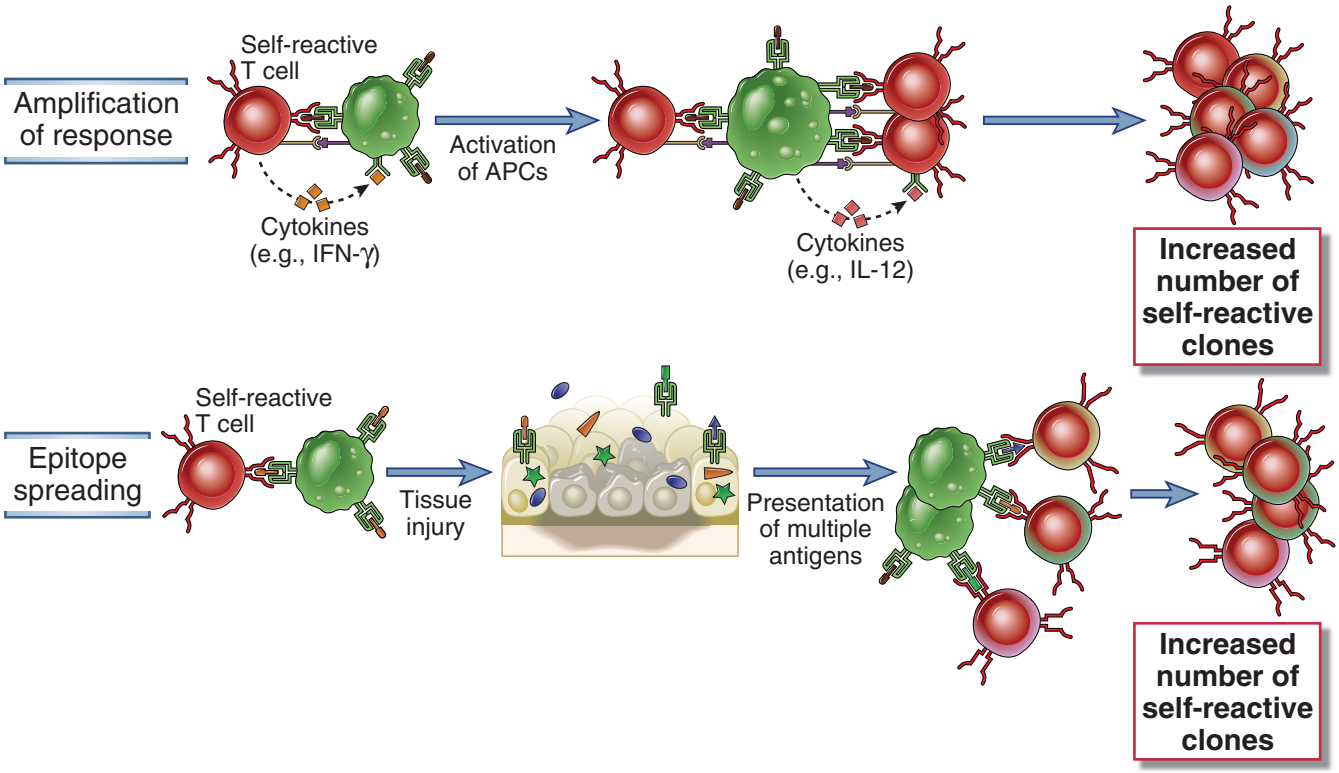


FIGURE 14–11 Mechanisms of chronicity of autoimmune diseases. Once an autoimmune reaction develops, amplification mechanisms (such as cytokines, shown as an illustrative example) promote activation of autoreactive lymphocytes, and release of self antigens from damaged cells and tissues leads to epitope spreading.

composed of self nucleoproteins and specific antibodies typically produces systemic diseases, such as systemic lupus erythematosus (SLE). In contrast, autoantibody or T cell responses against self antigens with restricted tissue distribution lead to organ-specific diseases, such as myasthenia gravis, type 1 diabetes, and multiple sclerosis.

- ***Various effector mechanisms are responsible for tissue injury in different autoimmune diseases.*** These mechanisms include immune complexes, circulating autoantibodies, and autoreactive T lymphocytes and are discussed in Chapter 18. The clinical and pathologic features of the disease are usually determined by the nature of the dominant autoimmune response.
- ***Autoimmune diseases tend to be chronic, progressive, and self-perpetuating.*** The reasons for these features are that the self antigens that trigger these reactions are persistent, and once an immune response starts, many amplification mechanisms are activated that perpetuate the response (Fig. 14-11). In addition, a response initiated against one self antigen that injures tissues may result in the release and alterations of other tissue antigens, activation of lymphocytes specific for these other antigens, and exacerbation of the disease. This phenomenon is called **epitope spreading**, and it may explain why once an autoimmune disease has developed, it may become prolonged and self-perpetuating.

In the following section, we describe the general principles of the pathogenesis of autoimmune diseases, with an emphasis on susceptibility genes, infections, and other factors that contribute to the development of autoimmunity. The pathogenesis and features of some illustrative autoimmune diseases are described in Chapter 18.

Genetic Basis of Autoimmunity

From the earliest studies of autoimmune diseases in patients and experimental animals, it has been appreciated that these diseases have a strong genetic component. For instance, type 1 diabetes shows a concordance of 35% to 50% in monozygotic twins and 5% to 6% in dizygotic twins, and other autoimmune diseases show similar evidence of a genetic contribution. Linkage analyses in families, genome-wide association studies and large scale resequencing efforts are revealing new information about the genes that may play causal roles in the development of autoimmunity and chronic inflammatory disorders. From these studies, several general features of genetic susceptibility have become apparent.

Most autoimmune diseases are complex polygenic traits, in which affected individuals inherit multiple genetic polymorphisms that contribute to disease susceptibility and these genes act with environmental factors to cause the diseases. Some of these polymorphisms are associated with several autoimmune diseases, suggesting that the causative genes influence general mechanisms

of immune regulation and self-tolerance. Other loci are associated with particular diseases, suggesting that they may affect organ damage or autoreactive lymphocytes of particular specificities. Each genetic polymorphism makes a small contribution to the development of particular autoimmune diseases and is also found in healthy individuals but at a lower frequency than in patients with the diseases. It is postulated that in individual patients, multiple such polymorphisms are coinherited and together account for development of the disease. Understanding the interplay of multiple genes with one another and with environmental factors is one of the continuing challenges in the field.

The best-characterized genes associated with autoimmune diseases and our current understanding of how they may contribute to loss of self-tolerance are described here.

Association of MHC Alleles with Autoimmunity

Among the genes that are associated with autoimmunity, the strongest associations are with MHC genes. In fact, in many autoimmune diseases, such as type 1 diabetes, 20 or 30 disease-associated genes have been identified; in most of these diseases, the HLA locus alone contributes half or more of the genetic susceptibility. HLA typing of large groups of patients with various autoimmune diseases has shown that some HLA alleles occur at higher frequency in these patients than in the general population. From such studies, one can calculate the *odds ratio* for development of a disease in individuals who inherit various HLA alleles (often referred to as the relative risk in the older literature) (Table 14-3). The strongest such association is between ankylosing spondylitis, an inflammatory, presumably autoimmune, disease of vertebral joints, and the class I HLA allele B27. Individuals who are HLA-B27 positive have an odds ratio of more than 100 for development of ankylosing spondylitis. Neither the mechanism of this disease nor the basis of its association with HLA-B27 is known. The association of class II HLA-DR and HLA-DQ alleles with autoimmune diseases has received great attention, mainly because class II MHC molecules are involved in the selection and activation of $CD4^+$ T cells, and $CD4^+$ T cells regulate both humoral and cell-mediated immune responses to protein antigens.

Several features of the association of HLA alleles with autoimmune diseases are noteworthy.

- An HLA-disease association may be identified by serologic typing of one HLA locus, but the actual association may be with other alleles that are linked to the typed allele and inherited together. For instance, individuals with a particular HLA-DR allele (hypothetically DR1) may show a higher probability of inheriting a particular HLA-DQ allele (hypothetically DQ2) than the probability of inheriting these alleles separately and randomly (i.e., at equilibrium) in the population. Such inheritance is an example of linkage disequilibrium. A disease may be found to be DR1 associated by HLA typing, but the causal association may actually be with the coinherited DQ2. This realization has emphasized the concept of "extended HLA haplotypes," which refers to sets of linked genes, both classical HLA and adjacent non-HLA genes, that tend to be inherited together as a single unit.
- In many autoimmune diseases, the disease-associated nucleotide polymorphisms encode amino acids in the peptide-binding clefts of the MHC molecules. This finding is not surprising because polymorphic residues of MHC molecules are located within and adjacent to the clefts, and the structure of the clefts is the key determinant of both functions of MHC molecules, namely, antigen presentation and recognition by T cells (see Chapter 6). These results support the general concept that MHC molecules influence the development of autoimmunity by controlling T cell selection and activation.
- Disease-associated HLA sequences are found in healthy individuals. In fact, if all individuals bearing a particular disease-associated HLA allele are monitored prospectively, most will never acquire the disease. Therefore, expression of a particular HLA gene is not by itself the cause of any autoimmune disease, but it may be one of several factors that contribute to autoimmunity.

The mechanisms underlying the association of particular HLA alleles with various autoimmune diseases are still not clear. When positive associations of MHC alleles with disease are noted, the disease-associated MHC molecule may present a particular self peptide and activate pathogenic T cells, and this has been established in a few

TABLE 14–3 Association of HLA Alleles with Autoimmune Disease

Disease	HLA Allele	Odds Ratio[1]
Rheumatoid arthritis (anti-CCP Ab positive)[2]	DRB1, 1 SE allele[3]	4
	DRB1, 2 SE alleles	12
Type 1 diabetes	DRB*0301-DQA1*0501-DQB1*0201 haplotype	4
	DRB1*0401-DQA1*0301-DQB1*0302 haplotype	8
	DRB1*0301/0401 heterozygotes	35
Multiple sclerosis	DRB1*1501	3
Systemic lupus erythematosus	DRB1*0301	2
	DRB1*1501	1.3
Ankylosing spondylitis	B*27 (mainly B*2705 and *2702)	100-200
Celiac disease	DQA1*0501-DB1*0201 haplotype	7

[1]The odds ratio approximates values of increased risk of the disease associated with inheritance of particular HLA alleles. The data are from European-derived populations.
[2]Anti-CCP Ab, antibodies directed against cyclic citrullinated peptides. Data are from patients who test positive for these antibodies in the serum.
[3]SE refers to shared epitope, so called because the susceptibility alleles map to one region of the DRB1 protein (positions 70-74).
(Courtesy Dr. Michelle Fernando, Imperial College, London.)

cases. When a particular allele is shown to be protective (a negative association with disease), it is surmised that this allele might induce negative selection of some developing and potentially pathogenic T cells, thus creating a "hole in the repertoire," or it might promote the development of regulatory T cells.

Polymorphisms in Non-HLA Genes Associated with Autoimmunity

Linkage analyses of autoimmune diseases identified a few disease-associated genes and many chromosomal regions in which the identity of the associated genes was suspected but not established. The technique of genome-wide association studies has greatly extended analysis of the genetic basis of complex diseases, and we now know several genes that are associated with autoimmune diseases (Table 14-4). Before the genes that are most clearly validated are discussed, it is important to summarize some of the general features of these genes.

- Many of the polymorphisms associated with various autoimmune diseases are in genes that influence the development and regulation of immune responses. Although this conclusion appears predictable, it has reinforced the utility of the approaches being used to identify disease-associated genes.
- Different polymorphisms may either protect against disease development or increase the incidence of the disease. The statistical methods used for genome-wide association studies have revealed both types of associations.
- Disease-associated polymorphisms are often located in noncoding regions of the genes. This unexpected result suggests that the polymorphisms may affect the expression of the encoded proteins.

Some of the genes associated with human autoimmune diseases, which have been defined by linkage analyses and genome-wide association studies, are described briefly next.

- ***PTPN22.*** A gain-of-function variant of the protein tyrosine phosphatase PTPN22, that replaces an arginine at position 620 with a tryptophan, is associated with rheumatoid arthritis, type 1 diabetes, autoimmune thyroiditis, and other autoimmune diseases. This activated phosphatase results in weaker T cell receptor and B cell receptor signaling and could thus contribute to defective central or peripheral tolerance in both B and T cells. A partial defect in tolerance in individuals with the tryptophan variant could predispose them to autoimmunity.
- ***NOD2.*** Polymorphisms in this gene are associated with Crohn's disease, one type of inflammatory bowel disease. NOD2 is a cytoplasmic sensor of bacterial wall peptidoglycans (see Chapter 4) and is expressed in

TABLE 14–4 Selected Non-HLA Genetic Associations with Autoimmune Diseases

Chromosomal Region	Gene of Interest	Function	Diseases
Genes Involved in Immune Regulation			
1p13	*PTPN22*	Protein tyrosine phosphatase; role in T and B cell receptors signaling	RA, T1D, IBD
1p12	*CD2/CD58*	Costimulation of T cells	RA, MS
1p31	*IL23R*	Component of IL-23 receptor; role in generation and maintenance of T_H17 cells	IBD, PS, AS
1q32	*IL10*	Downregulates expression of costimulators, MHC molecules, IL-12 in dendritic cells; inhibits T_H1 responses	IBD, SLE, T1D
2q33	*CTLA4*	Inhibitory receptor of T cells, effector molecule of regulatory T cells	T1D, RA
4q26	*IL2/IL21*	Growth and differentiation factors for T cells; IL-2 is involved in maintenance of functional Tregs	IBD, CeD, RA, T1D, MS
5q33	*IL12B*	p40 subunit of IL-12 (T_H1-inducing cytokine) and IL-23 (T_H17-inducing cytokine)	IBD, PS
8p23	*BLK*	B lymphocyte tyrosine kinase, involved in B cell activation	SLE, RA
10p15	*IL2RA*	IL-2 receptor α chain (CD25); role in T cell activation and maintenance of regulatory T cells	MS, T1D
Genes Involved in Responses to Microbes			
16q12	*NOD2*	Cytoplasmic sensor of bacteria	IBD
2q37	*ATG16*	Autophagy (destruction of microbes, maintenance of epithelial cell integrity)	IBD
7q32, 2q24	*IRF5, IFIH1*	Type I interferon responses to viruses	SLE

AS, ankylosing spondylitis; CeD, celiac diseases; IBD, inflammatory bowel disease; MS, multiple sclerosis; PS, psoriasis; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; T1D, type 1 diabetes.

Data from Zenewicz L, C Abraham, RA Flavell, and J Cho. Unraveling the genetics of autoimmunity. Cell 140:791-797, 2010, with permission of the publisher.

multiple cell types, including intestinal epithelial cells. It is thought that the disease-associated polymorphism reduces the function of NOD2, which cannot provide effective defense against intestinal microbes. As a result, these microbes are able to traverse the epithelium and initiate a chronic inflammatory reaction in the intestinal wall, which is the hallmark of inflammatory bowel disease (see Chapter 13).

- ***Insulin.*** Polymorphisms in the insulin gene that encode variable numbers of repeat sequences are associated with type 1 diabetes. These polymorphisms may affect the thymic expression of insulin. It is postulated that if the protein is expressed at low levels in the thymus because of a genetic polymorphism, developing T cells specific for insulin may not be negatively selected. These cells survive in the mature immune repertoire and are capable of attacking insulin-producing islet β cells and causing diabetes.
- ***CD25.*** Polymorphisms affecting expression of CD25, the α chain of the IL-2 receptor, are associated with multiple sclerosis, type 1 diabetes, and other autoimmune diseases. It is not clear if these changes in CD25 expression affect the maintenance of regulatory T cells or the IL-2–induced generation of effector and memory T cells; defects in regulation and excessive effector and memory responses may both contribute to autoimmunity.
- ***IL-23 receptor (IL-23R).*** Some polymorphisms in the receptor for IL-23 protect against the development of inflammatory bowel disease and the skin disease psoriasis. IL-23 is one of the cytokines involved in the development of T_H17 cells that trigger inflammatory reactions (see Chapters 9 and 10). It may be that these polymorphisms in the IL-23R affect T_H17 responses to microbes encountered in the intestinal tract and thus the development of intestinal and cutaneous inflammation.
- ***ATG16.*** Polymorphisms in this gene are also associated with inflammatory bowel disease. ATG16 is one of a family of proteins involved in autophagy, a cellular response to nutrient deprivation in which a starved cell "eats" its own organelles to provide substrates for energy generation and metabolism. The process of autophagy may play a role in the maintenance of intact intestinal epithelial cells or the destruction of microbes that have entered the cytoplasm, but how the *ATG16* polymorphism contributes to inflammatory bowel disease is not known.

There has been a tremendous increase in the number of polymorphisms identified in inflammatory diseases, largely because of genome-wide association studies. However, these studies do not necessarily identify a causal gene but may point to a region where a putative causal gene is located. Genome-wide association studies are not suitable for the identification of rare variants that may be highly penetrant and may actually be the cause of the disease. The advent of whole genome sequencing is likely to reveal even more single nucleotide polymorphisms (SNPs) in various diseases, so the list is certain to grow. One of the great challenges in the field of genetics of complex diseases, including autoimmune and inflammatory diseases, is to correlate the genetic polymorphisms with phenotypic changes. Until this is done, it will be difficult to elucidate the roles of these genes in the pathogenesis of the diseases.

Single-Gene Abnormalities That Cause Autoimmunity

Studies with mouse models and patients have identified several genes that strongly influence the maintenance of tolerance to self antigens (Table 14-5). Unlike the polymorphisms in complex diseases described before, these single-gene defects are examples of mendelian disorders in which the mutation is rare but has a high penetrance, so that most individuals carrying the mutation are affected. Many of these genes were mentioned earlier in the chapter, when we discussed the mechanisms of self-tolerance. Although these genes are associated with rare

TABLE 14–5 Examples of Single-Gene Mutations That Cause Autoimmune Diseases

Gene	Phenotype of Mutant or Knockout Mouse	Mechanism of Failure of Tolerance	Human Disease?
AIRE	Destruction of endocrine organs by antibodies, lymphocytes	Failure of central tolerance	Autoimmune polyendocrine syndrome (APS)
C4	SLE	Defective clearance of immune complexes; failure of B cell tolerance?	SLE
CTLA-4	Lymphoproliferation; T cell infiltrates in multiple organs, especially heart; lethal by 3-4 weeks	Failure of anergy in $CD4^+$ T cells; defective function of regulatory T cells	CTLA-4 polymorphisms associated with several autoimmune diseases
Fas/FasL	Anti-DNA and other autoantibodies; immune complex nephritis; arthritis; lymphoproliferation	Defective deletion of anergic self-reactive B cells; reduced deletion of mature $CD4^+$ T cells	Autoimmune lymphoproliferative syndrome (ALPS)
FoxP3	Multiorgan lymphocytic infiltrates, wasting	Deficiency of functional regulatory T cells	IPEX
IL-2, IL-2Rα/β	Inflammatory bowel disease; anti-erythrocyte and anti-DNA autoantibodies	Defective development, survival, or function of regulatory T cells	None known
SHP-1	Multiple autoantibodies	Failure of negative regulation of B cells	None known

AIRE, autoimmune regulator gene; *IL-2,* interleukin-2; IPEX, immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome; *SHP-1,* SH2-containing phosphatase 1; SLE, systemic lupus erythematosus.

autoimmune diseases, their identification has provided valuable information about the importance of various molecular pathways in the maintenance of self-tolerance. The known genes contribute to the established mechanisms of central tolerance (*AIRE*), generation of regulatory T cells (*FoxP3, IL-2, IL-2R*), anergy and the function of regulatory T cells (*CTLA-4*), and peripheral deletion of T and B lymphocytes (*Fas, FasL*). Here we describe two other genes that are associated with autoimmune diseases in humans.

- ***Genes encoding complement proteins.*** Genetic deficiencies of several complement proteins, including C1q, C2, and C4 (see Chapter 12), are associated with lupus-like autoimmune diseases. The postulated mechanism of this association is that complement activation promotes the clearance of circulating immune complexes and apoptotic cell bodies, and in the absence of complement proteins, these complexes accumulate in the blood and are deposited in tissues and the antigens of dead cells persist.
- ***FcγRIIB.*** Polymorphisms in this inhibitory Fc receptor (see Chapter 11) are associated with SLE in humans, and genetic deletion of this receptor in mice results in a lupus-like autoimmune disease. The likely mechanism of the disease is a failure to control antibody-mediated feedback inhibition of B cells.

Role of Infections in Autoimmunity

Viral and bacterial infections may contribute to the development and exacerbation of autoimmunity. In patients and in some animal models, the onset of autoimmune diseases is often associated with or preceded by infections. (One notable and unexplained exception is the NOD [non-obese diabetic] mouse strain, a model of type 1 diabetes, in which infections tend to ameliorate insulitis and diabetes.) In most of these cases, the infectious microorganism is not present in lesions and is not even detectable in the individual when autoimmunity develops. Therefore, the lesions of autoimmunity are not due to the infectious agent itself but result from host immune responses that may be triggered or dysregulated by the microbe.

Infections may promote the development of autoimmunity by two principal mechanisms (Fig. 14-12).

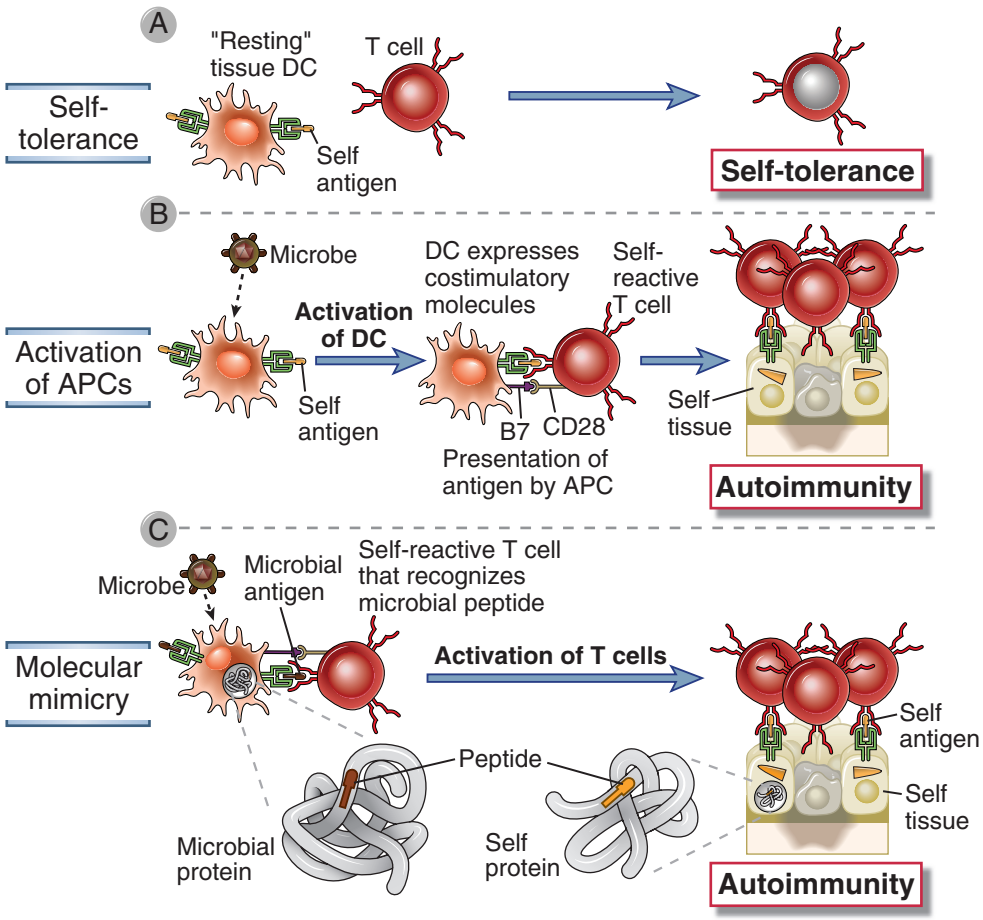


FIGURE 14–12 Role of infections in the development of autoimmunity. A, Normally, encounter of a mature self-reactive T cell with a self antigen presented by a costimulator-deficient resting tissue antigen-presenting cell (APC) results in peripheral tolerance by anergy. (Other possible mechanisms of self-tolerance are not shown.) **B,** Microbes may activate the APCs to express costimulators, and when these APCs present self antigens, the self-reactive T cells are activated rather than rendered tolerant. **C,** Some microbial antigens may cross-react with self antigens (molecular mimicry). Therefore, immune responses initiated by the microbes may activate T cells specific for self antigens.

- Infections of particular tissues may induce local innate immune responses that recruit leukocytes into the tissues and result in the activation of tissue APCs. These APCs begin to express costimulators and secrete T cell–activating cytokines, resulting in the breakdown of T cell tolerance. Thus, the infection results in the activation of T cells that are not specific for the infectious pathogen; this type of response is called **bystander activation**. The importance of aberrant expression of costimulators is suggested by experimental evidence that immunization of mice with self antigens together with strong adjuvants (which mimic microbes) results in the breakdown of self-tolerance and the development of autoimmune disease. In other experimental models, viral antigens expressed in tissues such as islet β cells induce T cell tolerance, but systemic infection of the mice with the virus results in the failure of tolerance and autoimmune destruction of the insulin-producing cells.

  Microbes may also engage Toll-like receptors (TLRs) on dendritic cells, leading to the production of lymphocyte-activating cytokines, and on autoreactive B cells, leading to autoantibody production. A role of TLR signaling in autoimmunity has been demonstrated in mouse modes of SLE.
- Infectious microbes may contain antigens that cross-react with self antigens, so immune responses to the microbes may result in reactions against self antigens. This phenomenon is called **molecular mimicry** because the antigens of the microbe cross-react with, or mimic, self antigens. One example of an immunologic cross-reaction between microbial and self antigens is rheumatic fever, which develops after streptococcal infections and is caused by antistreptococcal antibodies that cross-react with myocardial proteins. These antibodies are deposited in the heart and cause myocarditis. Molecular sequencing has revealed numerous short stretches of homologies between myocardial proteins and streptococcal protein.

  The significance of limited homologies between microbial and self antigens in common autoimmune diseases remains to be established, and it has been difficult to prove that a microbial protein can actually cause a disease that resembles a spontaneous autoimmune disease. On the basis of transgenic mouse models, it has been suggested that molecular mimicry is involved in triggering autoimmunity when the frequency of autoreactive lymphocytes is low; in this situation, the microbial mimic of the self antigen serves to expand the number of self-reactive lymphocytes above some pathogenic threshold. When the frequency of self-reactive lymphocytes is high, the role of microbes may be to induce tissue inflammation, to recruit self-reactive lymphocytes into the tissue, and to provide second signals for the activation of these bystander lymphocytes.

Some infections may protect against the development of autoimmunity. Epidemiologic studies suggest that reducing infections increases the incidence of type 1 diabetes and multiple sclerosis, and experimental studies show that diabetes in NOD mice is greatly retarded if the mice are infected. It seems paradoxical that infections can be triggers of autoimmunity and also inhibit autoimmune diseases. How they may reduce the incidence of autoimmune diseases is unknown.

Other Factors in Autoimmunity

The development of autoimmunity is related to several factors in addition to susceptibility genes and infections.

- ***Anatomic alterations in tissues, caused by inflammation (possibly secondary to infections), ischemic injury or trauma, may lead to the exposure of self antigens that are normally concealed from the immune system.*** Such sequestered antigens may not have induced self-tolerance. Therefore, if previously hidden self antigens are released, they can interact with immunocompetent lymphocytes and induce specific immune responses. Examples of anatomically sequestered antigens include intraocular proteins and sperm. Post-traumatic uveitis and orchitis are thought to be due to autoimmune responses against self antigens that are released from their normal locations by trauma.
- ***Hormonal influences play a role in some autoimmune diseases.*** Many autoimmune diseases have a higher incidence in females than in males. For instance, SLE affects women about 10 times more frequently than men. The SLE-like disease of (NZB × NZW)F_1 mice develops only in females and is retarded by androgen treatment. Whether this predominance results from the influence of sex hormones or other gender-related factors is not known.

Autoimmune diseases are among the most challenging scientific and clinical problems in immunology. The current knowledge of pathogenic mechanisms remains incomplete, so theories and hypotheses continue to outnumber facts. The application of new technical advances and the rapidly improving understanding of self-tolerance will, it is hoped, lead to clearer and more definitive answers to the enigmas of autoimmunity.

SUMMARY

- Immunologic tolerance is unresponsiveness to an antigen induced by the exposure of specific lymphocytes to that antigen. Tolerance to self antigens is a fundamental property of the normal immune system, and the failure of self-tolerance leads to autoimmune diseases. Antigens may be administered in ways that induce tolerance rather than immunity, and this may be exploited for the prevention and treatment of transplant rejection and autoimmune and allergic diseases.
- Central tolerance is induced in the generative lymphoid organs (thymus and bone marrow) when immature lymphocytes encounter self antigens present in these organs. Peripheral tolerance occurs

when mature lymphocytes recognize self antigens in peripheral tissues under particular conditions.

- In T lymphocytes, central tolerance (negative selection) occurs when immature thymocytes with high-affinity receptors for self antigens recognize these antigens in the thymus. Some immature T cells that encounter self antigens in the thymus die and others develop into $FoxP3^+$ regulatory T lymphocytes, which function to control responses to self antigens in peripheral tissues.
- Several mechanisms account for peripheral tolerance in mature T cells. In $CD4^+$ T cells, anergy is induced by antigen recognition without adequate costimulation or by engagement of inhibitory receptors like CTLA-4 and PD-1. Regulatory T cells inhibit immune responses in part by producing immunosuppressive cytokines. T cells that encounter self antigens without other stimuli or that are repeatedly stimulated die by apoptosis.
- In B lymphocytes, central tolerance is induced when immature B cells recognize multivalent self antigens in the bone marrow. The usual result is the acquisition of a new specificity, called receptor editing, or apoptotic death of the immature B cells. Mature B cells that recognize self antigens in the periphery in the absence of T cell help may be rendered anergic and ultimately die by apoptosis or become functionally unresponsive because of the activation of inhibitory receptors.
- Autoimmunity results from a failure of self-tolerance. Autoimmune reactions may be triggered by environmental stimuli, such as infections, in genetically susceptible individuals.
- Most autoimmune diseases are polygenic, and numerous susceptibility genes contribute to disease development. The greatest contribution is from MHC genes; other genes are believed to influence the selection or regulation of self-reactive lymphocytes.
- Infections may predispose to autoimmunity by several mechanisms, including enhanced expression of costimulators in tissues and cross-reactions between microbial antigens and self antigens. Some infections may protect individuals from autoimmunity, by unknown mechanisms.

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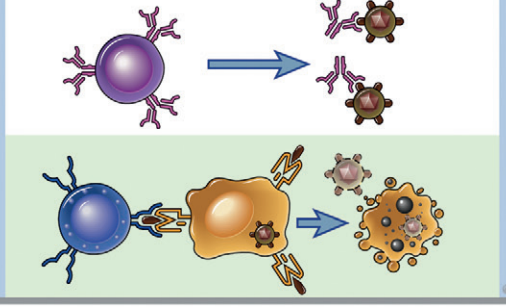
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CHAPTER

15

Immunity to Microbes

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SUMMARY, 363

In the preceding chapters, we have described the components of the immune system and the generation and functions of immune responses. Throughout, we have referred to protection against infections as the major physiologic function of the immune system, and discussed immune responses in the context of responses to microbes. In this chapter, we will integrate this information and discuss the main features of immunity to different types of pathogenic microorganisms as well as the mechanisms microbes use to resist immune defenses.

The development of an infectious disease in an individual involves complex interactions between the microbe and the host. The key events during infection include entry of the microbe, invasion and colonization of host tissues, evasion of host immunity, and tissue injury or functional impairment. Microbes produce disease by killing host cells or by liberating toxins that can cause tissue damage and functional derangements even without extensive colonization of host tissues. In some infections, the host response is the culprit, being the main cause of tissue injury and disease. Many features of microorganisms determine their virulence, and many diverse mechanisms contribute to the pathogenesis of infectious diseases. The topic of microbial pathogenesis is beyond the scope of this book and will not be discussed here. Rather, our discussion focuses on host immune responses to pathogenic microorganisms.

GENERAL FEATURES OF IMMUNE RESPONSES TO MICROBES

Although antimicrobial host defense reactions are numerous and varied, there are several important general features of immunity to microbes.

- ***Defense against microbes is mediated by the effector mechanisms of innate and adaptive immunity.*** The innate immune system provides early defense, and the adaptive immune system provides a more sustained and stronger response. Many pathogenic microbes have evolved to resist innate immunity, and protection against such infections is critically dependent on adaptive immune responses. Adaptive immune responses

to microbes are more specific than innate responses. Adaptive responses induce large numbers of effector cells that function to eliminate the microbes and also generate memory cells that protect the individual from repeated infections.

- ***The immune system responds in distinct and specialized ways to different types of microbes to most effectively combat these infectious agents.*** Because microbes differ greatly in patterns of host colonization and invasion, their elimination requires diverse effector systems. The specialization of adaptive immunity allows the host to respond optimally to different types of microbes. The generation of T_H1, T_H2, and T_H17 subsets of effector $CD4^+$ T cells and the production of different isotypes of antibodies are excellent examples of the specialization of adaptive immunity. Both have been described in earlier chapters; their importance in defense against different types of microbes is mentioned in this chapter.
- ***The survival and pathogenicity of microbes in a host are critically influenced by the ability of the microbes to evade or resist the effector mechanisms of immunity.*** Infectious microbes and the immune system have coevolved and are engaged in a constant struggle for survival. The balance between host immune responses and microbial strategies for resisting immunity often determines the outcome of infections. As we shall see later in this chapter, microorganisms have developed a variety of mechanisms for surviving in the face of powerful immunologic defenses.
- ***Many microbes establish latent, or persistent, infections in which the immune response controls but does not eliminate the microbe and the microbe survives without propagating the infection.*** Latency is a feature of infections by several viruses, especially DNA viruses of the herpesvirus and poxvirus families, and some intracellular bacteria. In latent viral infections, the viral DNA may be integrated into the DNA of infected cells, but no infectious virus is produced. In persistent bacterial infections such as tuberculosis, the bacteria may survive within the endosomal vesicles of infected cells. In all these situations, if the host's immune system becomes defective for any reason (such as cancer or therapy for cancer, immunosuppression to treat transplant rejection, or HIV infection), the latent microbe may be reactivated, resulting in an infection that causes significant clinical problems.
- ***In many infections, tissue injury and disease may be caused by the host response to the microbe and its products rather than by the microbe itself.*** Immunity, like many other defense mechanisms, is necessary for host survival but also has the potential for causing injury to the host.

This chapter considers the main features of immunity to five major categories of pathogenic microorganisms: extracellular bacteria, intracellular bacteria, fungi, viruses, and protozoan and multicellular parasites (Table 15-1). Our discussion of the immune responses to these microbes illustrates the diversity of antimicrobial immunity and the physiologic significance of the effector functions of lymphocytes discussed in earlier chapters.

IMMUNITY TO EXTRACELLULAR BACTERIA

Extracellular bacteria are capable of replicating outside host cells, for example, in the blood, in connective tissues, and in tissue spaces such as the lumens of the airways and gastrointestinal tract. Many different species of extracellular bacteria are pathogenic, and disease is caused by two principal mechanisms. First, these bacteria induce inflammation, which results in tissue destruction at the site of infection. Second, many of these bacteria produce toxins, which have diverse pathologic effects. The toxins may be endotoxins, which are components of bacterial cell walls, or exotoxins, which are actively secreted by the bacteria. The endotoxin of gram-negative bacteria, also called lipopolysaccharide (LPS), has been mentioned in earlier chapters as a potent activator of macrophages and dendritic cells. Many exotoxins are cytotoxic, and they kill cells by various biochemical mechanisms. Other exotoxins interfere with normal cellular functions without killing cells, and yet other exotoxins stimulate the production of cytokines that cause disease.

Innate Immunity to Extracellular Bacteria

The principal mechanisms of innate immunity to extracellular bacteria are complement activation, phagocytosis, and the inflammatory response.

- ***Complement activation.*** The major constituent of the cell walls of gram-positive bacteria, peptidoglycan, activates the alternative pathway of complement in the absence of antibody (see Chapter 12). LPS in the cell walls of gram-negative bacteria also activates complement by the alternative pathway. Bacteria that express mannose on their surface may bind mannose-binding lectin, which activates complement by the lectin pathway. One result of complement activation is opsonization and enhanced phagocytosis of the bacteria. In addition, the membrane attack complex lyses bacteria, especially *Neisseria* species that are particularly susceptible to lysis because of their thin cell walls, and complement byproducts stimulate inflammatory responses by recruiting and activating leukocytes.
- ***Activation of phagocytes and inflammation.*** Phagocytes use various surface receptors, including mannose receptors and scavenger receptors, to recognize extracellular bacteria, and they use Fc receptors and complement receptors to recognize bacteria opsonized with antibodies and complement proteins, respectively. Toll-like receptors (TLRs) and various cytoplasmic sensors of microbial products participate in the activation of phagocytes as a result of encounter with microbes. Some of these receptors function mainly to promote the phagocytosis of the microbes (e.g., mannose receptors, scavenger receptors); others stimulate the microbicidal activities of the phagocytes (mainly TLRs); and yet others promote both phagocytosis and activation of the phagocytes (Fc and complement receptors) (see Chapter 4). In addition, dendritic cells and phagocytes that are activated by the microbes secrete cytokines, which induce leukocyte infiltration

TABLE 15–1 Examples of Pathogenic Microbes

Microbe	Examples of Human Diseases	Mechanisms of Pathogenicity
Extracellular bacteria		
Staphylococcus aureus	Skin and soft tissue infections, lung abscess Systemic: toxic shock syndrome, food poisoning	Skin infections: acute inflammation induced by toxins; cell death caused by pore-forming toxins Systemic: enterotoxin ("superantigen")-induced cytokine production by T cells causing skin necrosis, shock, diarrhea
Streptococcus pyogenes (group A)	Pharyngitis Skin infections: impetigo, erysipelas; cellulitis Systemic: scarlet fever	Acute inflammation induced by various toxins, e.g., streptolysin O damages cell membranes
Streptococcus pyogenes (pneumococcus)	Pneumonia, meningitis	Acute inflammation induced by cell wall constituents; pneumolysin is similar to streptolysin O
Escherichia coli	Urinary tract infections, gastroenteritis, septic shock	Toxins act on intestinal epithelium chloride and water secretion; endotoxin (LPS) stimulates cytokine secretion by macrophages
Vibrio cholerae	Diarrhea (cholera)	Cholera toxin ADP ribosylates G protein subunit, which leads to increased cyclic AMP in intestinal epithelial cells and results in chloride secretion and water loss
Clostridium tetani	Tetanus	Tetanus toxin binds to the motor end plate at neuromuscular junctions and causes irreversible muscle contraction
Neisseria meningitidis (meningococcus)	Meningitis	Acute inflammation and systemic disease caused by potent endotoxin
Corynebacterium diphtheriae	Diphtheria	Diphtheria toxin ADP ribosylates elongation factor 2 and inhibits protein synthesis
Intracellular Bacteria		
Mycobacteria	Tuberculosis, leprosy	Macrophage activation resulting in granulomatous inflammation and tissue destruction
Listeria monocytogenes	Listeriosis	Listeriolysin damages cell membranes
Legionella pneumophila	Legionnaires' disease	Cytotoxin lyses cells and causes lung injury and inflammation
Fungi		
Candida albicans	Candidiasis	Unknown; binds complement proteins
Aspergillus fumigatus	Aspergillosis	Invasion and thrombosis of blood vessels causing ischemic necrosis and cell injury
Histoplasma capsulatum	Histoplasmosis	Lung infection caused by granulomatous inflammation
Viruses		
Polio	Poliomyelitis	Inhibits host cell protein synthesis (tropism for motor neurons in the anterior horn of the spinal cord)
Influenza	Influenza pneumonia	Inhibits host cell protein synthesis (tropism for peripheral nerves)
Rabies	Rabies encephalitis	Inhibits host cell protein synthesis (tropism for ciliated peripheral nerves)
Herpes simplex	Various herpes infections (skin, systemic)	Inhibits host cell protein synthesis; functional impairment of immune cells
Hepatitis B	Viral hepatitis	Host CTL response to infected hepatocytes
Epstein-Barr virus	Infectious mononucleosis; B cell proliferation, lymphomas	Acute infection: cell lysis (tropism for B lymphocytes) Latent infection: stimulates B cell proliferation
Human immunodeficiency virus (HIV)	Acquired immunodeficiency syndrome (AIDS)	Multiple: killing of $CD4^+$ T cells, functional impairment of immune cells (see Chapter 20)

Examples of pathogenic microbes of different classes are listed, with brief summaries of known or postulated mechanisms of tissue injury and disease. Examples of parasites are listed in Table 15-4. ADP, adenosine diphosphate; AMP, adenosine monophosphate; CTL, cytotoxic T lymphocyte; LPS, lipopolysaccharide.
This table was compiled with the assistance of Dr. Arlene Sharpe, Department of Pathology, Harvard Medical School and Brigham and Women's Hospital, Boston, Massachusetts.

into sites of infection (inflammation). The recruited leukocytes ingest and destroy the bacteria.

Adaptive Immunity to Extracellular Bacteria

Humoral immunity is a major protective immune response against extracellular bacteria, and it functions to block infection, to eliminate the microbes, and to neutralize their toxins (Fig. 15-1A). Antibody responses against extracellular bacteria are directed against cell wall antigens and secreted and cell-associated toxins, which may be polysaccharides or proteins. The polysaccharides are prototypic thymus-independent antigens, and humoral immunity is the principal mechanism of defense against polysaccharide-rich encapsulated bacteria. The effector mechanisms used by antibodies to combat these infections include neutralization, opsonization and phagocytosis, and activation of complement by the classical pathway (see Chapter 12). Neutralization is mediated by high-affinity IgG, IgM, and IgA isotypes, the latter mainly in the lumens of mucosal organs; opsonization by some subclasses of IgG; and complement activation by IgM and subclasses of IgG.

The protein antigens of extracellular bacteria also activate CD4⁺ helper T cells, which produce cytokines that induce local inflammation, enhance the phagocytic and microbicidal activities of macrophages and neutrophils, and stimulate antibody production (Fig. 15-1B). T_H17 responses induced by these microbes recruit neutrophils and monocytes and thus promote local inflammation at sites of bacterial infection. Defective T_H17 responses are associated with increased susceptibility to bacterial and fungal infections, with formation of multiple skin abscesses (localized infections). One cause of this disorder is mutations affecting the transcription factor STAT3, which is required for the development of T_H17 cells. This inherited disease is called Job's syndrome (because patients develop skin abscesses, thought to resemble the pestilence visited on Job in the biblical story) or hyper-IgE syndrome (because patients have increased levels of serum IgE, for unknown reasons). Bacteria also induce T_H1 responses, and interferon-γ (IFN-γ) produced by T_H1 cells activates macrophages to destroy phagocytosed microbes and may stimulate production of opsonizing and complement-binding antibody isotypes.

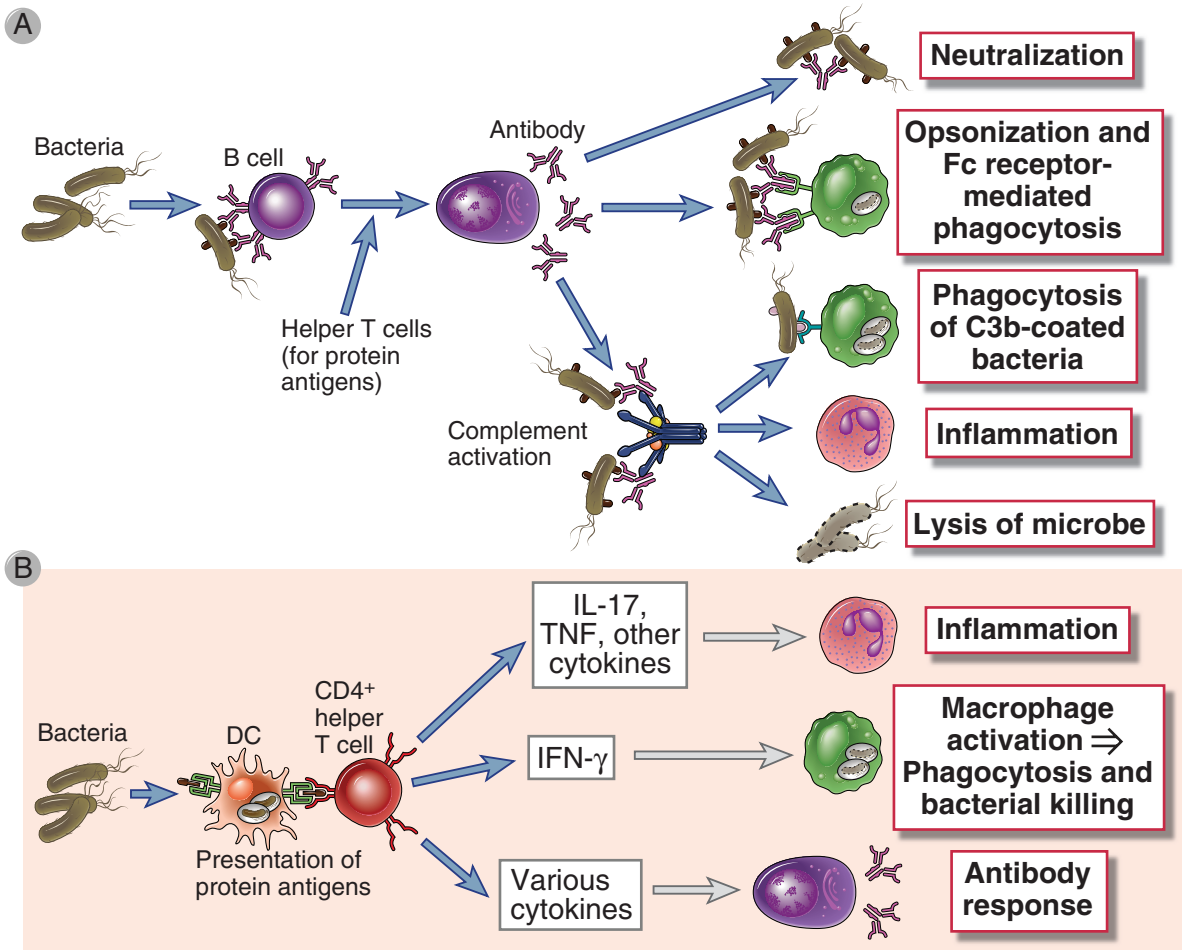


FIGURE 15–1 Adaptive immune responses to extracellular microbes. Adaptive immune responses to extracellular microbes such as bacteria and their toxins consist of antibody production **(A)** and the activation of $CD4^+$ helper T cells **(B)**. Antibodies neutralize and eliminate microbes and toxins by several mechanisms. Helper T cells produce cytokines that stimulate inflammation, macrophage activation, and B cell responses. DC, dendritic cell.

Injurious Effects of Immune Responses

The principal injurious consequences of host responses to extracellular bacteria are inflammation and septic shock. The same reactions of neutrophils and macrophages that function to eradicate the infection also cause tissue damage by local production of reactive oxygen species and lysosomal enzymes. These inflammatory reactions are usually self-limited and controlled. Cytokines secreted by leukocytes in response to bacterial products also stimulate the production of acute-phase proteins and cause the systemic manifestations of the infection (see Chapter 4). **Septic shock** is a severe pathologic consequence of disseminated infection by some gram-negative and gram-positive bacteria. It is a syndrome characterized by circulatory collapse and disseminated intravascular coagulation. The early phase of septic shock is caused by cytokines produced by macrophages that are activated by microbial components, including LPS and bacterial peptidoglycans. Tumor necrosis factor (TNF), IL-6, and IL-1 are the principal cytokine mediators of septic shock, but IFN-γ and interleukin-12 (IL-12) may also contribute (see Chapter 4). This early burst of large amounts of cytokines is sometimes called a cytokine storm. There is some evidence that the progression of septic shock is associated with defective immune responses, perhaps related to depletion or suppression of T cells, resulting in unchecked microbial spread.

Certain bacterial toxins stimulate all the T cells in an individual that express a particular family of V_β T cell receptor (TCR) genes. Such toxins are called **superantigens** because they resemble antigens in that they bind to TCRs and to class II MHC molecules (although not to the peptide-binding clefts) but activate many more T cells than do conventional peptide antigens (Fig. 15-2). Their importance lies in their ability to activate many T cells, with the subsequent production of large amounts of cytokines that can also cause a systemic inflammatory syndrome.

A late complication of the humoral immune response to bacterial infection may be the generation of disease-producing antibodies. The best defined examples are two rare sequelae of streptococcal infections of the throat or skin that are manifested weeks or even months after the infections are controlled. Rheumatic fever is a sequel to pharyngeal infection with some serologic types of β-hemolytic streptococci. Infection leads to the production of antibodies against a bacterial cell wall protein (M protein). Some of these antibodies cross-react with myocardial proteins and are deposited in the heart and subsequently cause inflammation (carditis). Poststreptococcal glomerulonephritis is a sequel to infection of the skin or throat with other serotypes of β-hemolytic streptococci. Antibodies produced against these bacteria form complexes with bacterial antigen, which may be deposited in kidney glomeruli and cause nephritis.

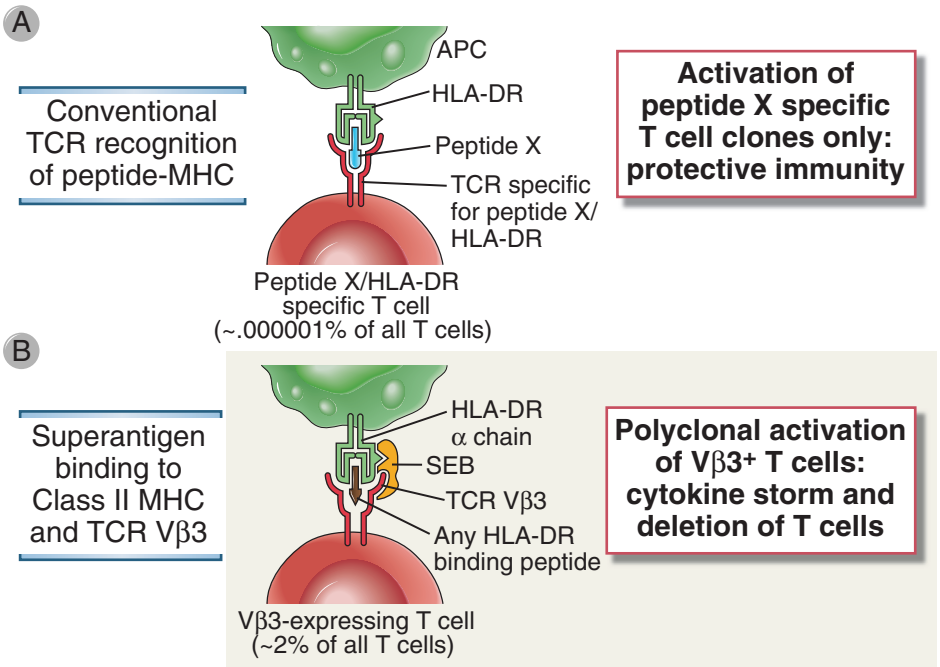


FIGURE 15–2 Polyclonal activation of T cells by bacterial superantigens. A, Conventional microbial T cell antigens, composed of a peptide bound to the peptide-binding groove of an MHC molecule, are recognized by a very small fraction of T cells in any one individual, and only these T cells are activated to become effector T cells that protect against the microbe. **B,** In contrast, a superantigen binds to class II MHC molecules outside the peptide-binding groove and simultaneously binds to the variable region of any TCR β chain, as long as it belongs to a particular V_β family, regardless of the peptide-MHC specificity of the TCR. In this way, superantigens activate T cells to secrete cytokines and also induce apoptosis of T cells. Different superantigens bind to TCRs of different V_β families. Because thousands of clones of T cells will express a TCR β chain from a particular V_β family, superantigens can induce massive cytokine release (cytokine storm) and cause deletion of many T cells. In the example shown, staphylococcal enterotoxin B (SEB) is the superantigen, which binds mainly to HLA-DR and the V_β segments of TCRs belonging to the $V_\beta 3$ family. APC, antigen-presenting cell.

TABLE 15–2 Mechanisms of Immune Evasion by Bacteria

Mechanism of Immune Evasion	Examples
Extracellular bacteria	
Antigenic variation	*Neisseria gonorrhoeae, Escherichia coli, Salmonella typhimurium*
Inhibition of complement activation	Many bacteria
Resistance to phagocytosis	Pneumococcus
Scavenging of reactive oxygen species	Catalase-positive staphylococci
Intracellular bacteria	
Inhibition of phagolysosome formation	*Mycobacterium tuberculosis, Legionella pneumophila*
Inactivation of reactive oxygen and nitrogen species	*Mycobacterium leprae* (phenolic glycolipid)
Disruption of phagosome membrane, escape into cytoplasm	*Listeria monocytogenes* (hemolysin protein)

Immune Evasion by Extracellular Bacteria

The virulence of extracellular bacteria has been linked to a number of mechanisms that resist innate immunity (Table 15-2), including antiphagocytic mechanisms and inhibition of complement or inactivation of complement products. Bacteria with polysaccharide-rich capsules resist phagocytosis and are therefore much more virulent than homologous strains lacking a capsule. The capsules of many pathogenic gram-positive and gram-negative bacteria contain sialic acid residues that inhibit complement activation by the alternative pathway.

A mechanism used by bacteria to evade humoral immunity is genetic variation of surface antigens. Some surface antigens of bacteria such as gonococci and *Escherichia coli* are contained in their pili, which are the structures responsible for bacterial adhesion to host cells. The major antigen of the pili is a protein called pilin. The pilin genes of gonococci undergo extensive gene conversion, because of which the progeny of one organism can produce up to 10^6 antigenically distinct pilin molecules. This ability to alter antigens helps the bacteria evade attack by pilin-specific antibodies, although its principal significance for the bacteria may be to select for pili that are more adherent to host cells so that the bacteria are more virulent. In other bacteria, such as *Haemophilus influenzae,* changes in the production of glycosidases lead to chemical alterations in surface LPS and other polysaccharides, which enable the bacteria to evade humoral immune responses against these antigens.

IMMUNITY TO INTRACELLULAR BACTERIA

A characteristic of facultative intracellular bacteria is their ability to survive and even to replicate within phagocytes. Because these microbes are able to find a niche where they are inaccessible to circulating antibodies, their elimination requires the mechanisms of cell-mediated immunity (Fig. 15-3). As we shall discuss later in this section, in many intracellular bacterial infections the host response also causes tissue injury.

Innate Immunity to Intracellular Bacteria

The innate immune response to intracellular bacteria is mediated mainly by phagocytes and natural killer (NK) cells. Phagocytes, initially neutrophils and later macrophages, ingest and attempt to destroy these microbes, but pathogenic intracellular bacteria are resistant to degradation within phagocytes. Products of these bacteria are recognized by TLRs and cytoplasmic proteins of the NOD-like receptor (NLR) family, resulting in activation of the phagocytes (see Chapter 4). Intracellular bacteria activate NK cells by inducing expression of NK cell–activating ligands on infected cells and by stimulating dendritic cell and macrophage production of IL-12 and IL-15, both of which are NK cell–activating cytokines. The NK cells produce IFN-γ, which in turn activates macrophages and promotes killing of the phagocytosed bacteria. Thus, NK cells provide an early defense against these microbes, before the development of adaptive immunity. In fact, mice with severe combined immunodeficiency, which lack T and B cells, are able to transiently control infection with the intracellular bacterium *Listeria monocytogenes* by NK cell–derived IFN-γ production. However, innate immunity usually fails to eradicate these infections, and eradication requires adaptive cell-mediated immunity.

Adaptive Immunity to Intracellular Bacteria

The major protective immune response against intracellular bacteria is T cell–mediated immunity. Individuals with deficient cell-mediated immunity, such as patients with acquired immunodeficiency syndrome (AIDS), are extremely susceptible to infections with intracellular bacteria (and viruses). The mechanisms of cell-mediated immunity were studied in the 1950s in mice, in examining protection against the intracellular bacterium *L. monocytogenes*. This form of immunity could be adoptively transferred to naive animals with lymphoid cells but not with serum from infected or immunized animals (see Chapter 10, Fig. 10-6).

As we discussed in Chapter 10, cell-mediated immunity consists of two types of reactions: $CD4^+$ T cells recruit phagocytes and activate them through the actions of CD40 ligand and IFN-γ, resulting in killing of phagocytosed microbes, and $CD8^+$ cytotoxic T lymphocytes (CTLs) kill infected cells. Both $CD4^+$ T cells and $CD8^+$ T cells respond to protein antigens of phagocytosed microbes, which are displayed as peptides associated with class II and class I major histocompatibility complex (MHC) molecules, respectively. $CD4^+$ T cells differentiate into T_H1 effectors under the influence of IL-12, which is produced by macrophages and dendritic cells. The T cells express CD40 ligand and secrete IFN-γ, and these two stimuli activate macrophages to produce several microbicidal substances, including reactive oxygen species, nitric oxide, and lysosomal enzymes. IFN-γ also stimulates the

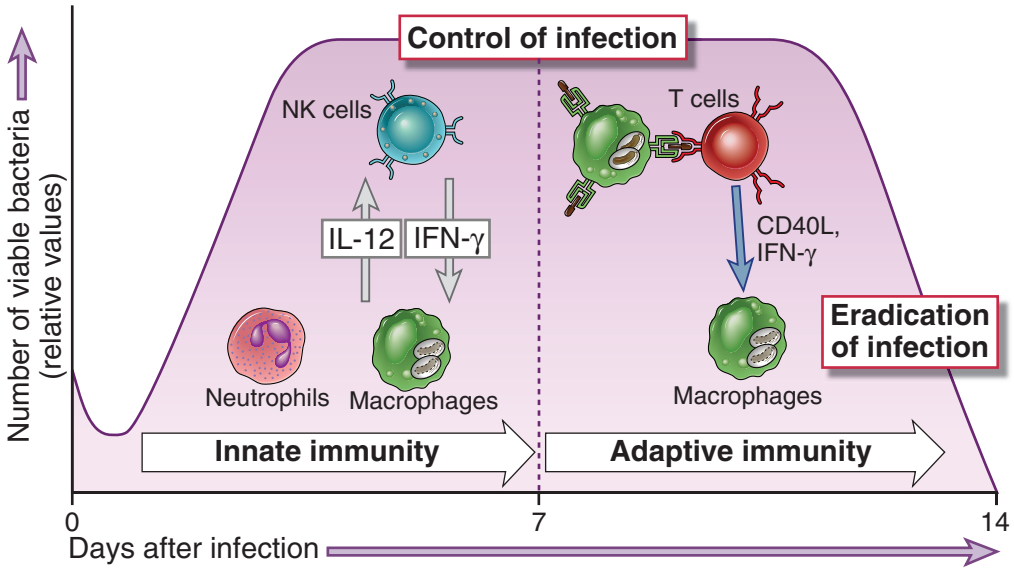


FIGURE 15–3 Innate and adaptive immunity to intracellular bacteria. The innate immune response to intracellular bacteria consists of phagocytes and NK cells, interactions among which are mediated by cytokines (IL-12 and IFN-γ). The typical adaptive immune response to these microbes is cell-mediated immunity, in which T cells activate phagocytes to eliminate the microbes. Innate immunity may control bacterial growth, but elimination of the bacteria requires adaptive immunity. These principles are based largely on analysis of *Listeria monocytogenes* infection in mice; the numbers of viable bacteria shown on the y-axis are relative values of bacterial colonies that can be grown from the tissues of infected mice. *(Data from Unanue ER. Studies in listeriosis show the strong symbiosis between the innate cellular system and the T-cell response. Immunological Reviews 158: 11-25, 1997.)*

production of antibody isotypes (e.g., IgG2a in mice) that activate complement and opsonize bacteria for phagocytosis, thus aiding the effector functions of macrophages. The stimuli for the production of these antibodies in humans are not as well defined. The importance of IL-12 and IFN-γ in immunity to intracellular bacteria has been demonstrated in experimental models and in congenital immunodeficiencies. For instance, individuals with inherited mutations in receptors for IFN-γ or IL-12 are highly susceptible to infections with atypical mycobacteria.

Phagocytosed bacteria stimulate $CD8^+$ T cell responses if bacterial antigens are transported from phagosomes into the cytosol or if the bacteria escape from phagosomes and enter the cytoplasm of infected cells. In the cytoplasm, the microbes are no longer susceptible to the microbicidal mechanisms of phagocytes, and for eradication of the infection, the infected cells have to be killed by CTLs. Thus, the effectors of cell-mediated immunity, namely, $CD4^+$ T cells that activate macrophages and $CD8^+$ CTLs, function cooperatively in defense against intracellular bacteria (Fig. 15-4).

The macrophage activation that occurs in response to intracellular microbes is capable of causing tissue injury. This injury may be the result of delayed-type hypersensitivity (DTH) reactions to microbial protein antigens (see Chapter 18). Because intracellular bacteria have evolved to resist killing within phagocytes, they often persist for long periods and cause chronic antigenic stimulation and T cell and macrophage activation, which may result in the formation of granulomas surrounding the microbes (see Chapter 18, Fig. 18-8). The histologic hallmark of infection with some intracellular bacteria is granulomatous inflammation. This type of inflammatory reaction may serve to localize and prevent spread of the microbes, but it is also associated with severe functional impairment caused by tissue necrosis and fibrosis.

Tuberculosis is an example of an infection with an intracellular bacterium in which protective immunity and pathologic hypersensitivity coexist, and the host response contributes significantly to the pathology. In a primary infection with *M. tuberculosis,* bacilli multiply slowly in the lungs and cause only mild inflammation. The infection is contained by alveolar macrophages (and probably dendritic cells). More than 90% of infected patients remain asymptomatic, but bacteria survive in the lungs, mainly in macrophages. By 6 to 8 weeks after infection, the macrophages have traveled to the draining lymph nodes, and $CD4^+$ T cells are activated; $CD8^+$ T cells may also be activated later. These T cells produce IFN-γ, which activates macrophages and enhances their ability to kill phagocytosed bacilli. TNF produced by T cells and macrophages also plays a role in local inflammation and macrophage activation. The T cell reaction is adequate to control bacterial spread. However, *M. tuberculosis* is capable of surviving within macrophages because components of its cell wall inhibit the fusion of phagocytic vacuoles with lysosomes. Continuing T cell activation leads to the formation of granulomas, which attempt to wall off the bacteria and are often associated with central necrosis, called caseous necrosis, which is caused by macrophage products such as lysosomal enzymes and reactive oxygen species. Necrotizing granulomas and the fibrosis (scarring) that accompanies granulomatous inflammation are the principal causes of tissue injury and clinical disease in tuberculosis. Previously infected persons show cutaneous DTH reactions to skin challenge with a bacterial antigen preparation (purified protein derivative, or PPD). Bacilli may survive for

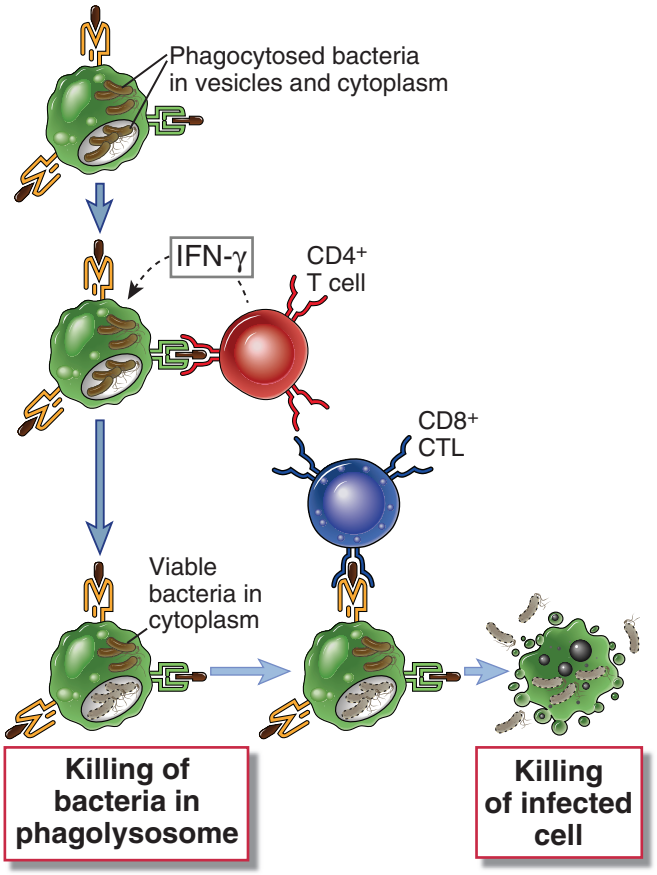
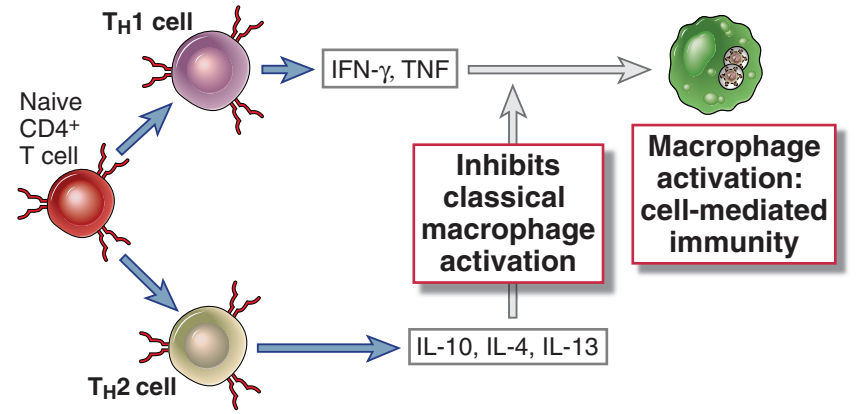


FIGURE 15–4 Cooperation of CD4⁺ and CD8⁺ T cells in defense against intracellular microbes. Intracellular bacteria such as *L. monocytogenes* are phagocytosed by macrophages and may survive in phagosomes and escape into the cytoplasm. $CD4^+$ T cells respond to class II MHC–associated peptide antigens derived from the intravesicular bacteria. These T cells produce IFN-γ, which activates macrophages to destroy the microbes in phagosomes. $CD8^+$ T cells respond to class I–associated peptides derived from cytosolic antigens and kill the infected cells.

many years and are contained without any pathologic consequences but may be reactivated at any time, especially if the immune response becomes unable to control the infection.

Differences among individuals in the patterns of T cell responses to intracellular microbes are important determinants of disease progression and clinical outcome (Fig. 15-5). An example of this relationship between the type of T cell response and disease outcome is leprosy, which is caused by *Mycobacterium leprae*. There are two polar forms of leprosy, the lepromatous and tuberculoid forms, although many patients fall into less clear intermediate groups. In lepromatous leprosy, patients have high specific antibody titers but weak cell-mediated responses to *M. leprae* antigens. Mycobacteria proliferate within macrophages and are detectable in large numbers. The bacterial growth and persistent but inadequate macrophage activation result in destructive lesions in the skin and underlying tissue. In contrast, patients with tuberculoid leprosy have strong cell-mediated immunity but low antibody levels. This pattern of immunity is reflected in granulomas that form around nerves and produce peripheral sensory nerve defects and secondary traumatic skin lesions but less with tissue destruction and a paucity of bacteria in the lesions. One possible reason for the differences in these two forms of disease caused by the same organism may be that there are different patterns of T cell differentiation and cytokine production in individuals. Some studies indicate that patients with the tuberculoid form of the disease produce IFN-γ and IL-2 in lesions (indicative of T_H1 cell activation), whereas patients with lepromatous leprosy produce less IFN-γ and perhaps more IL-4 and IL-10 (suggestive of T_H2 cells). In lepromatous leprosy, both the deficiency of IFN-γ and the macrophage-suppressive effects of IL-10 and possibly IL-4 may result in weak cell-mediated immunity and failure to control bacterial spread. The role of T_H1- and T_H2-derived cytokines in determining



Infection	Response	Outcome
Leishmania major	Most mouse strains: T_H1	⇒ Recovery
	BALB/c mice: T_H2	⇒ Disseminated infection
Mycobacterium leprae	Some patients: T_H1	⇒ Tuberculoid leprosy
	Some patients: Defective T_H1 or dominant T_H2	⇒ Lepromatous leprosy (high bacterial count)

FIGURE 15–5 Role of T cells and cytokines in determining the outcome of infections. Naive $CD4^+$ T lymphocytes may differentiate into T_H1 cells, which activate phagocytes to kill ingested microbes, and T_H2 cells, which inhibit this classical pathway of macrophage activation. The balance between these two T cell subsets may influence the outcome of infections, as illustrated by *Leishmania* infection in mice and *mycobacterium leprae* in humans.

the outcome of infection has been most clearly demonstrated in infection by the protozoan parasite *Leishmania major* in different strains of inbred mice (discussed later in this chapter).

Immune Evasion by Intracellular Bacteria

Different intracellular bacteria have developed various strategies to resist elimination by phagocytes (see Table 15-2). These include inhibiting phagolysosome fusion or escaping into the cytosol, thus hiding from the microbicidal mechanisms of lysosomes, and directly scavenging or inactivating microbicidal substances such as reactive oxygen species. The outcome of infection by these organisms often depends on whether the T cell–stimulated antimicrobial mechanisms of macrophages or microbial resistance to killing gains the upper hand. Resistance to phagocyte-mediated elimination is also the reason that such bacteria tend to cause chronic infections that may last for years, often recur after apparent cure, and are difficult to eradicate.

IMMUNITY TO FUNGI

Fungal infections, also called mycoses, are important causes of morbidity and mortality in humans. Some fungal infections are endemic, and these infections are usually caused by fungi that are present in the environment and whose spores enter humans. Other fungal infections are said to be opportunistic because the causative agents cause mild or no disease in healthy individuals but may infect and cause severe disease in immunodeficient persons. Compromised immunity is the most important predisposing factor for clinically significant fungal infections. Neutrophil deficiency as a result of bone marrow suppression or damage is frequently associated with such infections. A recent increase has been noted in opportunistic fungal infections secondary to an increase in immunodeficiency disease caused mainly by HIV and by therapy for disseminated cancer and transplant rejection. A serious opportunistic fungal infection associated with AIDS is *Pneumocystis jiroveci* pneumonia, but many others contribute to the morbidity and mortality caused by immune deficiencies.

Different fungi infect humans and may live in extracellular tissues and within phagocytes. Therefore, the immune responses to these microbes are often combinations of the responses to extracellular and intracellular bacteria. However, less is known about antifungal immunity than about immunity against bacteria and viruses. This lack of knowledge is partly due to the paucity of animal models for mycoses and partly due to the fact that these infections typically occur in individuals who are incapable of mounting effective immune responses.

Innate and Adaptive Immunity to Fungi

The principal mediators of innate immunity against fungi are neutrophils and macrophages. Patients with neutropenia are extremely susceptible to opportunistic fungal infections. Phagocytes and dendritic cells sense fungal organisms by TLRs and lectin-like receptors called dectins (see Chapter 4). Neutrophils presumably liberate fungicidal substances, such as reactive oxygen species and lysosomal enzymes, and phagocytose fungi for intracellular killing. Virulent strains of *Cryptococcus neoformans* inhibit the production of cytokines such as TNF and IL-12 by macrophages and stimulate production of IL-10, thus inhibiting macrophage activation.

Cell-mediated immunity is the major mechanism of adaptive immunity against fungal infections. *Histoplasma capsulatum*, a facultative intracellular parasite that lives in macrophages, is eliminated by the same cellular mechanisms that are effective against intracellular bacteria. $CD4^+$ and $CD8^+$ T cells cooperate to eliminate the yeast forms of *C. neoformans*, which tend to colonize the lungs and brain in immunodeficient hosts. Many extracellular fungi elicit strong T_H17 responses, which are driven in part by the activation of dendritic cells by fungal glucans binding to dectin-1, a receptor for this fungal polysaccharide, and this results in the production of T_H17-inducing cytokines (IL-6, IL-23) from the dendritic cells (see Chapter 9). The T_H17 cells stimulate inflammation, and the recruited neutrophils and monocytes destroy the fungi. *Candida* infections often start at mucosal surfaces, and cell-mediated immunity is believed to prevent spread of the fungi into tissues. T_H1 responses are protective in intracellular fungal infections, such as histoplasmosis, but these responses may elicit granulomatous inflammation, which is an important cause of host tissue injury in these infections (see Chapter 18). Fungi also elicit specific antibody responses that are of protective value.

IMMUNITY TO VIRUSES

Viruses are obligatory intracellular microorganisms that live inside cells, using components of the nucleic acid and protein synthetic machinery of the host to replicate and spread. Viruses typically infect various cell types by using normal cell surface molecules as receptors to enter the cells. After entering cells, viruses can cause tissue injury and disease by any of several mechanisms. Viral replication interferes with normal cellular protein synthesis and function and leads to injury and ultimately death of the infected cell. This result is one type of cytopathic effect of viruses, and the infection is said to be lytic because the infected cell is lysed. Viruses may also cause latent infections, discussed later.

Innate and adaptive immune responses to viruses are aimed at blocking infection and eliminating infected cells (Fig. 15-6). Infection is prevented by type I interferons as part of innate immunity and neutralizing antibodies contributing to adaptive immunity. Once infection is established, infected cells are eliminated by NK cells in the innate response and CTLs in the adaptive response.

Innate Immunity to Viruses

The principal mechanisms of innate immunity against viruses are inhibition of infection by type I interferons and NK cell–mediated killing of infected cells. Infection by many viruses is associated with production of type I

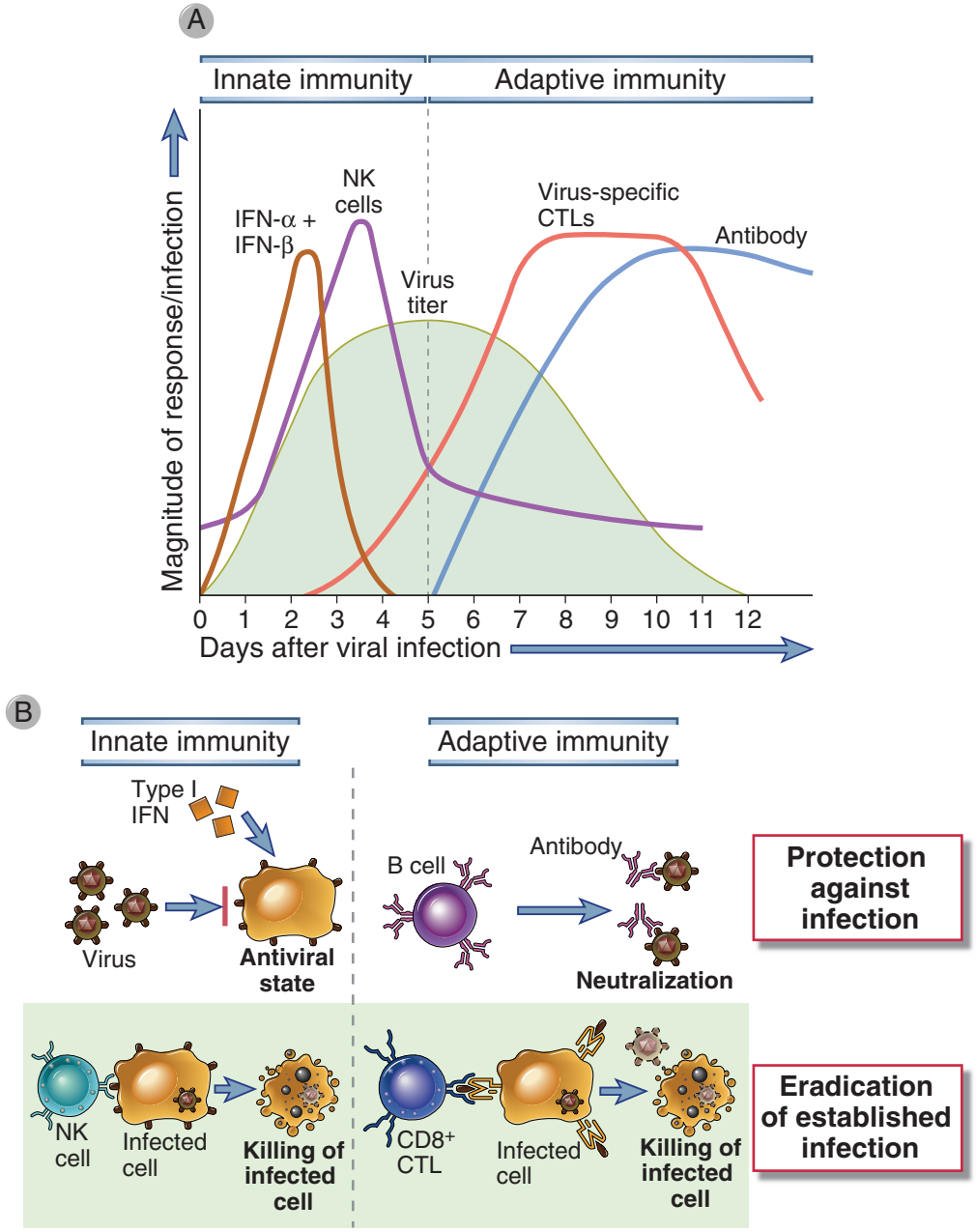


FIGURE 15–6 Innate and adaptive immune responses against viruses. A, Kinetics of innate and adaptive immune responses to a virus infection. **B,** Mechanisms by which innate and adaptive immunity prevent and eradicate virus infections. Innate immunity is mediated by type I interferons, which prevent infection, and NK cells, which eliminate infected cells. Adaptive immunity is mediated by antibodies and CTLs, which also block infection and kill infected cells, respectively.

interferons by infected cells, especially dendritic cells of the plasmacytoid type (see Chapter 4). Several biochemical pathways trigger interferon production (Fig. 15-7). These include recognition of viral RNA and DNA by endosomal TLRs and activation of cytoplasmic RIG-like receptors by viral RNA. These pathways converge on the activation of protein kinases, which in turn activate the IRF transcription factors that stimulate interferon gene transcription. Type I interferons function to inhibit viral replication in both infected and uninfected cells by inducing an "antiviral state." The mechanisms by which interferons block viral replication were discussed in Chapter 4 (see Fig. 4-15).

NK cells kill cells infected with a variety of viruses and are an important mechanism of immunity against viruses early in the course of infection, before adaptive immune responses have developed. NK cells also recognize infected cells in which the virus has shut off class I MHC expression as an escape mechanism from CTLs because the absence of class I releases NK cells from a normal state of inhibition (see Fig. 4-6, Chapter 4).

Adaptive Immunity to Viruses

Adaptive immunity against viral infections is mediated by antibodies, which block virus binding and entry into

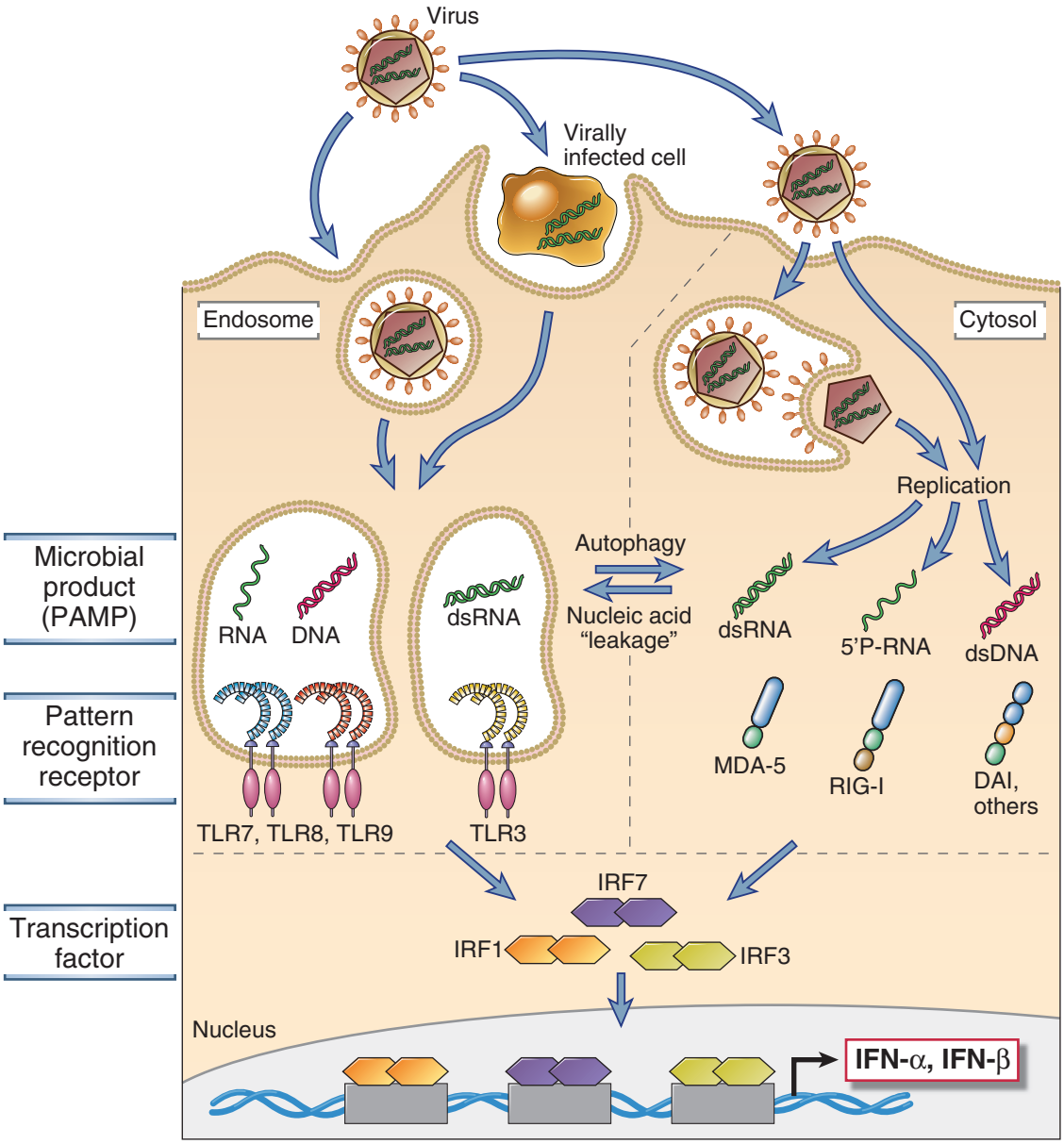


FIGURE 15–7 Mechanisms of induction of type I interferons by viruses. Viral nucleic acids and proteins are recognized by several cellular receptor families (TLRs and the family of cytosolic RIG-like receptors, or RLRs, which include MDA-5, RIG-I, DAI and others), which activate transcription factors (the IRF proteins) that stimulate the production of type I interferons, IFN-α and IFN-β. This process and the actions of interferons are described in more detail in Chapter 4.

host cells, and by CTLs, which eliminate the infection by killing infected cells (see Fig. 15-6). The most effective antibodies are high-affinity antibodies produced in T-dependent germinal center reactions (see Chapter 11). Antibodies are effective against viruses only during the extracellular stage of the lives of these microbes. Viruses may be extracellular early in the course of infection, before they infect host cells, or when they are released from infected cells by virus budding or if the cells are killed. Antiviral antibodies bind to viral envelope or capsid antigens and function mainly as neutralizing antibodies to prevent virus attachment and entry into host cells. Thus, antibodies prevent both initial infection and cell-to-cell spread. Secreted antibodies of the IgA isotype are important for neutralizing viruses within the respiratory and intestinal tracts. Oral immunization against poliomyelitis works by inducing mucosal immunity. In addition to neutralization, antibodies may opsonize viral particles and promote their clearance by phagocytes. Complement activation may also participate in antibody-mediated viral immunity, mainly by promoting phagocytosis and possibly by direct lysis of viruses with lipid envelopes.

The importance of humoral immunity in defense against viral infections is supported by the observation that resistance to a particular virus, induced by either infection or vaccination, is often specific for the serologic (antibody-defined) type of the virus. An example is influenza virus, in which exposure to one serologic type does not confer resistance to other serotypes of the virus.

Neutralizing antibodies block viral infection of cells and spread of viruses from cell to cell, but once the viruses enter cells and begin to replicate intracellularly, they are inaccessible to antibodies. Therefore, humoral immunity induced by previous infection or vaccination is able to protect individuals from viral infection but cannot by itself eradicate established infection.

Elimination of viruses that reside within cells is mediated by CTLs, which kill the infected cells. As we have mentioned in previous chapters, the principal physiologic function of CTLs is surveillance against viral infection. Most virus-specific CTLs are $CD8^+$ T cells that recognize cytosolic, usually endogenously synthesized, viral peptides presented by class I MHC molecules. If the infected cell is a tissue cell and not a professional antigen-presenting cell (APC), such as a dendritic cell, the infected cell may be phagocytosed by the dendritic cell, which processes the viral antigens and presents them to naive $CD8^+$ T cells. This process of cross-presentation, or cross-priming, was described in Chapter 6 (see Fig. 6-20). Full differentiation of $CD8^+$ CTLs often requires cytokines produced by $CD4^+$ helper cells or costimulators expressed on infected cells (see Chapter 9). As discussed in Chapter 9, $CD8^+$ T cells undergo massive proliferation during viral infection, and most of the proliferating cells are specific for a few viral peptides. Some of the activated T cells differentiate into effector CTLs, which can kill any infected nucleated cell. The antiviral effects of CTLs are mainly due to killing of infected cells, but other mechanisms include activation of nucleases within infected cells that degrade viral genomes and secretion of cytokines such as IFN-γ, which activates phagocytes and may have some antiviral activity.

The importance of CTLs in defense against viral infection is demonstrated by the increased susceptibility to such infections seen in patients and animals deficient in T lymphocytes and by the experimental observation that mice can be protected against some virus infections by adoptive transfer of virus-specific, class I–restricted CTLs. Furthermore, many viruses are able to alter their surface antigens, such as envelope glycoproteins, and thus escape attack by antibodies. However, infected cells may produce some viral proteins that are invariant, so that CTL-mediated defense remains effective against such viruses.

In latent infections, viral DNA persists in host cells but the virus does not replicate or kill infected cells. Latency is often a state of balance between infection and the immune response. CTLs are generated in response to the virus that can control the infection but not eradicate it. As a result, the virus persists in infected cells, sometimes for the life of the individual. Any deficiency in the host immune response can result in reactivation of the latent infection, with expression of viral genes that are responsible for cytopathic effects and for spread of the virus. These cytopathic effects may include lysis of infected cells or uncontrolled proliferation of the cells. Such latent infections are common with Epstein-Barr virus and several other DNA viruses of the herpesvirus family.

In some viral infections, tissue injury may be caused by CTLs. An experimental model of a disease in which the pathology is due to the host immune response is lymphocytic choriomeningitis virus (LCMV) infection in mice, which induces inflammation of the spinal cord meninges. LCMV infects meningeal cells, but it is noncytopathic and does not injure the infected cells directly. The virus stimulates the development of virus-specific CTLs that kill infected meningeal cells during a physiologic attempt to eradicate the infection. Therefore, meningitis develops in normal mice with intact immune systems, but T cell-deficient mice do not develop disease and instead become carriers of the virus. This observation appears to contradict the usual situation, in which immunodeficient individuals are more susceptible to infectious diseases than normal individuals are. Hepatitis B virus infection in humans shows some similarities to murine LCMV in that immunodeficient persons who become infected do not develop the disease but become carriers who can transmit the infection to otherwise healthy persons. The livers of patients with acute and chronic active hepatitis contain large numbers of $CD8^+$ T cells, and hepatitis virus–specific, class I MHC–restricted CTLs can be isolated from liver biopsy specimens and propagated in vitro.

Immune responses to viral infections may be involved in producing disease in other ways. A consequence of persistent infection with some viruses, such as hepatitis B, is the formation of circulating immune complexes composed of viral antigens and specific antibodies (see Chapter 18). These complexes are deposited in blood vessels and lead to systemic vasculitis. Some viral proteins contain amino acid sequences that are also present in some self antigens. It has been postulated that because of this "molecular mimicry," antiviral immunity can lead to immune responses against self antigens.

Immune Evasion by Viruses

Viruses have evolved numerous mechanisms for evading host immunity (Table 15-3).

- ***Viruses can alter their antigens and are thus no longer targets of immune responses.*** The antigens affected are most commonly surface glycoproteins that are recognized by antibodies, but T cell epitopes may also undergo variation. The principal mechanisms of antigenic variation are point mutations and reassortment of RNA genomes (in RNA viruses), leading to antigenic drift and antigenic shift. These processes are of great importance in the spread of influenza virus. The two major antigens of the virus are the trimeric viral hemagglutinin (the viral "spike" protein) and neuraminidase. Viral genomes undergo mutations in the genes that encode these surface proteins, and the variation that occurs as a result is called antigenic drift. Influenza viruses that normally inhabit different host species can recombine in host cells, and these reassorted viruses can differ quite dramatically from prevalent strains (Fig. 15-8). These reassortment processes result in a major change in antigenic structure called antigenic shift, which creates distinct viruses such as the avian flu or the swine flu viruses. Because of antigenic variation, a virus may become resistant to immunity generated in the population by previous infections. The

influenza pandemics that occurred in 1918, 1957, and 1968 were due to different strains of the virus, and the H1N1 pandemic of 2009 was due to a strain in which the strands of the RNA genome were reassorted among strains endemic in pigs, fowl, and humans. Subtler viral variants arise more frequently. There are so many serotypes of rhinovirus that specific immunization against the common cold may not be a feasible preventive strategy. Human immunodeficiency virus 1 (HIV-1), the virus that causes AIDS, is also capable of tremendous antigenic variation (see Chapter 20). In these situations, prophylactic vaccination may have to be directed against invariant viral proteins.

TABLE 15–3 Mechanisms of Immune Evasion by Viruses

Mechanism of Immune Evasion	Examples
Antigenic variation	Influenza, rhinovirus, HIV
Inhibition of antigen processing	
Blockade of TAP transporter	Herpes simplex
Removal of class I molecules from the ER	Cytomegalovirus
Production of cytokine receptor homologues	Vaccinia, poxviruses (IL-1, IFN-γ) Cytomegalovirus (chemokine)
Production of immunosuppressive cytokine	Epstein-Barr (IL-10)
Infection and death or functional impairment of immune cells	HIV

Representative examples of different mechanisms used by viruses to resist host immunity are listed. ER, endoplasmic reticulum; HIV, human immunodeficiency virus; TAP, transporter associated with antigen processing.

- ***Some viruses inhibit class I MHC–associated presentation of cytosolic protein antigens.*** Viruses make a variety of proteins that block different steps in antigen processing, transport and presentation (Fig. 15-9). Inhibition of antigen presentation blocks the assembly and expression of stable class I MHC molecules and the display of viral peptides. As a result, cells infected by such viruses cannot be recognized or killed by $CD8^+$ CTLs. However, it is difficult to prove that the viral genes encoding proteins that inhibit antigen presentation are actually virulence genes, required for the infectivity or pathogenicity of the viruses. NK cells may have evolved as an adaptation to this viral evasion strategy because NK cells are activated by infected cells, especially in the absence of class I MHC molecules. There is emerging evidence that some viruses may produce proteins that act as ligands for NK cell inhibitory receptors and thus inhibit NK cell activation. These are excellent examples of the constant

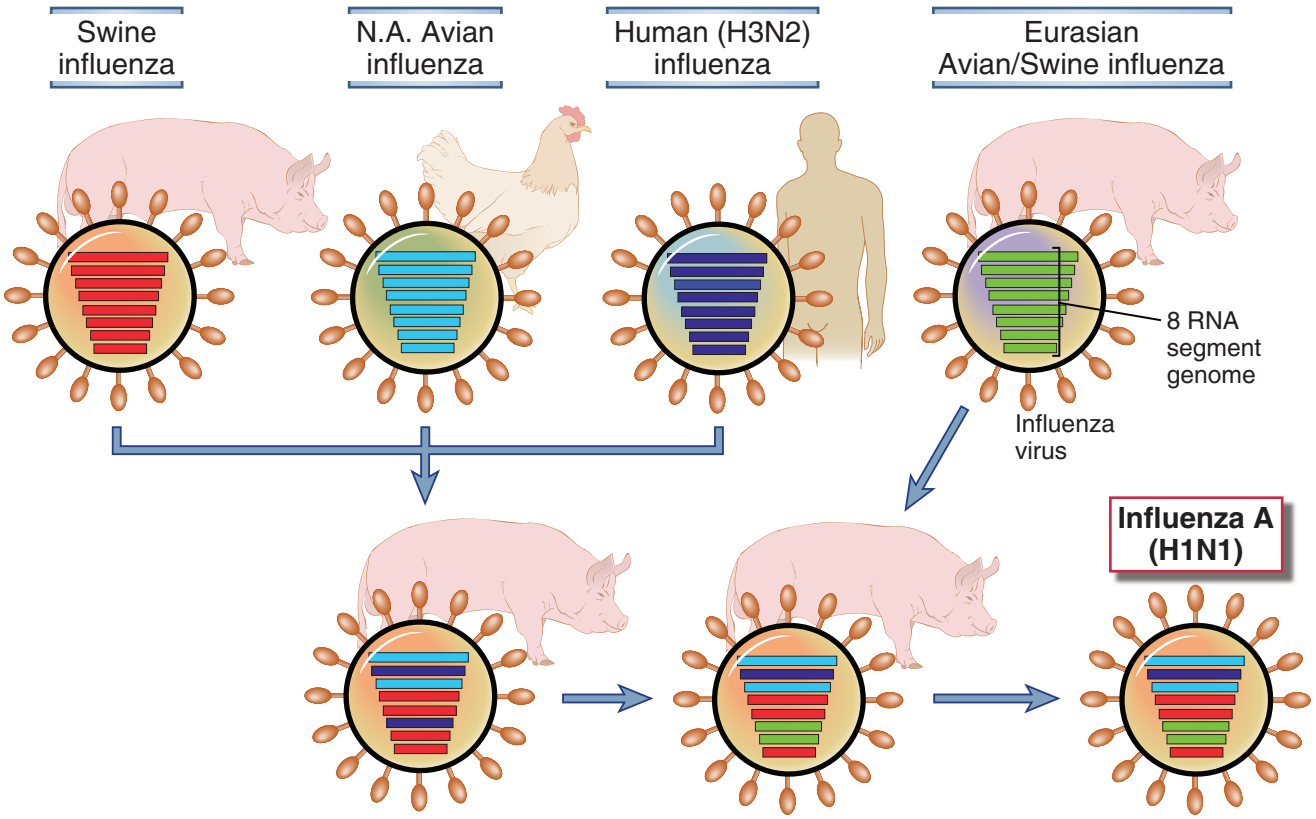


FIGURE 15–8 Generation of new influenza virus strains by genetic recombination (antigenic shift). The genome of the influenza virus is composed of eight separate RNA strands, which allows genetic recombination by reassortment of the segments in various hosts, such as a pig, bird, or humans, that are simultaneously infected with two different strains. These genetic reassortments create new viruses that are antigenically distinct from their precursors and thus are able to evade immune detection in large numbers of newly infected hosts. In the example shown, H1N1 influenza virus, which was responsible for the pandemic of 2009, was generated by reassortment of swine, avian, and human viruses in pigs and then passed back to humans.

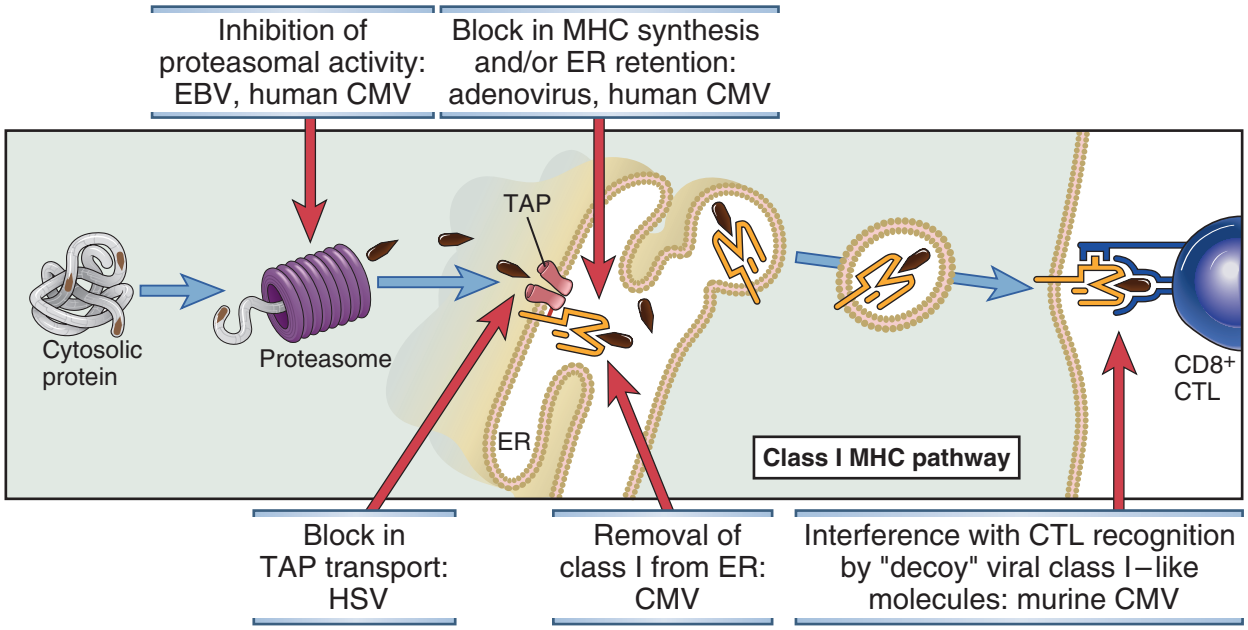


FIGURE 15–9 Mechanisms by which viruses inhibit antigen processing and presentation. The pathway of class I MHC–associated antigen presentation is shown, with examples of viruses that block different steps in this pathway. CMV, cytomegalovirus; EBV, Epstein-Barr virus; ER, endoplasmic reticulum; HSV, herpes simplex virus; TAP, transporter associated with antigen processing.

evolutionary struggle between microbes and host immunity.

- ***Some viruses produce molecules that inhibit the immune response.*** Poxviruses encode molecules that are secreted by infected cells and bind to several cytokines, including IFN-γ, TNF, IL-1, IL-18, and chemokines. The secreted cytokine-binding proteins may function as competitive antagonists of the cytokines. Epstein-Barr virus produces a protein that is homologous to the cytokine IL-10, which inhibits activation of macrophages and dendritic cells and may thus suppress cell-mediated immunity. These examples probably represent a small fraction of immunosuppressive viral molecules. Identification of these molecules raises the intriguing possibility that viruses have acquired genes encoding endogenous inhibitors of immune responses during their passage through human hosts and have thus evolved to infect and colonize humans.
- ***Some chronic viral infections are associated with failure of CTL responses,*** which allows viral persistence. Studies of a chronic infection with lymphocytic choriomeningitis in mice have shown that this type of immune deficit may result from activation of inhibitory T cell pathways, such as the PD-1 pathway, which normally functions to maintain T cell tolerance to self antigens (see Chapter 14). Reduced T cell responses resulting from HIV infection may also be partly because of PD-1–mediated T cell unresponsiveness. Thus, viruses may have evolved to exploit normal mechanisms of immune regulation and to activate these pathways in T cells. This phenomenon has been called exhaustion, implying that immune responses against the virus are initiated but then shut off prematurely.
- ***Viruses may infect and either kill or inactivate immunocompetent cells.*** The obvious example is HIV, which survives by infecting and eliminating $CD4^+$ T cells, the key inducers of immune responses to protein antigens.

IMMUNITY TO PARASITES

In infectious disease terminology, parasitic infection refers to infection with animal parasites such as protozoa, helminths, and ectoparasites (e.g., ticks and mites). Such parasites currently account for greater morbidity and mortality than any other class of infectious organisms, particularly in developing countries. It is estimated that about 30% of the world's population suffers from parasitic infestations. Malaria alone affects more than 100 million people worldwide and is responsible for 1 to 2 million deaths annually. The magnitude of this public health problem is the principal reason for the great interest in immunity to parasites and for the development of immunoparasitology as a distinct branch of immunology.

Most parasites go through complex life cycles, part of which occurs in humans (or other vertebrates) and part of which occurs in intermediate hosts, such as flies, ticks, and snails. Humans are usually infected by bites from infected intermediate hosts or by sharing a particular habitat with an intermediate host. For instance, malaria and trypanosomiasis are transmitted by insect bites, and schistosomiasis is transmitted by exposure to water in which infected snails reside. Most parasitic infections are chronic because of weak innate immunity and the ability of parasites to evade or resist elimination by adaptive immune responses. Furthermore, many antiparasite drugs are not effective at killing the organisms. Individuals living in endemic areas require repeated chemotherapy because of continued exposure, and such treatment is often not possible because of expense and logistic

problems. Therefore, the development of prophylactic vaccines for parasites has long been considered an important goal for developing countries.

Innate Immunity to Parasites

Although different protozoan and helminthic parasites have been shown to activate different mechanisms of innate immunity, these organisms are often able to survive and replicate in their hosts because they are well adapted to resisting host defenses. The principal innate immune response to protozoa is phagocytosis, but many of these parasites are resistant to phagocytic killing and may even replicate within macrophages. Some protozoa express surface molecules that are recognized by TLRs and activate phagocytes. *Plasmodium* species (the protozoa that are responsible for malaria), *Toxoplasma gondii* (the agent that causes toxoplasmosis), and *Cryptosporidium* species (the major parasite that causes diarrhea in HIV-infected patients) all express glycosyl phosphatidylinositol lipids that can activate TLR2 and TLR4. Phagocytes may also attack helminthic parasites and secrete microbicidal substances to kill organisms that are too large to be phagocytosed. However, many helminths have thick teguments that make them resistant to the cytocidal mechanisms of neutrophils and macrophages, and they are too large to be ingested by phagocytes. Some helminths may activate the alternative pathway of complement, although as we shall discuss later, parasites recovered from infected hosts appear to have developed resistance to complement-mediated lysis.

Adaptive Immunity to Parasites

Different protozoa and helminths vary greatly in their structural and biochemical properties, life cycles, and pathogenic mechanisms. It is therefore not surprising that different parasites elicit distinct adaptive immune responses (Table 15-4). Some pathogenic protozoa have evolved to survive within host cells, so protective immunity against these organisms is mediated by mechanisms similar to those that eliminate intracellular bacteria and viruses. In contrast, metazoa such as helminths survive in extracellular tissues, and their elimination is often dependent on special types of antibody responses.

The principal defense mechanism against protozoa that survive within macrophages is cell-mediated immunity, particularly macrophage activation by T_H1 cell–derived cytokines. Infection of mice with *Leishmania major,* a protozoan that survives within the endosomes of macrophages, is the best documented example of how dominance of T_H1 or T_H2 responses determines disease resistance or susceptibility (see Fig. 15-5). Resistance to the infection is associated with activation of *Leishmania*-specific T_H1 $CD4^+$ T cells, which produce IFN-γ and thereby activate macrophages to destroy intracellular parasites. Conversely, activation of T_H2 cells by the protozoa results in increased parasite survival and exacerbation of lesions because of the macrophage-suppressive actions of T_H2 cytokines, notably IL-4. A good example of this difference is seen in *Leishmania* infections in different inbred mouse strains. Most inbred strains of mice are resistant to infection with *L. major,* but inbred BALB/c and some related strains of mice are highly susceptible and die if they are infected with large numbers of parasites. After infection, the resistant strains produce large amounts of IFN-γ in response to leishmanial antigens, whereas the strains that are susceptible to fatal leishmaniasis produce more IL-4 in response to the parasite. Promoting the T_H1 response or inhibiting the T_H2 response in susceptible strains increases their resistance to the infection. Multiple genes appear to control the balance of protective and harmful immune responses to intracellular parasites in inbred mice and presumably in humans as well. Attempts to identify these genes are ongoing in many laboratories.

Protozoa that replicate inside various host cells and lyse these cells stimulate specific antibody and CTL responses, similar to cytopathic viruses. An example of such an organism is the malaria parasite, which resides mainly in red blood cells and in hepatocytes during its life cycle. It was thought for many years that antibodies were the major protective mechanism against malaria, and early attempts at vaccinating against this infection focused on generating antibodies. It is now apparent that the CTL response against parasites residing in hepatocytes is an important defense against the spread of this intracellular protozoan. The cytokine IFN-γ has been shown to be protective in many protozoal infections, including malaria, toxoplasmosis, and cryptosporidiosis.

Defense against many helminthic infections is mediated by the activation of T_H2 cells, which results in production of IgE antibodies and activation of eosinophils. Helminths stimulate differentiation of naive $CD4^+$ helper T cells to the T_H2 subset of effector cells, which secrete

TABLE 15–4 Immune Responses to Disease-Causing Parasites

Parasite	Diseases	Principal Mechanisms of Protective Immunity
Protozoa		
Plasmodium species	Malaria	Antibodies and $CD8^+$ CTLs
Leishmania donovani	Leishmaniasis (mucocutaneous disseminated)	$CD4^+$ T_H1 cells activate macrophages to kill phagocytosed parasites
Trypanosoma brucei	African trypanosomiasis	Antibodies
Entamoeba histolytica	Amebiasis	Antibodies, phagocytosis
Metazoa		
Schistosoma species	Schistosomiasis	Killing by eosinophils, macrophages
Filaria, e.g., *Wuchereria bancrofti*	Filariasis	Cell-mediated immunity; role of antibodies?

Selected examples of parasites and immune responses to them are listed.

IL-4 and IL-5. IL-4 stimulates the production of IgE, which binds to the Fcε receptor of eosinophils and mast cells, and IL-5, which stimulates the development of eosinophils and activates eosinophils. IgE, mast cell and eosinophil-mediated effector mechanisms are described in Chapter 19. The combined actions of mast cells and eosinophils also contribute to expulsion of the parasites from the intestine, so-called barrier immunity (see Chapter 10, Fig. 10-9). The expulsion of some intestinal nematodes may be due to IL-4–dependent mechanisms that do not require IgE, such as increased peristalsis.

Adaptive immune responses to parasites can also contribute to tissue injury. Some parasites and their products induce granulomatous responses with concomitant fibrosis. *Schistosoma mansoni* eggs deposited in the liver stimulate $CD4^+$ T cells, which in turn activate macrophages and induce DTH reactions. DTH reactions result in the formation of granulomas around the eggs; an unusual feature of these granulomas, especially in mice, is their association with T_H2 responses. (Granulomas are generally induced by T_H1 responses against persistent antigens; see Chapter 18.) Such T_H2-induced granulomas may result from the process of "alternative macrophage activation" that is induced by IL-4 and IL-13 (see Chapter 10). The granulomas serve to contain the schistosome eggs, but severe fibrosis associated with this chronic cell-mediated immune response leads to cirrhosis, disruption of venous blood flow in the liver, and portal hypertension. In lymphatic filariasis, lodging of the parasites in lymphatic vessels leads to chronic cell-mediated immune reactions and ultimately to fibrosis. Fibrosis results in lymphatic obstruction and severe lymphedema. Chronic and persistent parasitic infestations are often associated with the formation of complexes of parasite antigens and specific antibodies. The complexes can be deposited in blood vessels and kidney glomeruli and produce vasculitis and nephritis, respectively (see Chapter 18). Immune complex disease is a complication of schistosomiasis and malaria.

Immune Evasion by Parasites

Parasites evade protective immunity by reducing their immunogenicity and by inhibiting host immune responses. Different parasites have developed remarkably effective ways of resisting immunity (Table 15-5).

TABLE 15–5 Mechanisms of Immune Evasion by Parasites

Mechanism of Immune Evasion	Examples
Antigenic variation	Trypanosomes, *Plasmodium*
Acquired resistance to complement, CTLs	Schistosomes
Inhibition of host immune responses	Filaria (secondary to lymphatic obstruction), trypanosomes
Antigen shedding	Entamoeba

CTL, cytotoxic T lymphocyte.

- Parasites change their surface antigens during their life cycle in vertebrate hosts. Two forms of antigenic variation are well defined. The first is a stage-specific change in antigen expression, such that the mature tissue stages of parasites produce antigens different from those of the infective stages. For example, the infective sporozoite stage of malaria parasites is antigenically distinct from the merozoites that reside in the host and are responsible for chronic infection. By the time the immune system has responded to infection by sporozoites, the parasite has differentiated, expresses new antigens, and is no longer a target for immune elimination. The second and more remarkable example of antigenic variation in parasites is the continuous variation of major surface antigens seen in African trypanosomes such as *Trypanosoma brucei* and *Trypanosoma rhodesiense*. Continuous antigenic variation in trypanosomes is mainly due to programmed variation in expression of the genes encoding the major surface antigen. Infected patients show waves of blood parasitemia, and each wave consists of parasites expressing a surface antigen that is different from the previous wave. Thus, by the time the host produces antibodies against the parasite, an antigenically different organism has grown out. More than a hundred such waves of parasitemia can occur in an infection. One consequence of antigenic variation in parasites is that it is difficult to effectively vaccinate individuals against these infections.
- Parasites become resistant to immune effector mechanisms during their residence in vertebrate hosts. Perhaps the best examples are schistosome larvae, which travel to the lungs of infected animals and during this migration develop a tegument that is resistant to damage by complement and by CTLs. The biochemical basis of this change is not known.
- Protozoan parasites may conceal themselves from the immune system either by living inside host cells or by developing cysts that are resistant to immune effectors. Some helminthic parasites reside in intestinal lumens and are sheltered from cell-mediated immune effector mechanisms. Parasites may also shed their antigenic coats, either spontaneously or after binding specific antibodies. Shedding of antigens renders the parasites resistant to subsequent antibody-mediated attack. *Entamoeba histolytica* is a protozoan parasite that sheds antigens and can also convert to a cyst form in the lumen of the large intestine.
- Parasites inhibit host immune responses by multiple mechanisms. T cell anergy to parasite antigens has been observed in severe schistosomiasis involving the liver and spleen and in filarial infections. The mechanisms of immunologic unresponsiveness in these infections are not well understood. In lymphatic filariasis, infection of lymph nodes with subsequent architectural disruption may contribute to deficient immunity. Some parasites, such as *Leishmania,* stimulate the development of regulatory T cells, which suppress the immune response enough to allow persistence of the parasites. More nonspecific and generalized immunosuppression is observed in malaria and African trypanosomiasis. This immune deficiency has been

attributed to the production of immunosuppressive cytokines by activated macrophages and T cells and defects in T cell activation.

The consequences of parasitic infestations for health and economic development are devastating. Attempts to develop effective vaccines against these infections have been actively pursued for many years. Although the progress has been slower than one would have hoped, elucidation of the fundamental mechanisms of immune responses to and immune evasion by parasites holds promise for the future.

STRATEGIES FOR VACCINE DEVELOPMENT

The birth of immunology as a science dates from Edward Jenner's successful vaccination against smallpox in 1796. The importance of prophylactic immunization against infectious diseases is best illustrated by the fact that worldwide programs of vaccination have led to the complete or nearly complete eradication of many of these diseases in developed countries (see Chapter 1, Table 1-1). The fundamental principle of vaccination is to administer a killed or attenuated form of an infectious agent, or a component of a microbe, that does not cause disease but elicits an immune response that provides protection against infection by the live, pathogenic microbe.

The success of vaccination in eradicating infectious disease is dependent on several properties of the microbes. Vaccines are effective if the infectious agent does not establish latency, if it does not undergo much or any antigenic variation, and if it does not interfere with the host immune response. It is difficult to effectively vaccinate against microbes such as HIV, which establishes latent infection, is highly variable, and disables key components of the immune system. Vaccines are most effective against infections that are limited to human hosts and do not have animal reservoirs.

Most vaccines in use today work by inducing humoral immunity. Antibodies are the only immune mechanism that prevents infections, by neutralizing and clearing microbes before they gain their foothold in the host. The best vaccines are those that stimulate the development of long-lived plasma cells that produce high-affinity antibodies as well as memory B cells. These aspects of humoral immune responses are best induced by the germinal center reaction (see Chapter 11), which requires help provided by protein antigen-specific $CD4^+$ T cells.

In the following section, we summarize the approaches to vaccination that have been tried (Table 15-6) and their major value and limitations.

TABLE 15–6 Vaccine Approaches

Type of Vaccine	Examples
Live attenuated or killed bacteria	Bacillus Calmette-Guérin, cholera
Live attenuated viruses	Polio, rabies
Subunit (antigen) vaccines	Tetanus toxoid, diphtheria toxoid
Conjugate vaccines	*Haemophilus influenzae,* pneumococcus
Synthetic vaccines	Hepatitis (recombinant proteins)
Viral vectors	Clinical trials of HIV antigens in canarypox vector
DNA vaccines	Clinical trials ongoing for several infections

Attenuated and Inactivated Bacterial and Viral Vaccines

Vaccines composed of intact nonpathogenic microbes are made by treating the microbes in such a way that they can no longer cause disease (i.e., their virulence is attenuated) or by killing the microbes while retaining their immunogenicity. The great advantage of attenuated microbial vaccines is that they elicit all the innate and adaptive immune responses (both humoral and cell mediated) that the pathogenic microbe would, and they are therefore the ideal way of inducing protective immunity. Live, attenuated bacteria were first shown by Louis Pasteur to confer specific immunity. The attenuated or killed bacterial vaccines in use today generally induce limited protection and are effective for only short periods. Live, attenuated viral vaccines are usually more effective; polio, measles, and yellow fever are three good examples. The most frequently used approach for producing such attenuated viruses is repeated passage in cell culture. More recently, temperature-sensitive and deletion mutants have been generated to achieve the same goal. Viral vaccines often induce long-lasting specific immunity, so immunization of children is sufficient for lifelong protection. Some attenuated viral vaccines (e.g., polio) may cause disease in immune-compromised hosts, and for this reason inactivated poliovirus vaccines are now more commonly used. The major concern with attenuated viral or bacterial vaccines is safety.

A widely used inactivated vaccine of considerable public health importance is the influenza vaccine. Influenza viruses grown in chicken eggs are used in two types of vaccines. The most common vaccine is a trivalent inactivated (killed) vaccine that is used in the "flu shot" that is given intramuscularly. Three of the most frequently encountered influenza strains are selected every year and incorporated in this vaccine. A second type of influenza vaccine involves the same three strains, but the vaccine is made up of live attenuated viruses and is used as a nasal spray.

Purified Antigen (Subunit) Vaccines

Subunit vaccines are composed of antigens purified from microbes or inactivated toxins and are usually administered with an adjuvant. One effective use of purified antigens as vaccines is for the prevention of diseases caused by bacterial toxins. Toxins can be rendered harmless without loss of immunogenicity, and such "toxoids" induce strong antibody responses. Diphtheria and tetanus are two infections whose life-threatening consequences have been largely controlled because of immunization of children

with toxoid preparations. Vaccines composed of bacterial polysaccharide antigens are used against pneumococcus and *H. influenzae*. Because polysaccharides are T-independent antigens, they tend to elicit low-affinity antibody responses and may be poorly immunogenic in infants (who do not mount strong T cell–independent antibody responses). High-affinity antibody responses may be generated against polysaccharide antigens even in infants by coupling the polysaccharides to proteins to form conjugate vaccines. Such vaccines work like hapten-carrier conjugates and are a practical application of the principle of T-B cell cooperation (see Chapter 11). The currently used *H. influenzae,* pneumococcal, and meningococcal vaccines are conjugate vaccines. Purified protein vaccines stimulate helper T cells and antibody responses, but they do not generate potent CTLs. The reason for poor CTL development is that, unlike with attenuated microbial vaccines, exogenous proteins (and peptides) are inefficient at entering the class I MHC pathway of antigen presentation and cannot readily displace peptides from surface class I molecules. As a result, protein vaccines are not recognized efficiently by class I–restricted $CD8^+$ T cells.

Synthetic Antigen Vaccines

A goal of vaccine research has been to identify the most immunogenic microbial antigens or epitopes, to synthesize these in the laboratory, and to use the synthetic antigens as vaccines. It is possible to deduce the protein sequences of microbial antigens from nucleotide sequence data and to prepare large quantities of proteins by recombinant DNA technology. Vaccines made of recombinant DNA-derived antigens are now in use for hepatitis virus, herpes simplex virus, foot-and-mouth disease virus (a major pathogen for livestock), human papillomavirus, and rotavirus. In the case of the most widely used human papillomavirus vaccine, recombinant viral proteins from four viral strains (HPV 6, 11, 16, and 18) are made in yeast and combined with an adjuvant. HPV 6 and 11 are common causes of warts, and HPV 16 and 18 are the most common HPV strains linked to cervical cancer. This antiviral vaccine is therefore also a preventive cancer vaccine.

Live Viral Vaccines Involving Recombinant Viruses

Another approach for vaccine development is to introduce genes encoding microbial antigens into a noncytopathic virus and to infect individuals with this virus. Thus, the virus serves as a source of the antigen in an inoculated individual. The great advantage of viral vectors is that they, like other live viruses, induce the full complement of immune responses, including strong CTL responses. This technique has been used most commonly with vaccinia virus vectors. Inoculation of such recombinant viruses into many species of animals induces both humoral and cell-mediated immunity against the antigen produced by the foreign gene (and, of course, against vaccinia virus antigens as well). A potential problem with recombinant viruses is that the viruses may infect host cells, and even though they are not pathogenic, they may produce antigens that stimulate CTL responses that kill the infected host cells. These and other safety concerns have limited widespread use of viral vectors for vaccine delivery.

DNA Vaccines

An interesting method of vaccination was developed on the basis of an unexpected observation. Inoculation of a plasmid containing complementary DNA (cDNA) encoding a protein antigen leads to strong and long-lived humoral and cell-mediated immune responses to the antigen. It is likely that APCs, such as dendritic cells, are transfected by the plasmid and the cDNA is transcribed and translated into immunogenic protein that elicits specific responses. The unique feature of DNA vaccines is that they provide the only approach, other than live viruses, for eliciting strong CTL responses because the DNA-encoded proteins are synthesized in the cytosol of transfected cells. Furthermore, bacterial plasmids are rich in unmethylated CpG nucleotides and are recognized by a TLR (TLR9) on dendritic cells and other cells, thereby eliciting an innate immune response that enhances adaptive immunity (see Chapter 4). Therefore, plasmid DNA vaccines could be effective even when administered without adjuvants. The ease of manipulating cDNAs to express many diverse antigens, the ability to store DNA without refrigeration for use in the field, and the ability to coexpress other proteins that may enhance immune responses (such as cytokines and costimulators) make this technique promising. However, DNA vaccines have not been as effective as hoped in clinical trials, and the factors that determine the efficacy of these vaccines, especially in humans, are still not fully defined.

Adjuvants and Immunomodulators

The initiation of T cell–dependent immune responses against protein antigens requires that the antigens be administered with adjuvants. Most adjuvants elicit innate immune responses, with increased expression of costimulators and production of cytokines such as IL-12 that stimulate T cell growth and differentiation. Heat-killed bacteria are powerful adjuvants that are commonly used in experimental animals. However, the severe local inflammation that such adjuvants trigger precludes their use in humans. Much effort is currently being devoted to development of safe and effective adjuvants for use in humans. Several are in clinical practice, including aluminum hydroxide gel (which appears to promote B cell responses) and lipid formulations that are ingested by phagocytes. An alternative to adjuvants is to administer natural substances that stimulate T cell responses together with antigens. For instance, IL-12 incorporated in vaccines promotes strong cell-mediated immunity. As mentioned, plasmid DNA has intrinsic adjuvant-like activities, and it is possible to incorporate costimulators (e.g., B7 molecules) or cytokines into plasmid DNA vaccines. These interesting ideas remain experimental.

Passive Immunization

Protective immunity can also be conferred by passive immunization, for instance, by transfer of specific

antibodies. In the clinical situation, passive immunization is most commonly used for rapid treatment of potentially fatal diseases caused by toxins, such as tetanus, and for protection from rabies and hepatitis. Antibodies against snake venom can be lifesaving when administered after poisonous snakebites. Passive immunity is short-lived because the host does not respond to the immunization, and protection lasts only as long as the injected antibody persists. Moreover, passive immunization does not induce memory, so an immunized individual is not protected against subsequent exposure to the toxin or microbe.

SUMMARY

- The interaction of the immune system with infectious organisms is a dynamic interplay of host mechanisms aimed at eliminating infections and microbial strategies designed to permit survival in the face of powerful defenses. Different types of infectious agents stimulate distinct types of immune responses and have evolved unique mechanisms for evading immunity. In some infections, the immune response is the cause of tissue injury and disease.
- Innate immunity against extracellular bacteria is mediated by phagocytes and the complement system (the alternative and lectin pathways).
- The principal adaptive immune response against extracellular bacteria consists of specific antibodies that opsonize the bacteria for phagocytosis and activate the complement system. Toxins produced by such bacteria are neutralized by specific antibodies. Some bacterial toxins are powerful inducers of cytokine production, and cytokines account for much of the systemic disease associated with severe, disseminated infections with these microbes.
- Innate immunity against intracellular bacteria is mediated mainly by macrophages. However, intracellular bacteria are capable of surviving and replicating within host cells, including phagocytes, because they have developed mechanisms for resisting degradation within phagocytes.
- Adaptive immunity against intracellular bacteria is principally cell mediated and consists of activation of macrophages by $CD4^+$ T cells (as in DTH) as well as killing of infected cells by $CD8^+$ CTLs. The characteristic pathologic response to infection by intracellular bacteria is granulomatous inflammation.
- Protective responses to fungi consist of innate immunity, mediated by neutrophils and macrophages, and adaptive cell-mediated and humoral immunity. Fungi are usually readily eliminated by phagocytes and a competent immune system, because of which disseminated fungal infections are seen mostly in immunodeficient persons.
- Innate immunity against viruses is mediated by type I interferons and NK cells. Neutralizing antibodies protect against virus entry into cells early in the course of infection and later if the viruses are released from killed infected cells. The major defense mechanism against established infection is CTL-mediated killing of infected cells. CTLs may contribute to tissue injury even when the infectious virus is not harmful by itself. Viruses evade immune responses by antigenic variation, inhibition of antigen presentation, and production of immunosuppressive molecules.
- Parasites such as protozoa and helminths give rise to chronic and persistent infections because innate immunity against them is weak and parasites have evolved multiple mechanisms for evading and resisting specific immunity. The structural and antigenic diversity of pathogenic parasites is reflected in the heterogeneity of the adaptive immune responses that they elicit. Protozoa that live within host cells are destroyed by cell-mediated immunity, whereas helminths are eliminated by IgE antibody and eosinophil-mediated killing as well as by other leukocytes. Parasites evade the immune system by varying their antigens during residence in vertebrate hosts, by acquiring resistance to immune effector mechanisms, and by masking and shedding their surface antigens.
- Vaccination is a powerful strategy for preventing infections. The most effective vaccines are those that stimulate the production of high-affinity antibodies and memory cells. Many approaches for vaccinating are in clinical use and being tried for various infections.

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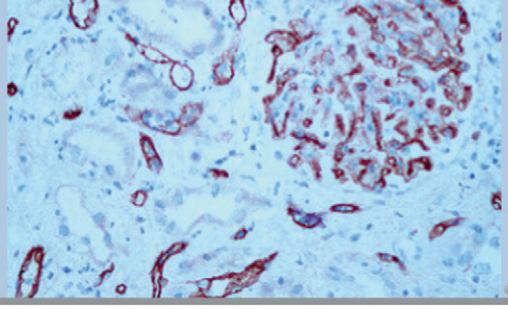
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CHAPTER

16

Transplantation Immunology

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Transplantation is a widely used treatment for replacement of nonfunctioning organs and tissues with healthy organs or tissues. Technically, transplantation is the process of taking cells, tissues, or organs, called a **graft**, from one individual and placing them into a (usually) different individual. The individual who provides the graft is called the **donor**, and the individual who receives the graft is called either the **recipient** or the **host**. If the graft is placed into its normal anatomic location, the procedure is called orthotopic transplantation; if the graft is placed in a different site, the procedure is called heterotopic transplantation. **Transfusion** refers to the transfer of circulating blood cells or plasma from one individual to another. Clinical transplantation to treat human diseases has increased steadily during the past 45 years, and transplantation of kidneys, hearts, lungs, livers, pancreata, and bone marrow is widely used today (Fig. 16-1). More than 30,000 kidney, heart, lung, liver, and pancreas transplantations are currently performed in the United States each year. In addition, transplantation of many other organs or cells, including stem cells, is now being attempted.

Transplantation of cells or tissues from one individual to a genetically nonidentical individual invariably leads to rejection of the transplant due to an adaptive immune response. Rejection has been a major barrier to successful transplantation of tissues. This problem was first appreciated when attempts to replace damaged skin on burn patients with skin from unrelated donors proved to be uniformly unsuccessful. During a matter of 1 to 2 weeks, the transplanted skin would undergo necrosis and fall off. The failure of the grafts led Peter Medawar and many other investigators to study skin transplantation in animal models. These experiments established that the failure of skin grafting was caused by an inflammatory reaction called **rejection**. The conclusion that graft rejection is the result of an adaptive immune response came from experiments demonstrating that the process had characteristics of memory and specificity and was mediated by lymphocytes (Fig. 16-2). For instance, rejection occurs in 7 to 14 days after the first transplant from a donor to a recipient (called first-set rejection) and more rapidly after the second transplant from the same donor to this recipient (called second-set rejection), implying that the recipient developed memory for the grafted tissue. Individuals who have rejected a graft from one donor show accelerated rejection of another graft from the same donor but not from a different donor, demonstrating that the rejection process is immunologically specific. These

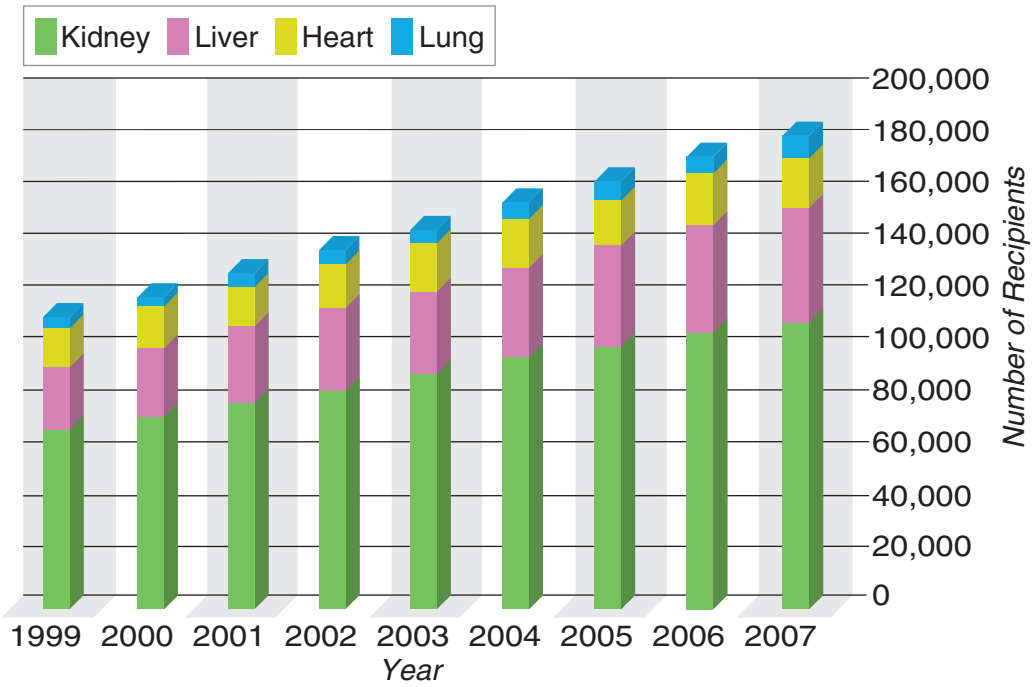


FIGURE 16–1 People in the United States living with functioning organ grafts, 1999-2007. *(Data from OPTN/SRTR Annual Report 2009. Available at: http://www.ustransplant.org/csr/current/fastfacts.aspx. Accessed April 2010.)*

experimental results were recapitulated in clinical transplantation. Perhaps the most compelling evidence showing that allograft rejection is an adaptive immune response was the finding that the ability to rapidly reject a transplant can be transferred with lymphocytes from a sensitized to a naive host.

Transplant immunologists have developed a special vocabulary to describe the kinds of cells and tissues encountered in the transplant setting. A graft transplanted from one individual to the same individual is called an **autologous graft**. A graft transplanted between two genetically identical or syngeneic individuals is called a **syngeneic graft**. A graft transplanted between two genetically different individuals of the same species is called an **allogeneic graft** (or **allograft**). A graft transplanted between individuals of different species is called a **xenogeneic graft** (or **xenograft**). The molecules that are recognized as foreign on allografts are called **alloantigens**, and those on xenografts are called **xenoantigens**. The lymphocytes and antibodies that react with alloantigens or xenoantigens are described as being **alloreactive** or **xenoreactive**, respectively.

The immunology of transplantation is important for several reasons. First, immunologic rejection remains one of the major problems in clinical transplantation. Second, although transplantation of tissues is not a normal phenomenon, the immune response to allogeneic molecules has been a useful model for studying the mechanisms of lymphocyte activation. Third, many immunosuppressive therapies that have proved to be useful for a variety of immunologic and inflammatory diseases were first tested and shown to be effective for treatment of graft rejection, which is a clinically important immunologic reaction that can be measured rapidly and with precision. Most of this chapter focuses on allogeneic transplantation because it is far more commonly practiced and better understood than xenogeneic transplantation, which is discussed briefly at the end of the chapter. We consider both the basic immunology and some aspects of the clinical practice of transplantation. We conclude the chapter with a discussion of hematopoietic stem cell transplantation, which raises special issues not usually encountered with solid organ transplants.

IMMUNE RESPONSES TO ALLOGRAFTS

Alloantigens elicit both cellular and humoral immune responses. In this section of the chapter, we discuss the molecular and cellular mechanisms of allorecognition, with an emphasis on the nature of graft antigens that stimulate allogeneic responses and the properties of the responding lymphocytes.

Recognition of Alloantigens

Recognition of transplanted cells as self or foreign is determined by polymorphic genes, called histocompatibility genes, which differ among different members of a species. This conclusion is based on the results of experimental transplantation between inbred strains of mice, and in some cases, the results have been confirmed in human transplantation. Remember that all the animals of an inbred strain are genetically identical, and they are homozygous for all genes (except the sex chromosomes in males). The basic rules of transplantation immunology, which are derived from such animal experiments, are the following (Fig. 16-3).

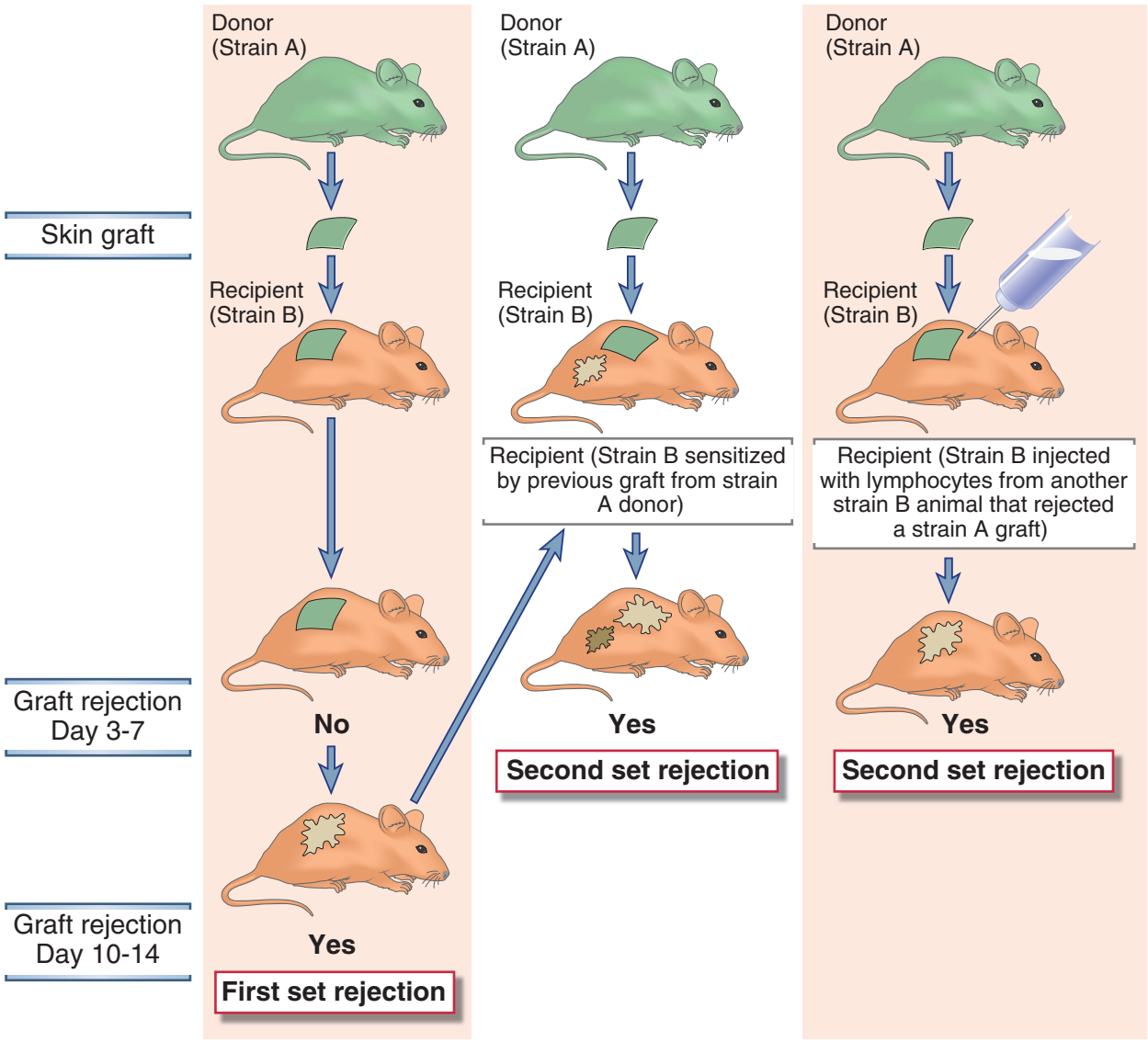


FIGURE 16–2 First- and second-set allograft rejection. Results of the experiments shown indicate that graft rejection displays the features of adaptive immune responses, namely, memory and mediation by lymphocytes. An inbred strain B mouse will reject a graft from an inbred strain A mouse with first-set kinetics *(left panel).* An inbred strain B mouse sensitized by a previous graft from an inbred strain A mouse will reject a second graft from an inbred strain A mouse with second-set kinetics *(middle panel),* demonstrating memory. An inbred strain B mouse injected with lymphocytes from another strain B mouse that has rejected a graft from a strain A mouse will reject a graft from a strain A mouse with second-set kinetics *(right panel),* demonstrating the role of lymphocytes in mediating rejection and memory. An inbred strain B mouse sensitized by a previous graft from a strain A mouse will reject a graft from a third unrelated strain with first-set kinetics, thus demonstrating another feature of adaptive immunity, specificity (not shown). Syngeneic grafts are never rejected (not shown).

- Cells or organs transplanted between genetically identical individuals (identical twins or members of the same inbred strain of animals) are never rejected.
- Cells or organs transplanted between genetically nonidentical people or members of two different inbred strains of a species are almost always rejected.
- The offspring of a mating between two different inbred strains of animal will typically not reject grafts from either parent. In other words, an $(A \times B)F_1$ animal will not reject grafts from an A or B strain animal. (This rule is violated by bone marrow transplantation, which we will discuss later in the chapter.)
- A graft derived from the offspring of a mating between two different inbred strains will almost always be rejected by either parent. In other words, a graft from an $(A \times B)F_1$ animal will be rejected by either an A or a B strain animal.

Such results suggested that the molecules in the grafts that are responsible for eliciting rejection must be polymorphic and their expression is codominant. Polymorphic refers to the fact that these graft antigens differ among the individuals of a species (other than identical twins) or between different inbred strains of animals. Codominant expression means that every individual inherits genes encoding these molecules from both parents and both parental alleles are expressed. Therefore, $(A \times B)F_1$ animals express both A and B alleles

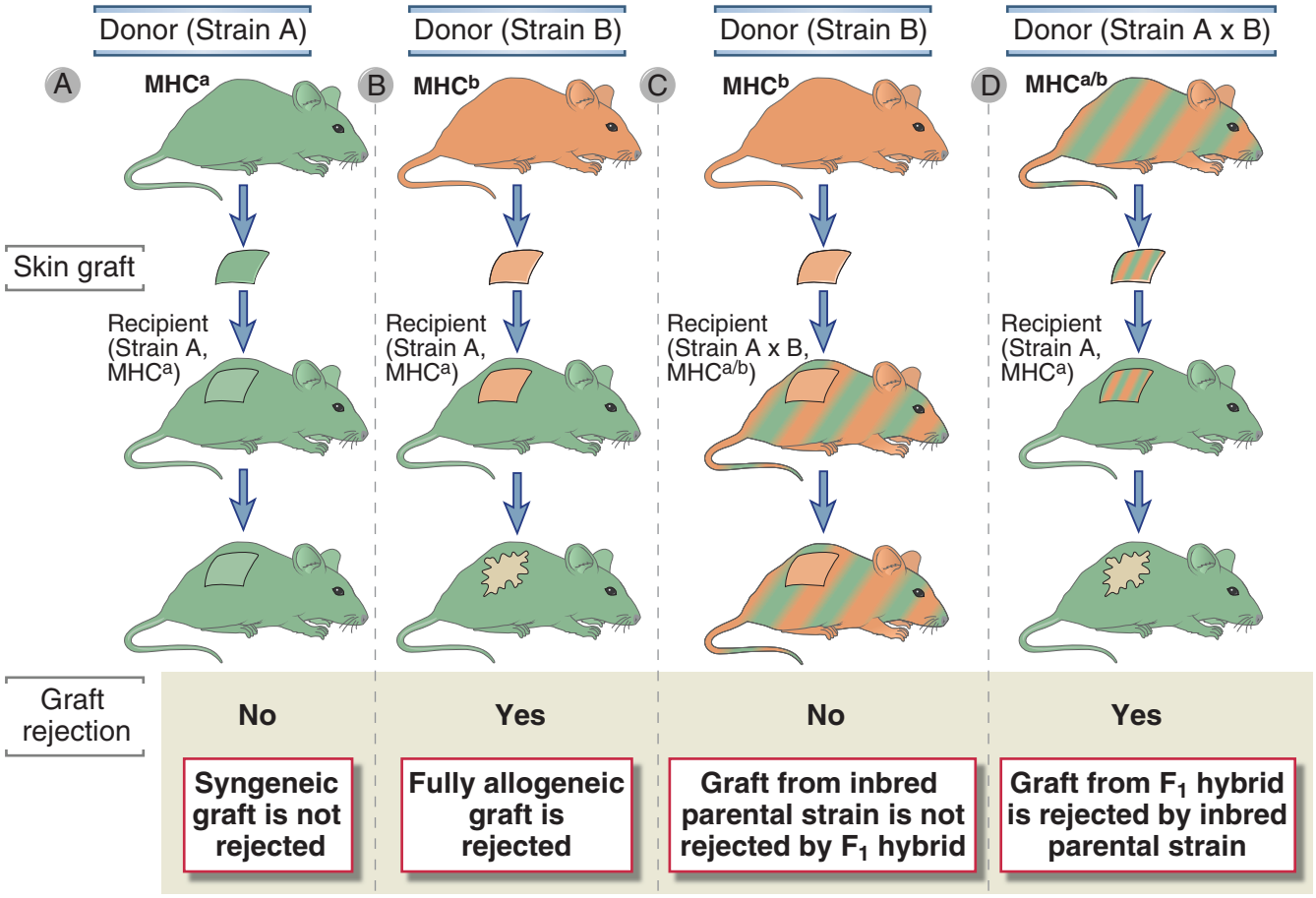


FIGURE 16–3 The genetics of graft rejection. In the illustration, the two different mouse colors represent inbred strains with different MHC haplotypes. Inherited MHC alleles from both parents are codominantly expressed in the skin of an A × B offspring, and therefore these mice are represented by both colors. Syngeneic grafts are not rejected **(A).** Allografts are always rejected **(B)**. Grafts from an A or B parent will not be rejected by an (A × B)F1 offspring **(C)**, but grafts from the offspring will be rejected by either parent **(D)**. These phenomena are due to the fact that MHC gene products are responsible for graft rejection; grafts are rejected only if they express an MHC type (represented by green or orange) that is not expressed by the recipient mouse.

and see both A and B tissues as self, whereas inbred A or B animals express only one allele and see (A × B)F_1 tissues as partly foreign. This is why an (A × B)F_1 animal does not reject either A or B strain grafts and why both A and B strain recipients reject an (A × B)F_1 graft.

The molecules responsible for almost all strong (rapid) rejection reactions are called major histocompatibility complex (MHC) molecules. George Snell and colleagues used pairs of congenic strains of inbred mice, which were bred to be genetically identical to each other except for genes needed for graft rejection, to identify the polymorphic genes that encode the molecular targets of allograft rejection. This approach led to the identification of MHC genes as the underlying genetic basis of graft rejection. Transplants of most tissues between any pair of individuals, except identical twins, will be rejected because MHC molecules, the major polymorphic targets of graft rejection, are expressed on virtually all tissues. As discussed in Chapter 6, the normal function of MHC molecules is to present peptides derived from protein antigens in a form that can be recognized by T cells. The role of MHC molecules as the antigens that cause graft rejection is a consequence of the nature of T cell antigen recognition, as we will discuss later. Recall that human MHC molecules are called human leukocyte antigens (HLA), and in the context of human transplantation, the terms MHC and HLA are used interchangeably.

Allogeneic MHC molecules of a graft may be presented for recognition by the T cells of the recipient in two fundamentally different ways, called direct and indirect (Fig. 16-4). Initial studies showed that the T cells of a graft recipient recognize intact, unprocessed MHC molecules in the graft, and this is called **direct presentation** of alloantigens. Subsequent studies showed that sometimes, the recipient T cells recognize graft MHC molecules only in the context of the recipient's MHC molecules, implying that the recipient's MHC molecules must be presenting allogenic graft MHC proteins to recipient T cells. This process is called **indirect presentation**, and it is essentially the same as the presentation of any foreign (e.g., microbial) protein antigen. Not only MHC molecules but other alloantigens in a graft that are different between the donor and recipient can also be presented to host T cells by the indirect pathway. We discuss the mechanisms of direct and indirect presentation separately.

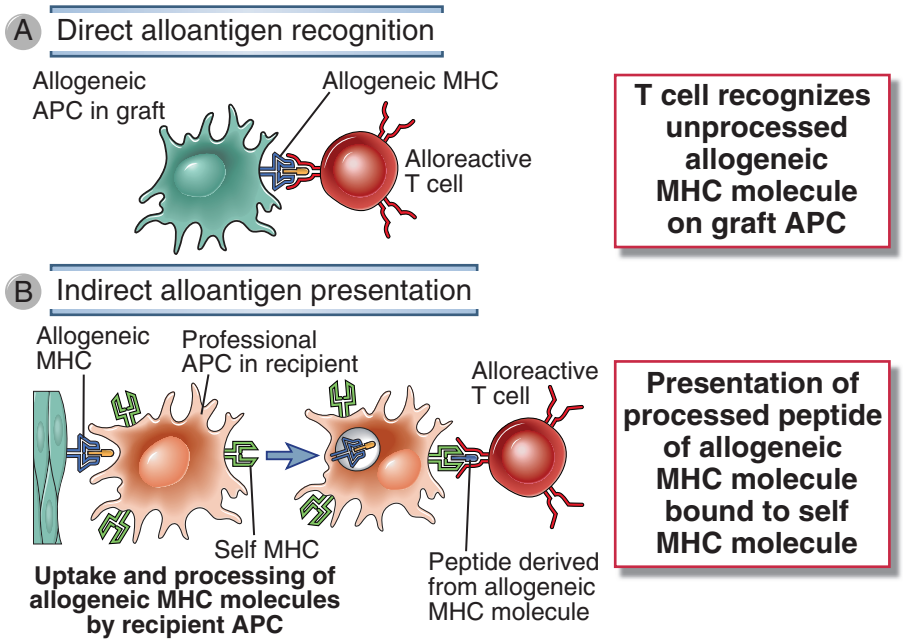


FIGURE 16–4 Direct and indirect alloantigen recognition. A, Direct alloantigen recognition occurs when T cells bind directly to an intact allogeneic MHC molecule on a graft (donor) antigen-presenting cell (APC). **B,** Indirect alloantigen recognition occurs when allogeneic MHC molecules from graft cells are taken up and processed by recipient APCs and peptide fragments of the allogeneic MHC molecules containing polymorphic amino acid residues are bound and presented by recipient (self) MHC molecules.

Direct Presentation of MHC Alloantigens

In direct presentation, an intact MHC molecule is displayed by donor antigen-presenting cells (APCs) in the graft and recognized by recipient T cells without a need for host APCs. It may seem puzzling that T cells that are normally selected during their maturation to be self MHC restricted are capable of recognizing foreign (allogeneic or xenogeneic) MHC molecules. In fact, as we will discuss in more detail later, the frequency of T cells in a normal individual that recognize a single allogeneic MHC molecule is as high as 1% to 2% of all T cells, which is 100 to 1000 times greater than the frequency of T cells specific for any microbial peptide displayed by self MHC molecules. There are several likely explanations for this surprisingly strong recognition of foreign MHC molecules.

- ***The structure of all T cell receptors (TCRs) is inherently biased to recognize MHC molecules, even before selection in the thymus.*** In other words, TCR genes have evolved to encode a protein structure that has some, probably low, intrinsic affinity for MHC molecules. During T cell development in the thymus, positive selection results in survival of T cells with weak self MHC reactivity, and among these T cells, there may be many with strong reactivity to allogeneic MHC molecules. Also, negative selection in the thymus efficiently eliminates T cells with high affinity for self MHC (see Chapters 8 and 14), but it does not necessarily eliminate T cells that bind strongly to allogeneic MHC molecules, simply because these molecules are not present in the thymus. The result is that the mature repertoire has an intrinsic weak affinity for self MHC molecules and includes many T cells that bind allogeneic MHC molecules with high affinity.
- ***The structure of an allogeneic MHC molecule is similar enough to self MHC that many self MHC–restricted T cells recognize the foreign MHC molecule.*** In other words, an allogeneic MHC molecule with a bound peptide can mimic the determinant formed by a self MHC molecule plus a particular foreign peptide (Fig. 16-5). Direct allorecognition is an example of an immunologic cross-reaction in which a T cell that was selected to be self MHC restricted is able to recognize the structurally similar allogeneic MHC molecules. A single allogeneic MHC molecule may resemble many combinations of self MHC plus different bound peptides because of amino acid differences between the allogeneic and self MHC molecules. In this case, multiple T cells specific for the various self MHC–peptide complexes may cross-react with the single allogeneic MHC molecule.
- ***Many peptides may combine with a single MHC molecule and further expand the number of T cells that can recognize these combinations.*** MHC molecules that are expressed on cell surfaces normally contain bound peptides, and the peptides form part of the structure recognized by the alloreactive T cell, exactly like the role of peptides in the normal recognition of foreign antigens by self MHC–restricted T cells (Fig. 16-5C). Most of these peptides are likely to be self peptides that are the same in the donor and the recipient, but the donor peptides are displayed by allogeneic MHC molecules and therefore appear different from self peptide–self MHC complexes.
- All the MHC molecules on a donor APC will be foreign and will be recognized by alloreactive T cells; in contrast, in the case of an infection, less than 1% (and perhaps as few as 0.1%) of the MHC molecules on an APC normally present microbial peptides at any time and are recognized by T cells.

Direct allorecognition can generate both $CD4^+$ and $CD8^+$ T cells that recognize graft antigens and contribute to rejection. This aspect of the alloreactive T cell response is described later.

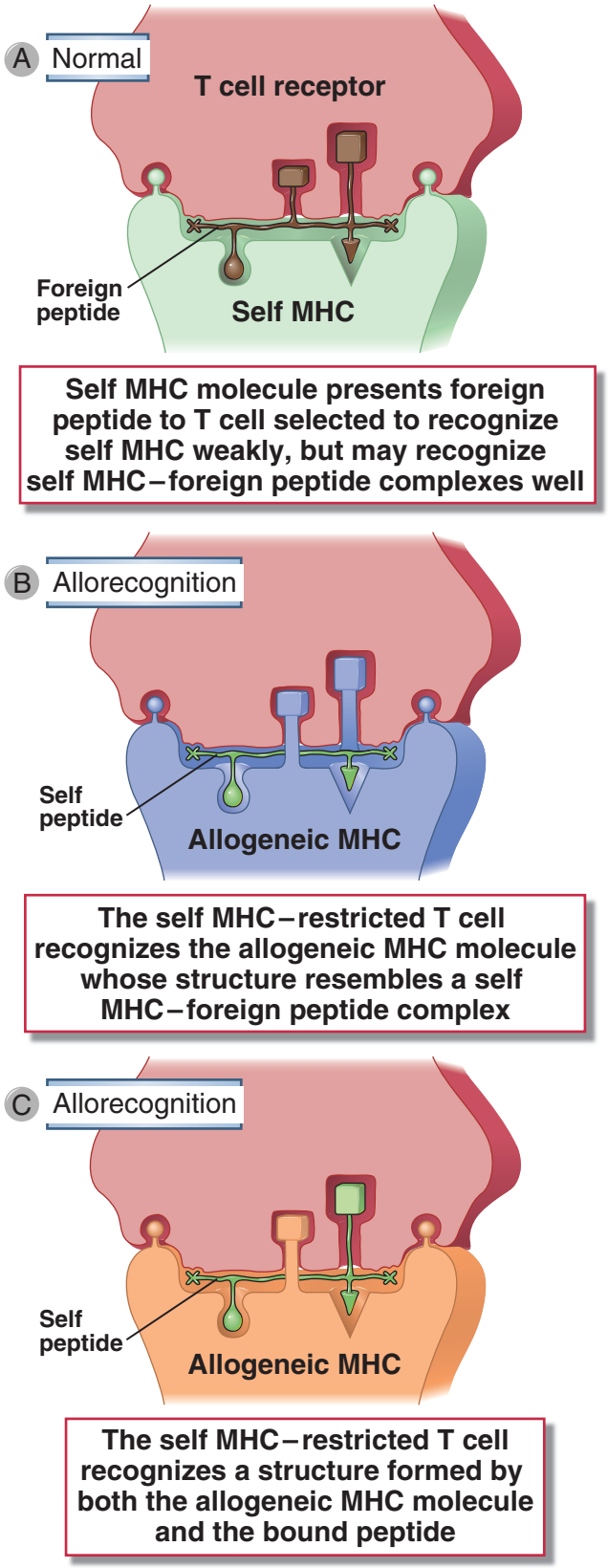


FIGURE 16–5 Molecular basis of direct recognition of allogeneic MHC molecules. Direct recognition of allogeneic MHC molecules may be thought of as a cross-reaction in which a T cell specific for a self MHC molecule–foreign peptide complex **(A)** also recognizes an allogeneic MHC molecule **(B, C)**. Nonpolymorphic donor peptides, labeled "self peptide," may not contribute to allorecognition **(B)** or they may **(C)**.

Indirect Presentation of Alloantigens

In the indirect pathway, donor (allogeneic) MHC molecules are captured and processed by recipient APCs that enter grafts, and peptides derived from the allogeneic MHC molecules are presented in association with self MHC molecules (see Fig. 16-4). Thus, peptides from the allogeneic MHC molecules are displayed by host APCs and recognized by T cells like conventional foreign protein antigens. Because allogeneic MHC molecules have amino acid sequences different from those of the host, they can generate foreign peptides associated with self MHC molecules on the surface of host APCs. In fact, MHC molecules are the most polymorphic proteins in the genome; therefore, each allogeneic MHC molecule may give rise to multiple foreign peptides, each recognized by different T cells. Indirect presentation may result in allorecognition by $CD4^+$ T cells because alloantigen is acquired by host APCs primarily through the endosomal vesicular pathway (i.e., as a consequence of phagocytosis) and is therefore presented by class II MHC molecules. Some antigens of phagocytosed graft cells appear to enter the class I MHC pathway of antigen presentation and are indirectly recognized by $CD8^+$ T cells. This phenomenon is an example of cross-presentation or cross-priming (see Chapter 6, Fig. 6-20), in which dendritic cells ingest antigens of another cell, from the graft, and present these antigens on class I MHC molecules to activate or "prime" $CD8^+$ T lymphocytes.

Evidence that indirect presentation of allogeneic MHC molecules plays a significant role in graft rejection was obtained from studies with knockout mice lacking class II MHC expression. For example, skin grafts from donor mice lacking class II MHC are able to induce recipient $CD4^+$ (i.e., class II restricted) T cell responses to the donor alloantigens, including peptides derived from donor class I MHC molecules. In these experiments, the donor class I MHC molecules are processed and presented by class II molecules on the recipient's APCs and stimulate the recipient's helper T cells. Evidence has also been obtained that indirect antigen presentation may contribute to late rejection of human allografts. For example, $CD4^+$ T cells from heart and liver allograft recipients recognize and are activated by peptides derived from donor MHC when presented by the patient's own APCs.

In the setting of any transplant between genetically nonidentical donor and recipient, there will be polymorphic antigens other than MHC molecules against which the recipient may mount an immune response. These antigens typically induce weak or slower (more gradual) rejection reactions than do MHC molecules and are therefore called **minor histocompatibility antigens**. Most minor histocompatibility antigens are proteins that are processed and presented to host T cells in association with self MHC molecules on host APCs (i.e., by the indirect pathway). The relevance of minor histocompatibility antigens in clinical solid organ transplantation is uncertain, mainly because there has been little success in identifying the relevant antigens. The male H-Y antigen appears to be a target of immune recognition by female recipients of male donor organs, and this correlates with a very slight increase in risk of rejection compared with sex-matched transplants. Antibodies specific for donor

alleles of the class I MHC–like molecule MIC-A are detectable in some renal allograft recipients, and the presence of the antibodies correlates with reduced graft survival. This has led to speculation that these proteins are also minor histocompatibility antigens of relevance to graft rejection. Minor histocompatibility antigens play a more significant role in stimulating graft-versus-host responses after hematopoietic stem cell transplantation, discussed later, but the nature of the relevant antigens in that setting is also not defined.

Activation of Alloreactive Lymphocytes

Allografts stimulate T and B cell responses that are similar to immune responses to conventional protein antigens but also have some special features. Here we discuss these common and unique aspects of the immune responses to alloantigens.

T Cell Recognition of Alloantigens

The T cell response to an organ graft may be initiated in the lymph nodes that drain the graft (Fig. 16-6). Most organs contain resident APCs such as dendritic cells. Transplantation of these organs into an allogeneic recipient provides APCs that express donor MHC molecules as well as costimulators. It is believed that these donor APCs migrate to regional lymph nodes and present, on their surface, unprocessed allogeneic MHC molecules to the recipient's T cells (the direct pathway of allorecognition). Host dendritic cells from the recipient may also migrate into the graft, pick up graft alloantigens, and transport these back to the draining lymph nodes, where they are displayed (the indirect pathway). Naive lymphocytes that normally traffic through the lymph node encounter these alloantigens and are induced to proliferate and differentiate into effector cells. This process is sometimes called **sensitization** to alloantigens. Effector T cells migrate back into the graft and mediate rejection.

As many as 1% to 2% of an individual's T cells are capable of recognizing and responding to a single foreign MHC molecule, and this high frequency of T cells reactive with allogeneic MHC molecules is one reason that allografts elicit strong immune responses. Recall that the frequency of T cells reactive with any foreign (e.g., microbial) antigen is only 1 in 10^5 or 10^6. The likely reasons that each allogeneic MHC molecule is directly recognized by so many different TCRs were discussed earlier.

Many of the T cells that respond to an allogeneic MHC molecule, even on first exposure, are memory T cells. It is likely that these memory cells were generated during previous exposure to other foreign (e.g., microbial) antigens and cross-react with allogeneic MHC molecules. These memory cells are not only expanded populations of antigen-specific cells but also more rapid and powerful responders than naive lymphocytes, and thus contribute to the strength of the alloreactive T cell response. Memory cells are also thought to be more resistant to immunosuppression than are naive lymphocytes, and the presence of large numbers of memory cells may lead to poor outcomes of transplantation.

Role of Costimulation in T Cell Responses to Alloantigens

In addition to recognition of alloantigen, costimulation of T cells primarily by B7 molecules on APCs is important for activating alloreactive T cells. Rejection of allografts, and stimulation of alloreactive T cells in a mixed lymphocyte reaction (described later), can be inhibited by agents that block B7 molecules. Allografts survive for longer periods when they are transplanted into knockout mice lacking B7-1 (CD80) and B7-2 (CD86) compared with transplants into normal recipients. As we will discuss later, blocking of B7 costimulation is a therapeutic strategy to inhibit graft rejection in humans as well. There is experimental evidence, largely from rodents, that several other T cell costimulatory pathways, including ICOS ligand/ICOS and Ox40 ligand/Ox40, contribute to acute allograft rejection, but the relevance of these pathways to human transplantation has not yet been examined.

The requirement for costimulation leads to the interesting question of why these costimulators are expressed by graft APCs in the absence of infection, which we have previously discussed as the physiologic stimulus for the expression of costimulators (see Chapter 9). A likely possibility is that the process of organ transplantation is associated with ischemic damage and death of some cells in the graft, during the time the organ is removed from the donor and before it is surgically connected to the circulatory system of the recipient. Several molecules expressed by or released from ischemically damaged cells (so-called damage-associated molecular patterns) stimulate innate immune responses that result in increased expression of costimulators on APCs (see Chapter 4). In fact, the clinical experience is that the ischemia time of an organ is a determinant of the frequency and severity of acute rejection, and one reason for this may be that ischemic death of graft cells stimulates subsequent antigraft immune responses.

The Mixed Lymphocyte Reaction

The response of alloreactive T cells to foreign MHC molecules can be analyzed in an in vitro reaction called the **mixed lymphocyte reaction** (MLR). The MLR is used as a predictive test of T cell–mediated graft rejection. Studies of the MLR were among the first to establish the role of class I and class II MHC molecules in activating distinct populations of T cells ($CD8^+$ and $CD4^+$, respectively).

The MLR is induced by culturing mononuclear leukocytes (which include T cells, B cells, natural killer [NK] cells, mononuclear phagocytes, and dendritic cells) from one individual with mononuclear leukocytes derived from another individual. In clinical practice, these cells are typically isolated from peripheral blood; in mouse or rat experiments, mononuclear leukocytes are usually purified from the spleen or lymph nodes. If the two individuals have differences in the alleles of the MHC genes, a large proportion of the mononuclear cells will proliferate during a period of 4 to 7 days. This proliferative response is called the allogeneic MLR (Fig. 16-7). If cells from two MHC-disparate individuals are mixed, each can react against the other and both will proliferate, thus resulting in a two-way MLR. To simplify the analysis, one of the two leukocyte populations can be rendered

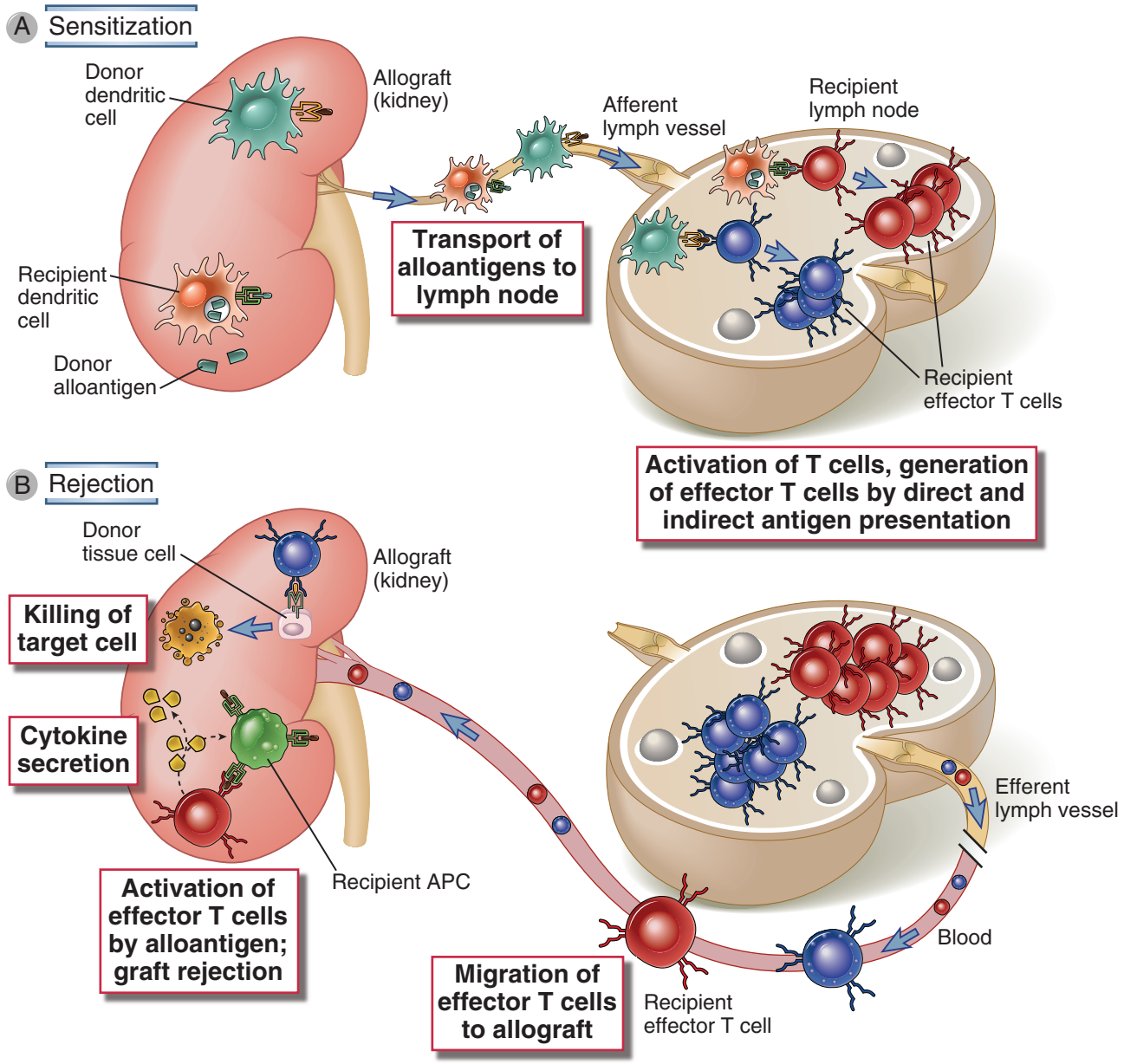


FIGURE 16–6 Activation of alloreactive T cells. A, In the case of direct allorecognition, donor dendritic cells in the allograft migrate to secondary lymphoid tissues, where they present allogeneic MHC molecules to host T cells. **B,** In the case of indirect allorecognition, recipient dendritic cells that have entered the allograft transport donor MHC proteins to secondary lymphoid tissues and present peptides derived from these MHC proteins to alloreactive host T cells. In both cases, the T cells become activated and differentiate into effector cells. The alloreactive effector T cells migrate into the allograft, become reactivated by alloantigen, and mediate damage. Lymphatic drainage of grafted organs is not well described, and therefore the location of the relevant lymph nodes is uncertain.

incapable of proliferation before culture, either by γ-irradiation or by treatment with the antimitotic drug mitomycin C. In this one-way MLR, the treated cells serve exclusively as stimulators, and the untreated cells, still capable of proliferation, serve as the responders. Among the T cells that respond in an MLR, the $CD4^+$ cells are specific for allogeneic class II MHC molecules and the $CD8^+$ cells for class I molecules.

Because of the high frequency of alloreactive T cells, primary responses to alloantigens are the only responses of naive T cells that can readily be detected in vitro. Responses of T cells to a protein antigen in vitro can be detected only if the T cells are from an individual who has previously been exposed to that antigen (e.g., by vaccination), because there are too few naive antigen–specific T cells to mount a detectable response. In contrast, naive T cells will proliferate vigorously when cultured with mononuclear cells from another individual in an MLR.

Effector Functions of Alloreactive T Cells

Alloreactive $CD4^+$ and $CD8^+$ T cells that are activated by graft alloantigens cause rejection by distinct mechanisms. The $CD4^+$ helper T cells differentiate into cytokine-producing effector cells that damage grafts by cytokine-mediated inflammation, similar to a delayed-type

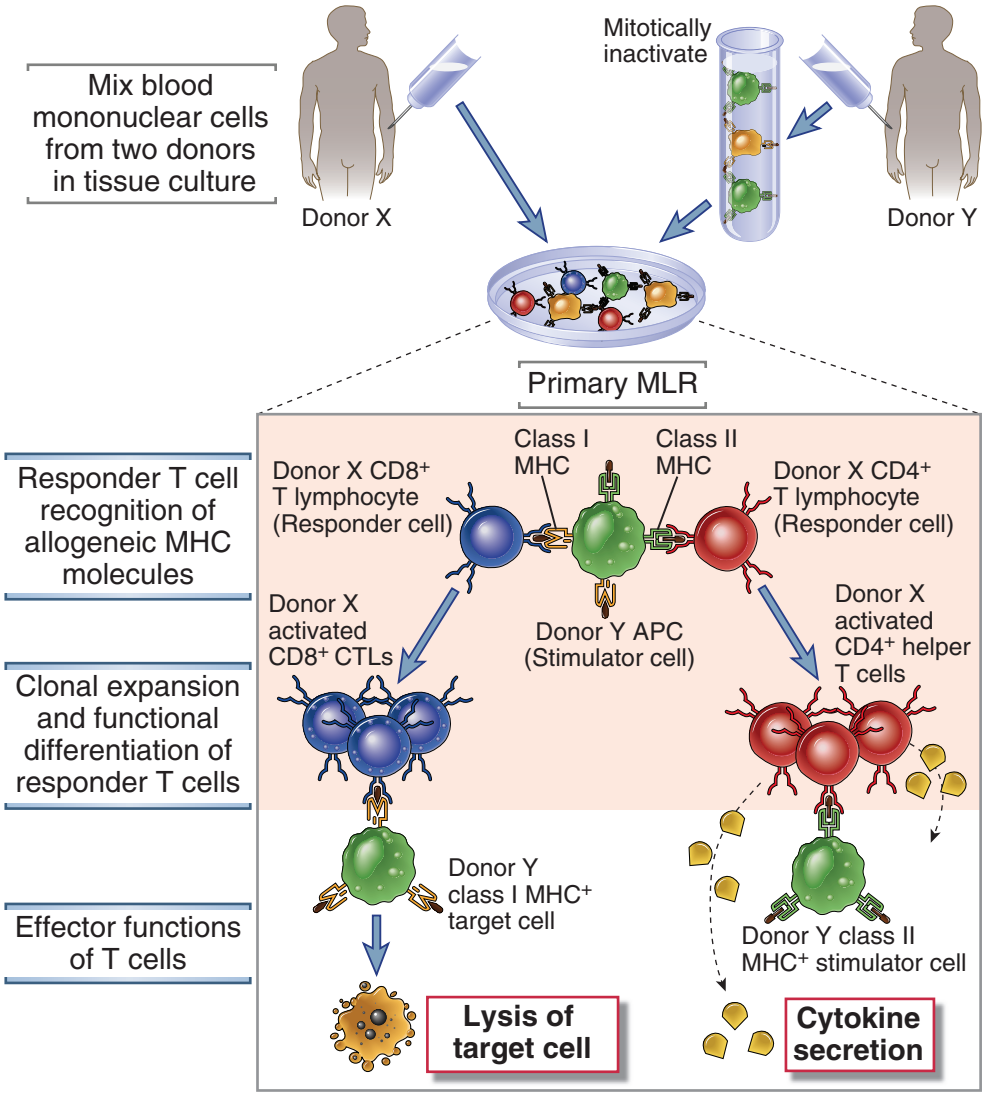


FIGURE 16–7 The mixed lymphocyte reaction (MLR). In a one-way primary MLR, stimulator cells (from donor Y) activate and cause the expansion of two types of responder T cells (from donor X). $CD4^+$ T cells from donor X react to donor Y class II molecules, and $CD8^+$ T lymphocytes from donor X react to donor Y class I MHC molecules. The $CD4^+$ T cells differentiate into cytokine-secreting helper T cells, and the $CD8^+$ T cells differentiate into CTLs. APC, antigen-presenting cell.

hypersensitivity (DTH) reaction (see Chapters 10 and 18). Alloreactive $CD8^+$ T cells differentiate into cytotoxic T lymphocytes (CTLs), which kill nucleated cells in the graft that express the allogeneic class I MHC molecules. CTLs also secrete inflammatory cytokines, which can contribute to graft damage.

Only CTLs that are generated by direct allogeneic MHC recognition can kill graft cells, whereas CTLs or helper T cells generated by either direct or indirect alloantigen recognition can cause cytokine-mediated damage to grafts. $CD8^+$ CTLs that are generated by direct allorecognition recognize graft alloantigens and can, therefore, kill graft cells that express these same alloantigens. In contrast, any $CD8^+$ CTLs that are generated by the indirect pathway are self MHC restricted, and they will not be able to kill the foreign graft cells because these cells do not express self MHC alleles displaying allogeneic peptides. Therefore, when alloreactive T cells are stimulated by the indirect pathway, the principal mechanism of rejection is not CTL-mediated killing of graft cells but inflammation caused by the cytokines produced by either $CD8^+$ or $CD4^+$ effector T cells. Presumably, these effector cells infiltrate the graft and recognize graft alloantigens being displayed by host APCs that have also entered the graft. The relative importance of the direct and indirect pathways in graft rejection is not definitively established. It may be that $CD8^+$ CTLs induced by direct recognition of alloantigens are most important for acute cellular rejection of allografts, in which killing of graft cells is a prominent component, whereas $CD4^+$ effector T cells stimulated by the indirect pathway play a greater role in chronic rejection. These differences may be of clinical significance because conventional immunosuppressive therapy for graft rejection seems to preferentially suppress $CD8^+$ CTL responses induced by direct allorecognition and is less effective against $CD4^+$ T cells activated by the indirect pathway.

Activation of Alloreactive B Cells and Production of Alloantibodies

Most high-affinity alloantibodies are produced by helper T cell–dependent activation of alloreactive B cells, much like antibodies against other protein antigens (see Chapter 11). The antigens most frequently recognized by alloantibodies in graft rejection are donor HLA molecules, including both class I and class II MHC proteins. The likely sequence of events leading to the generation of these alloantibody-producing cells is that naive B lymphocytes recognize foreign MHC molecules, internalize and process these proteins, and present peptides derived from them to helper T cells that were previously activated by the same peptides presented by dendritic cells. Thus, activation of alloreactive B cells is an example of indirect presentation of alloantigens. Anti-HLA antibodies contribute significantly to allograft rejection, as we will discuss below.

PATTERNS AND MECHANISMS OF ALLOGRAFT REJECTION

Thus far, we have described the molecular basis of alloantigen recognition and the cells involved in the recognition of and responses to allografts. We now turn to a consideration of the effector mechanisms responsible for the immunologic rejection of allografts. In different experimental models and in clinical transplantation, alloreactive $CD4^+$ and $CD8^+$ T cells and alloantibodies have been shown to be capable of mediating allograft rejection. These different immune effectors cause graft rejection by different mechanisms (Fig. 16-8), and all three effectors may contribute to rejection concurrently.

For historical reasons, graft rejection is classified on the basis of histopathologic features or the time course of rejection after transplantation rather than on the basis of immune effector mechanisms. Based on the experience of renal transplantation, the histopathologic patterns are called hyperacute, acute, and chronic (see Fig. 16-8). These patterns are associated with different dominant immune effector mechanisms.

Hyperacute Rejection

Hyperacute rejection is characterized by thrombotic occlusion of the graft vasculature that begins within minutes to hours after host blood vessels are anastomosed to graft vessels and is mediated by preexisting antibodies in the host circulation that bind to donor endothelial antigens (Fig. 16-8A). Binding of antibody to endothelium activates complement, and antibody and complement products together induce a number of changes in the graft endothelium that promote intravascular thrombosis. Complement activation leads to endothelial cell injury and exposure of subendothelial basement membrane proteins that activate platelets. The endothelial cells are stimulated to secrete high-molecular-weight forms of von Willebrand factor that cause platelet adhesion and aggregation. Both endothelial cells and platelets undergo membrane vesiculation, leading to shedding of lipid particles that promote coagulation. Endothelial cells lose the cell surface heparan sulfate proteoglycans that normally interact with antithrombin III to inhibit coagulation. These processes contribute to thrombosis and vascular occlusion (Fig. 16-9A), and the grafted organ suffers irreversible ischemic damage.

In the early days of transplantation, hyperacute rejection was often mediated by preexisting IgM alloantibodies, which are present at high titer before transplantation. Such "natural antibodies" are believed to arise in response to carbohydrate antigens expressed by bacteria that normally colonize the intestine. The best known examples of such alloantibodies are those directed against the ABO blood group antigens expressed on red blood cells, discussed later. ABO antigens are also expressed on vascular endothelial cells. Today, hyperacute rejection by anti-ABO antibodies is extremely rare because all donor and recipient pairs are selected so that they have the same ABO type. As we shall discuss later in this chapter, hyperacute rejection caused by natural antibodies is the major barrier to xenotransplantation and limits the use of animal organs for human transplantation.

Currently, hyperacute rejection of allografts, when it occurs, is usually mediated by IgG antibodies directed against protein alloantigens, such as donor MHC molecules, or against less well defined alloantigens expressed on vascular endothelial cells. Such antibodies generally arise as a result of previous exposure to alloantigens through blood transfusion, previous transplantation, or multiple pregnancies. If the titer of these alloreactive antibodies is low, hyperacute rejection may develop slowly, during several days. In this case, it is sometimes referred to as accelerated allograft rejection because the onset is still earlier than that typical for acute rejection. As we will discuss later in this chapter, patients in need of allografts are routinely screened before grafting for the presence of antibodies that bind to cells of a potential organ donor to avoid hyperacute rejection.

In rare cases in which grafts have to be done in ABO-incompatible recipients, survival may be improved by rigorous depletion of antibodies and B cells. Sometimes, if the graft is not rapidly rejected, it survives even in the presence of anti-graft antibody. One possible mechanism of this resistance to hyperacute rejection is increased expression of complement regulatory proteins on graft endothelial cells, a beneficial adaptation of the tissue that has been called *accommodation*.

Acute Rejection

Acute rejection is a process of injury to the graft parenchyma and blood vessels mediated by alloreactive T cells and antibodies. Before modern immunosuppression, acute rejection would often begin several days to a few weeks after transplantation. The delayed time of onset of acute rejection is because alloreactive effector T cells and antibodies take time to be generated from naive or resting memory T cells in response to the graft. In current clinical practice, episodes of acute rejection may occur at much later times, even years after transplantation, if immunosuppression is reduced for any number of reasons. Although the patterns of acute rejection are divided into cellular, mediated by T cells, and

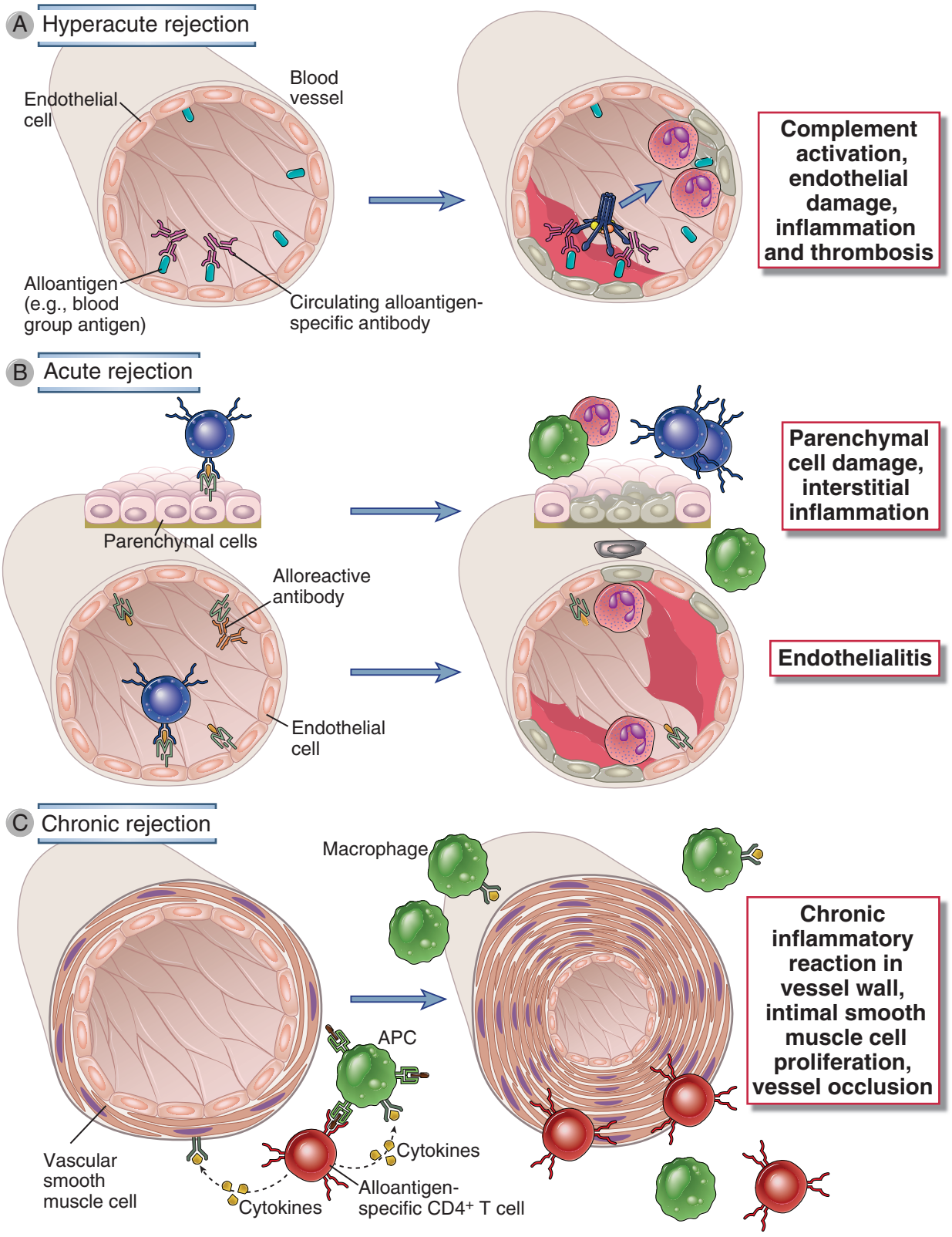


FIGURE 16–8 Immune mechanisms of graft rejection. A, In hyperacute rejection, preformed antibodies reactive with vascular endothelium activate complement and trigger rapid intravascular thrombosis and necrosis of the vessel wall. **B,** In acute rejection, $CD8^+$ T lymphocytes reactive with alloantigens on endothelial cells and parenchymal cells mediate damage to these cell types. Alloreactive antibodies formed after engraftment may also contribute to vascular injury. **C,** In chronic rejection with graft arteriosclerosis, injury to the vessel wall leads to intimal smooth muscle cell proliferation and luminal occlusion. This lesion may be caused by a chronic DTH reaction to alloantigens in the vessel wall.

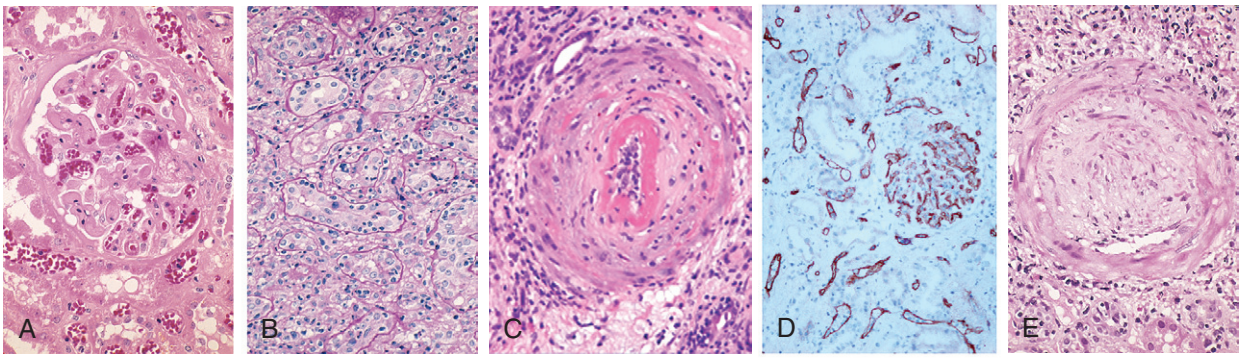


FIGURE 16–9 Histopathology of different forms of graft rejection. A, Hyperacute rejection of a kidney allograft with endothelial damage, platelet and thrombin thrombi, and early neutrophil infiltration in a glomerulus. **B,** Acute rejection of a kidney with inflammatory cells in the connective tissue around the tubules and between epithelial cells of the tubules. **C,** Acute antibody-mediated rejection of a kidney allograft with destructive inflammatory reaction destroying the endothelial layer of an artery. **D,** Complement C4d deposition in vessels in acute antibody-mediated rejection. **E,** Chronic rejection in a kidney allograft with graft arteriosclerosis. The vascular lumen is replaced by an accumulation of smooth muscle cells and connective tissue in the vessel intima. *(Courtesy of Dr. Helmut Rennke, Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts.)*

humoral, mediated by antibodies, both typically coexist in an acutely rejecting organ.

Acute Cellular Rejection

The principal mechanism of acute cellular rejection is CTL-mediated killing of cells in the graft (Fig. 16-8B). On histologic examination, this type of rejection is characterized by infiltrates of lymphocytes, which invade and destroy graft components (Fig. 16-9B). There are many lines of evidence that support the role of CTLs in acute cellular rejection. The cellular infiltrates present in grafts undergoing this type of rejection are markedly enriched for $CD8^+$ CTLs specific for graft alloantigens. In fact, the presence of mRNAs encoding CTL-specific genes (e.g., perforin and granzyme B) is sometimes used as a specific and sensitive indicator of clinical acute rejection. Experimentally, alloreactive $CD8^+$ CTLs can be used to adoptively transfer acute cellular graft rejection. The destruction of allogeneic cells in a graft is highly specific, a hallmark of CTL killing. The best evidence for this specificity has come from mouse skin graft experiments using chimeric grafts that contain two distinct cell populations, one syngeneic to the host and one allogeneic to the host. When these skin grafts are transplanted, the allogeneic cells are killed without injury to the "bystander" syngeneic cells.

In addition to direct killing of the graft cells by CTLs, activated $CD4^+$ helper T cells and CTLs produce cytokines that recruit and activate inflammatory cells, which also injure the graft.

In vascularized grafts such as kidney grafts, endothelial cells are major targets of acute rejection. Microvascular endothelialitis is a frequent early finding in grafts undergoing acute rejection episodes. Endothelialitis or intimal arteritis in medium-sized arteries also occurs at an early stage of acute rejection and is indicative of severe rejection, which, if left untreated, will likely result in acute graft failure. Both $CD8^+$ and $CD4^+$ T cells may contribute to endothelial injury.

Acute Antibody-Mediated Rejection

Alloantibodies cause acute rejection by binding to alloantigens, mainly HLA molecules, on vascular endothelial cells, causing endothelial injury and intravascular thrombosis that results in graft destruction (see Fig. 16-8B). The binding of the alloantibodies to the endothelial cell surface triggers local complement activation, which leads to lysis of the cells, recruitment and activation of neutrophils, and thrombus formation. In addition, alloantibody binding to the endothelial surface may directly alter endothelial function by inducing intracellular signals that enhance surface expression of proinflammatory and procoagulant molecules.

The histologic hallmark of this form of acute rejection is transmural necrosis of graft vessel walls with acute inflammation (Fig. 16-9C), which is different from the thrombotic occlusion without vessel wall necrosis seen in hyperacute rejection. Immunohistochemical identification of the C4d complement fragment in capillaries of renal allografts is used clinically as an indicator of activation of the classical complement pathway and humoral rejection (Fig. 16-9D). In a significant fraction of cases of antibody-mediated rejection, there is no C4d deposition detectable, suggesting that damage is caused by the complement-independent effects of alloantibody binding to endothelial cells, mentioned before.

Chronic Rejection and Graft Vasculopathy

As therapy for acute rejection has improved, the major cause of the failure of vascularized organ allografts has become chronic rejection. Since 1990, 1-year survival of kidney allografts has been better than 90% but the 10-year survival has remained about 60% despite advances in immunosuppressive therapy. Chronic rejection develops insidiously during months or years and may or may not be preceded by episodes of acute rejection. Chronic rejection of different transplanted organs is associated with distinct pathologic changes. In the kidney

and heart, chronic rejection results in vascular occlusion and interstitial fibrosis. Lung transplants undergoing chronic rejection show thickened small airways (bronchiolitis obliterans), and liver transplants show fibrotic and nonfunctional bile ducts (called the vanishing bile duct syndrome).

A dominant lesion of chronic rejection in vascularized grafts is arterial occlusion as a result of the proliferation of intimal smooth muscle cells, and the grafts eventually fail mainly because of the resulting ischemic damage (Fig. 16-8C). The arterial changes are called graft vasculopathy or accelerated graft arteriosclerosis (Fig. 16-9E). Graft vasculopathy is frequently seen in failed cardiac and renal allografts and can develop in any vascularized organ transplant within 6 months to a year after transplantation. The pathogenesis of the lesions remains poorly understood but likely involves a combination of immunologic and nonimmunologic processes. The likely mechanisms underlying the occlusive vascular lesions of chronic rejection are: activation of alloreactive T cells and secretion of cytokines that stimulate proliferation of vascular endothelial and smooth muscle cells; repair with fibrosis after repeated bouts of acute antibody-mediated or cellular rejection; and consequences of perioperative ischemia, toxic effects of immunosuppressive drugs, and even chronic viral infections. As the arterial lesions of graft arteriosclerosis progress, blood flow to the graft parenchyma is compromised, and the parenchyma is slowly replaced by nonfunctioning fibrous tissue. This process leads to congestive heart failure or arrhythmias in cardiac transplant patients or loss of function in glomeruli and ischemic renal failure in renal transplant patients.

PREVENTION AND TREATMENT OF ALLOGRAFT REJECTION

If the recipient of an allograft has a fully functional immune system, transplantation almost invariably results in some form of rejection. The strategies used in clinical practice and in experimental models to avoid or to delay rejection are general immunosuppression and minimizing the strength of the specific allogeneic reaction. An important goal in transplantation research is to find ways of inducing donor-specific tolerance, which would allow grafts to survive without nonspecific immunosuppression.

Immunosuppression to Prevent or to Treat Allograft Rejection

Immunosuppressive drugs that inhibit or kill T lymphocytes are the principal agents used to treat or prevent graft rejection. Several methods of immunosuppression are commonly used (Fig. 16-10).

Inhibitors of T Cell Signaling Pathways

The calcineurin inhibitors cyclosporine and FK506 (tacrolimus) inhibit transcription of certain genes in T cells,

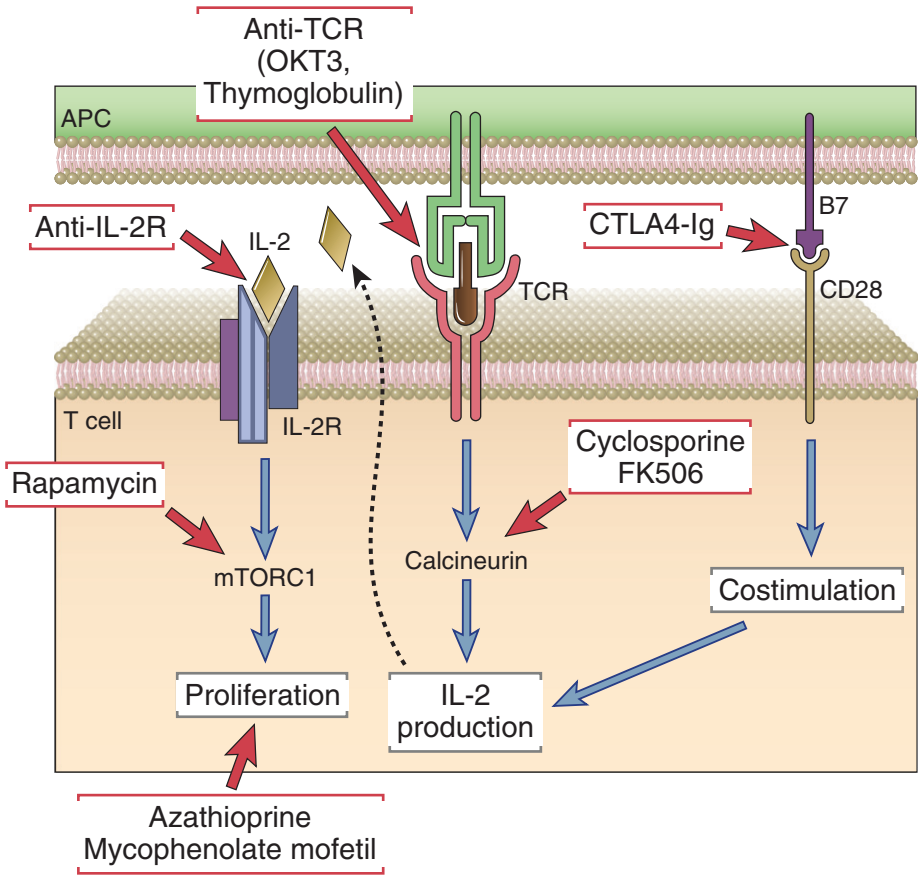


FIGURE 16–10 Mechanisms of action of immunosuppressive drugs. Each major category of drugs used to prevent or to treat allograft rejection is shown along with the molecular targets of the drugs.

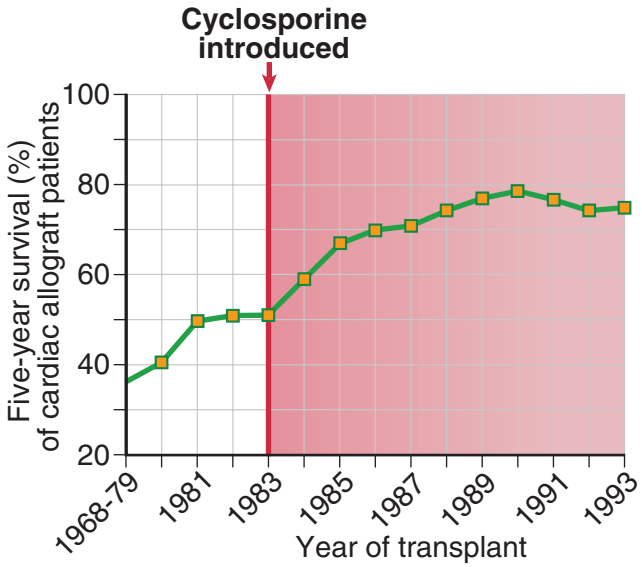


FIGURE 16–11 Influence of cyclosporine on graft survival. Five-year survival rates for patients receiving cardiac allografts increased significantly beginning when cyclosporine was introduced in 1983. *(Data from Transplant Patient DataSource, United Network for Organ Sharing, Richmond, Virginia. Available at: http://207.239.150.13/tpd/. Accessed February 16, 2000.)*

most notably those encoding cytokines such as IL-2. Cyclosporine is a fungal peptide that binds with high affinity to a ubiquitous cellular protein called cyclophilin. The complex of cyclosporine and cyclophilin binds to and inhibits the enzymatic activity of the calcium/calmodulin-activated serine/threonine phosphatase calcineurin (see Chapter 7). Because calcineurin is required to activate the transcription factor NFAT (nuclear factor of activated T cells), cyclosporine inhibits NFAT activation and the transcription of IL-2 and other cytokine genes. The net result is that cyclosporine blocks the IL-2–dependent proliferation and differentiation of T cells. FK506 is a macrolide lactone made by a bacterium that functions like cyclosporine. FK506 and its binding protein (called FKBP) share with the cyclosporine-cyclophilin complex the ability to bind calcineurin and inhibit its activity.

The introduction of cyclosporine into clinical practice ushered in the modern era of transplantation. Before the use of cyclosporine, the majority of transplanted hearts and livers were rejected. Now as a result of the use of cyclosporine, FK506, and other more recently introduced drugs, the majority of these allografts survive for more than 5 years (Fig. 16-11). Nevertheless, these drugs have limitations. For example, at doses needed for optimal immunosuppression cyclosporine causes kidney damage, and some rejection episodes are refractory to cyclosporine treatment. FK506 was initially used for liver transplant recipients, but it is now used widely for immunosuppression of kidney allograft recipients, including those who are not adequately controlled by cyclosporine. FK506 is also used topically for some inflammatory skin diseases.

The immunosuppressive drug rapamycin (sirolimus) inhibits growth factor–mediated T cell proliferation. Like FK506, rapamycin binds to FKBP, but the rapamycin-FKBP complex does not inhibit calcineurin. Instead, this complex binds to and inhibits a cellular enzyme called mammalian target of rapamycin complex 1 (mTORC1), which is a serine/threonine protein kinase required for translation of proteins that promote cell survival and proliferation. mTORC1 is negatively regulated by a protein complex called tuberous sclerosis complex 1 (TSC1)–TSC2 complex. Phosphotidyinositol 3-kinase (PI3K)–Akt signaling results in phosphorylation of TSC2 and release of mTOR regulation. Several growth factor receptor signaling pathways, including the IL-2 receptor pathway in T cells, activate mTOR through PI3K-Akt, leading to translation of proteins needed for cell cycle progression. Thus, by inhibiting mTORC1 function, rapamycin blocks IL-2–driven T cell proliferation. Combinations of cyclosporine (which blocks IL-2 synthesis) and rapamycin (which blocks IL-2–driven proliferation) are potent inhibitors of T cell responses. Interestingly, rapamycin inhibits the generation of effector T cells but does not impair the survival and functions of regulatory T cells as much, which may promote immune suppression of allograft rejection. mTORC1 is involved in dendritic cell functions, and therefore rapamycin may suppress T cell responses by interfering with dendritic cell function as well. mTORC1 is also involved in B cell proliferation and antibody responses, and therefore rapamycin may also be effective in preventing or treating antibody-mediated rejection. In addition to rapamycin, other mTOR inhibitors have been developed for immunosuppression of allograft recipients and for cancer therapy.

Other molecules involved in cytokine and T cell receptor signaling are also targets of immunosuppressive drugs that are in early trials for treatment or prevention of allograft rejection. These target molecules include JAK3, a kinase linked to signaling of various cytokine receptors, including IL-2, and protein kinase C, an essential kinase in T cell receptor signaling.

Antimetabolites

Metabolic toxins that kill proliferating T cells are used in combination with other drugs to treat graft rejection. These agents inhibit the proliferation of lymphocyte precursors during their maturation and also kill proliferating mature T cells that have been stimulated by alloantigens. The first such drug to be developed for the prevention and treatment of rejection was azathioprine. This drug is still used, but it is toxic to precursors of leukocytes in the bone marrow and enterocytes in the gut. The most widely used drug in this class is **mycophenolate mofetil (MMF)**. MMF is metabolized to mycophenolic acid, which blocks a lymphocyte-specific isoform of inosine monophosphate dehydrogenase, an enzyme required for de novo synthesis of guanine nucleotides. Because MMF selectively inhibits the lymphocyte-specific isoform of this enzyme, it has relatively few toxic effects on other cells. MMF is now routinely used, often in combination with cyclosporine or FK506, to prevent acute allograft rejection.

Function-Blocking or Depleting Antilymphocyte Antibodies

Antibodies that react with T cell surface structures and deplete or inhibit T cells are used to treat acute rejection

episodes. One widely used antibody is a mouse monoclonal antibody called OKT3 that is specific for human CD3. Polyclonal rabbit or horse antibodies specific for a mixture of human T cell surface proteins, so-called antithymocyte globulin, have also been in clinical use for many years to treat acute allograft rejections. These anti–T cell antibodies deplete circulating T cells either by activating the complement system to eliminate T cells or by opsonizing them for phagocytosis. T cells that escape elimination by OKT3 probably do so by endocytosis ("modulation") of CD3 off their surface, but such cells may be rendered nonfunctional.

Monoclonal antibodies are now in clinical use that are specific for CD25, the α subunit of the IL-2 receptor. These reagents presumably prevent T cell activation by blocking IL-2 binding to activated T cells and IL-2 signaling.

Another monoclonal antibody in use in clinical transplantation is a rat IgM specific for CD52, a cell surface protein expressed widely on most mature B and T cells whose function is not understood. Anti-CD52 was originally developed to treat B cell malignant neoplasms, and it was found to profoundly deplete most peripheral B and T cells for many weeks after injection into patients. In current trials, it has been administered just before and early after transplantation, with the hope that it may induce a prolonged state of graft tolerance as new lymphocytes develop in the presence of the allograft.

The major limitation to the use of monoclonal or polyclonal antibodies from other species is that humans given these agents produce anti-immunoglobulin (Ig) antibodies that eliminate the injected foreign Ig. For this reason, human-mouse chimeric ("humanized") antibodies (e.g., against CD3 and CD25), which are less immunogenic, have been developed.

Costimulatory Blockade

Drugs that block T cell costimulatory pathways reduce acute allograft rejection. The rationale for the use of these types of drugs is to prevent the delivery of costimulatory signals required for activation of T cells (see Chapter 9). A soluble high-affinity form of CTLA-4 fused to an IgG Fc domain binds to B7 molecules on APCs and prevents them from interacting with T cell CD28 (see Chapter 9, Fig. 9-7) and is near approval for use in allograft recipients. Clinical studies have shown that CTLA-4–Ig can be as effective as cyclosporine in preventing acute rejection. An antibody that binds to T cell CD40 ligand and prevents its interactions with CD40 on APCs (see Chapter 9) has also proved beneficial for preventing graft rejection in experimental animals. In some experimental protocols, simultaneous blockade of both B7 and CD40 appears to be more effective than either alone in promoting graft survival. However, the anti-CD40L antibody has a serious side effect of thrombotic complications, apparently related to the expression of CD40L on platelets.

Drugs Targeting Alloantibodies and Alloreactive B Cells

As we have learned more about the importance of alloantibodies in mediating acute and perhaps chronic rejection, therapies targeting antibodies and B cells that were developed for other diseases are now being used in transplant patients. For example, plasmapheresis is sometimes used to treat acute antibody-mediated rejection. In this procedure, a patient's blood is pumped through a machine that removes the plasma but returns the blood cells to the circulation. In this way, circulating antibodies, including pathogenic alloreactive antibodies, can be removed. Intravenous immune globulin (IVIG) therapy, which is used to treat several, often antibody-mediated, inflammatory diseases, is also being applied in the setting of acute antibody-mediated rejection. In IVIG therapy, pooled IgG from normal donors is injected intravenously into a patient. The mechanisms of action are not fully understood but likely involve binding of the injected IgG to the patient's Fc receptors on various cell types, thereby reducing alloantibody production and blocking effector functions of the patient's own antibodies. IVIG also enhances degradation of the patient's antibodies by competitively inhibiting their binding to the neonatal Fc receptor (see Chapter 12). A monoclonal antibody specific for the B cell surface protein CD20 very effectively depletes mature B cells from the circulation and secondary lymphoid organs. Anti-CD20 has been used for treatment of B cell lymphomas and for autoimmune diseases and is now in clinical trials for treatment of antibody-mediated allograft rejection. These antibody and B cell targeted therapies have been used in combination to effectively treat antibody-mediated rejection.

Anti-inflammatory Drugs

Anti-inflammatory agents, specifically corticosteroids, are frequently used to reduce the inflammatory reaction to organ allografts. The proposed mechanism of action of these natural hormones and their synthetic analogues is to block the synthesis and secretion of cytokines, including tumor necrosis factor (TNF) and IL-1, and other inflammatory mediators, such as prostaglandins, reactive oxygen species, and nitric oxide, produced by macrophages and other inflammatory cells. The net result of this therapy is reduced leukocyte recruitment, inflammation, and graft damage. Very high doses of corticosteroids may inhibit T cell secretion of cytokines or even kill some T cells, but it is unlikely that the levels of corticosteroids achieved in vivo act in this way. Newer anti-inflammatory agents are in clinical trials, including soluble cytokine receptors and anticytokine antibodies.

Inhibitors of Leukocyte Migration

A new therapeutic agent, called fingolimod (FTY720), works by binding to and blocking sphingosine 1-phosphate (S1P) receptors on lymphocytes. S1P is required for the egress of lymphocytes from lymphoid organs (see Chapter 3), and blocking its action leads to the sequestration of lymphocytes in lymph nodes. Fingolimod inhibits allograft rejection in animal models. This drug is not yet used for clinical transplantation, but it is approved for treatment of multiple sclerosis, an autoimmune disease of the central nervous system.

Anti-integrin antibodies have proved to be effective treatments for some autoimmune diseases because they block leukocyte recruitment from the circulation into inflamed tissues (see Chapter 3). There are some early

animal studies testing whether these drugs work to block allograft rejection, but so far there are too few data to predict if this approach will be useful.

Current immunosuppressive protocols have dramatically improved graft survival. Before the use of calcineurin inhibitors, the 1-year survival rate of unrelated cadaveric kidney grafts was between 50% and 60%, with a 90% rate for grafts from living related donors (which are better matched with the recipients). Since cyclosporine, FK506, rapamycin, and MMF have been introduced, the survival rate of unrelated cadaveric kidney grafts has increased to about 90% at 1 year. Heart transplantation, for which HLA matching is not practical, has also significantly benefited from the use of cyclosporine and now has a similar ~90% 1-year survival rate (see Fig. 16-11). Experience with other organs is more limited, but survival rates have also improved with modern immunosuppressive therapy, with 10-year patient survival rates of approximately 60% and 75% for pancreas and liver recipients, respectively, and 3-year patient survival rates of 70% to 80% for lung recipients.

Strong immunosuppression is usually started in allograft recipients at the time of transplantation with a combination of drugs, and after a few days, the drugs are changed for long-term maintenance of immunosuppression. For example, in the case of adult kidney transplantation, a patient may be initially induced with an anti–IL-2R or anti–T cell depleting antibody and a high-dose corticosteroid, and then maintained on a calcineurin inhibitor, an antimetabolite, and maybe low-dose steroids. Acute rejection, when it occurs, is managed by rapidly intensifying immunosuppressive therapy. In modern transplantation, chronic rejection has become a more common cause of allograft failure, especially in cardiac transplantation. Chronic rejection is more insidious than acute rejection, and it is much less reversible by immunosuppression.

Immunosuppressive therapy leads to increased susceptibility to various types of intracellular infections and virus-associated tumors. The major goal of immunosuppression to treat graft rejection is to reduce the generation and function of helper T cells and CTLs, which mediate acute cellular rejection. It is therefore not surprising that defense against viruses and other intracellular pathogens, the physiologic function of T cells, is also compromised in immunosuppressed transplant recipients. Reactivation of latent herpesviruses is a frequent problem in immunosuppressed patients, including cytomegalovirus, herpes simplex virus, varicella-zoster virus, and Epstein-Barr virus. For this reason, transplant recipients are now given prophylactic antiviral therapy for herpesvirus infections. Immunosuppressed allograft recipients are also at greater risk for a variety of so-called opportunistic infections, which normally do not occur in immunocompetent people, including fungal infections (*Pneumocystis jiroveci* pneumonia, histoplasmosis, coccidioidomycosis), protozoan infections (toxoplasmosis), and gastrointestinal parasitic infections (*Cryptosporidium* and *Microsporidium*). Immunosuppressed allograft recipients have a higher risk for development of neoplasias compared with the general population, including various forms of skin cancer. Some of the tumors that are more frequently found in allograft recipients are known to be caused by viruses, and therefore they may arise because of impaired antiviral immunity. These include uterine cervical carcinoma, which is related to human papillomavirus infection, and lymphomas caused by Epstein-Barr virus infection. The lymphomas found in allograft recipients as a group are called post-transplantation lymphoproliferative disorders, and most are derived from B lymphocytes.

Despite the risks of infections and neoplasias associated with the use of immunosuppressive drugs, the major limitation on the tolerated doses of most of these drugs, including calcineurin inhibitors, mTOR inhibitors, antimetabolites, and steroids, is direct toxicity to cells unrelated to immunosuppression. In some cases, the toxicities affect the same cells as rejection does, such as cyclosporine toxicity to renal tubular epithelial cells, which can complicate the interpretation of declining renal function in kidney allograft recipients.

Methods to Reduce the Immunogenicity of Allografts

In human transplantation, the major strategy to reduce graft immunogenicity has been to minimize alloantigenic differences between the donor and recipient. Several clinical laboratory tests are routinely performed to reduce the risk for immunologic rejection of allografts. These include ABO blood typing; the determination of HLA alleles expressed on donor and recipient cells, called tissue typing; the detection of preformed antibodies in the recipient that recognize HLA and other antigens representative of the donor population; and the detection of preformed antibodies in the recipient that bind to antigens of an identified donor's leukocytes, called cross-matching. Not all of these tests are done in all types of transplantation. We will now summarize each of these tests and discuss their significance.

To avoid hyperacute rejection, the ABO blood group antigens of the graft donor are selected to be identical to those of the recipient. This test is uniformly used in renal transplantation because kidney grafts will typically not survive if there are ABO incompatibilities between the donor and recipient. Natural IgM antibodies specific for allogeneic ABO blood group antigens (discussed earlier) will cause hyperacute rejection. Blood typing is performed by mixing a patient's red blood cells with standardized sera containing anti-A or anti-B antibodies. If the patient expresses either blood group antigen, the serum specific for that antigen will agglutinate the red cells. The biology of the ABO blood group system is discussed later in the chapter in the context of blood transfusion.

In kidney transplantation, the larger the number of MHC alleles that are matched between the donor and recipient, the better the graft survival (Fig. 16-12). HLA matching had a more profound influence on graft survival before modern immunosuppressive drugs were routinely used, but current data still show significantly greater survival of grafts when donor and recipient have fewer HLA allele mismatches. Past clinical experience with older typing methods had shown that of all the class I and class II loci, matching at HLA-A, HLA-B, and

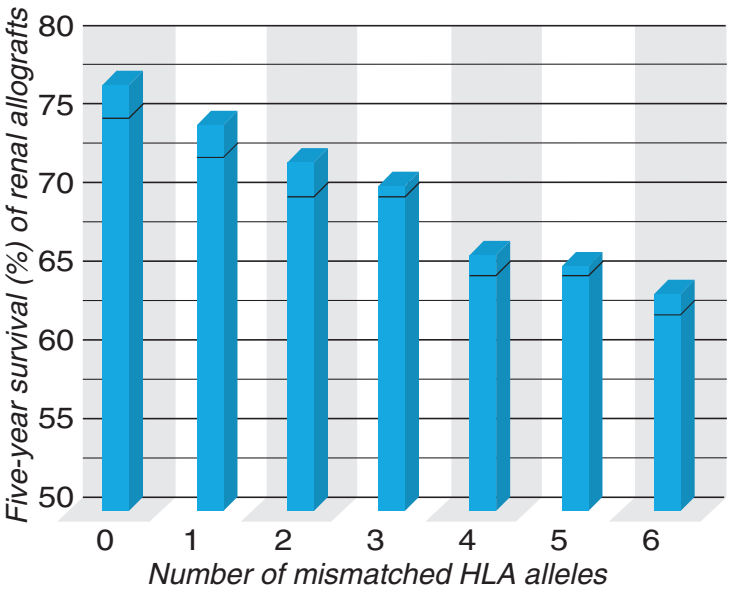


FIGURE 16–12 Influence of MHC matching on graft survival. Matching of MHC alleles between the donor and recipient significantly improves renal allograft survival. The data shown are for deceased donor (cadaver) grafts. HLA matching has less of an impact on survival of renal allografts from live donors, and some MHC alleles are more important than others in determining outcome. *(Data from Organ Procurement and Transplantation Network/Scientific Registry annual report, 2010.)*

HLA-DR is most important for predicting survival of kidney allografts. (HLA-C is not as polymorphic as HLA-A or HLA-B, and HLA-DR and HLA-DQ are in strong linkage disequilibrium, so matching at the DR locus often also matches at the DQ locus.) Although current typing protocols in many centers include HLA-C, DQ, and DP loci, most of the available data in predicting graft outcome refer only to HLA-A, HLA-B, and HLA-DR mismatches. Because two codominantly expressed alleles are inherited for each of these HLA genes, it is possible to have zero to six HLA mismatches of these three loci between the donor and recipient. Zero-antigen mismatches predict the best survival of living related donor grafts, and grafts with one-antigen mismatches do slightly worse. The survival of grafts with two to six HLA mismatches is significantly worse than that of grafts with zero- and one-antigen mismatches. HLA matching has an even greater impact on nonliving (unrelated) donor renal allografts. Therefore, attempts are made to reduce the number of differences in HLA alleles expressed on donor and recipient cells, which will have a modest effect in reducing the chance of rejection.

HLA matching in renal transplantation is possible because donor kidneys can be stored in organ banks before transplantation until a well-matched recipient can be identified, and because patients needing a kidney allograft can be maintained on dialysis until a well-matched organ is available. In the case of heart and liver transplantation, organ preservation is more difficult, and potential recipients are often in critical condition. For these reasons, HLA typing is not considered in pairing of potential donors and recipients, and the choice of donor and recipient is based only on ABO blood group matching and anatomic compatibility. In heart transplantation, the paucity of donors, the emergent need for transplantation, and the success of immunosuppression override the possible benefit of reducing HLA mismatches between donor and recipient. As we will discuss later, in bone marrow transplantation, HLA matching is essential to reduce the risk of graft-versus-host disease.

Most HLA haplotype determinations are now performed by polymerase chain reaction (PCR), replacing older serologic methods. MHC genes can be amplified by PCR with use of primers that bind to conserved sequences within the 5′ and 3′ ends of exons encoding the polymorphic regions of class I and class II MHC molecules. The amplified segment of DNA can then be sequenced. Thus, the actual nucleotide sequence, and therefore the predicted amino acid sequence, can be directly determined for the MHC alleles of any cell, providing precise molecular tissue typing. On the basis of these DNA sequencing efforts, the nomenclature of HLA alleles has changed to reflect the identification of many alleles not distinguished by previous serologic methods. Each allele defined by sequence has at least a four-digit number, but some alleles require six or eight digits for precise definition. The first two digits usually correspond to the older serologically defined allotype, and the third and fourth digits indicate the subtypes. Alleles with differences in the first four digits encode proteins with different amino acids. For example, HLA-DRB1*1301 is the sequence-defined 01 allele of the 13-allele family of the gene encoding the HLA-DR β1 protein.

Patients in need of allografts are also tested for the presence of preformed antibodies against donor MHC molecules or other cell surface antigens. Two types of tests are done to detect these antibodies. In the **panel reactive antibody** test, patients waiting for organ transplants are screened for the presence of preformed antibodies reactive with allogeneic HLA molecules prevalent in the population. These antibodies, which may be produced as a result of previous pregnancies, transfusions, or transplantation, can identify risk for hyperacute or acute vascular rejection. Small amounts of the patient's serum are mixed with multiple fluorescently labeled beads coated with defined MHC molecules, representative of the MHC alleles that may be present in an organ donor population. Each MHC allele is attached to a bead with a differently colored fluorescent label. Binding of the patient's antibodies to beads is determined by flow

cytometry. The results are reported as percent reactive antibody (PRA), which is the percentage of the MHC allele pool with which the patient's serum reacts. The PRA is determined on multiple occasions while a patient waits for an organ allograft. This is because the PRA can vary, as each panel is chosen at random and the patient's serum antibody titers may change over time.

If a potential donor is identified, the **crossmatching test** will determine whether the patient has antibodies that react specifically with that donor's cells. The test is performed by mixing the recipient's serum with the donor's blood lymphocytes. Complement-mediated cytotoxicity tests or flow cytometric assays can then be used to determine if antibodies in the recipient serum have bound to the donor cells. For example, complement is added to the mixture of cells and serum, and if preformed antibodies, usually against donor MHC molecules, are present in the recipient's serum, the donor cells are lysed. This would be a positive crossmatch, which indicates that the donor is not suitable for that recipient.

Methods to Induce Donor-Specific Tolerance

Allograft rejection may be prevented by making the host tolerant to the alloantigens of the graft. Tolerance in this setting means that the host does not injure the graft despite the absence or withdrawal of immunosuppressive and anti-inflammatory agents. It is presumed that tolerance to an allograft will involve the same mechanisms that are involved in peripheral tolerance to self antigens (see Chapter 14), namely, anergy, deletion, and active suppression of alloreactive T cells. Tolerance is desirable in transplantation because it is alloantigen specific and will therefore avoid the major problems associated with nonspecific immunosuppression, namely, immune deficiency leading to increased susceptibility to infection and development of tumors and drug toxicity. In addition, achieving graft tolerance may reduce chronic rejection, which has to date been unaffected by the commonly used immunosuppressive agents that prevent and reverse acute rejection episodes.

Various experimental approaches and clinical observations have shown that it should be possible to achieve tolerance to allografts. In experiments in mice, Medawar and colleagues found that if neonatal mice of one strain (the recipient) are given spleen cells of another strain (the donor), the recipients will subsequently accept skin grafts from the donor. Such tolerance is alloantigen specific because the recipients will reject grafts from mouse strains that express MHC alleles that differ from the donor's. Renal transplant patients who have received blood transfusions containing allogeneic leukocytes have a lower incidence of acute rejection episodes than do those who have not been transfused. The postulated explanation for this effect is that the introduction of allogeneic leukocytes by transfusion produces tolerance to alloantigens. One underlying mechanism for tolerance induction may be that the transfused donor cells contain immature dendritic cells, which induce unresponsiveness to donor alloantigens. Indeed, pretreatment of potential recipients with blood transfusions is now used as prophylactic therapy to reduce rejection. Some recipients of liver allografts are able to retain healthy grafts even after withdrawal of immunosuppression. The mechanism underlying this apparent "spontaneous" tolerance is not known, and it seems to be unique to liver grafts.

Several strategies are being tested to induce donor-specific tolerance in allograft recipients.

- ***Costimulatory blockade.*** It was postulated that recognition of alloantigens in the absence of costimulation would lead to T cell tolerance, and there is some experimental evidence in animals to support this. However, the clinical experience with agents that block costimulation is that they inhibit immune responses to the allograft but do not induce long-lived tolerance, and patients have to be maintained on the therapy.
- ***Hematopoietic chimerism.*** We mentioned earlier that transfusion of donor blood cells into the graft recipient inhibits rejection. If the transfused donor cells or progeny of the cells survive for extended periods in the recipient, the recipient becomes a chimera. Hematopoietic chimerism with long-term allograft tolerance has also been achieved in a small number of renal allograft patients by doing a bone marrow cell transplant from the donor at the same time as the organ allograft, but the risks of bone marrow transplantation and the availability of appropriate donors may limit the applicability of this approach.
- ***Transfer or induction of regulatory T cells.*** Attempts to generate donor-specific regulatory T cells in culture and to transfer these into graft recipients are ongoing. There has been some success reported in recipients of hematopoietic stem cell transplants, in whom infusions of regulatory T cells reduce graft-versus-host disease. An alternative approach is to activate regulatory T cells in vivo, and this is being attempted by administration of a weakly stimulatory anti-CD3 antibody in recipients of pancreatic islet transplants.
- ***Other approaches.*** Other strategies that have been tried in experimental models include the administration of soluble MHC proteins or peptides under conditions predicted to induce tolerance. It seems unlikely that such an approach will be broadly applicable in the clinical situation given the great polymorphism of HLA molecules.

XENOGENEIC TRANSPLANTATION

The use of solid organ transplantation as a clinical therapy is greatly limited by the lack of availability of donor organs. For this reason, the possibility of transplantation of organs from other mammals, such as pigs, into human recipients has kindled great interest.

A major immunologic barrier to xenogeneic transplantation is the presence of natural antibodies that cause hyperacute rejection. More than 95% of primates have natural IgM antibodies that are reactive with carbohydrate determinants expressed by cells of species that are evolutionarily distant, such as the pig. The majority of human anti-pig natural antibodies are directed at one particular carbohydrate determinant formed by the

action of a pig α-galactosyltransferase enzyme. This enzyme places an α-linked galactose moiety on the same substrate that in human and other primate cells is fucosylated to form the blood group H antigen. Species combinations that give rise to natural antibodies against each other are said to be discordant. Natural antibodies are rarely produced against carbohydrate determinants of closely related, concordant species, such as humans and chimpanzees. Thus, organs from chimpanzees or other higher primates might theoretically be accepted in humans. However, ethical and logistic concerns have limited such procedures. For reasons of anatomic compatibility, pigs are the preferred xenogeneic species for organ donation to humans.

Natural antibodies against xenografts induce hyperacute rejection by the same mechanisms as those seen in hyperacute allograft rejection. These mechanisms include the generation of endothelial cell procoagulants and platelet-aggregating substances, coupled with the loss of endothelial anticoagulant mechanisms. However, the consequences of activation of human complement on pig cells are typically more severe than the consequences of activation of complement by natural antibodies on human allogeneic cells, possibly because some of the complement regulatory proteins made by pig cells, such as decay-accelerating factor, are not able to interact with human complement proteins and thus cannot limit the extent of complement-induced injury (see Chapter 12). A strategy for reducing hyperacute rejection in xenotransplantation is to breed transgenic pigs that cannot express enzymes that synthesize pig antigens or express human proteins that inhibit human complement activation. For example, α-galactosyltransferase knockout pigs and transgenic pigs expressing human complement regulatory proteins have been generated, and transplants of organs from these animals into primates are resistant to hyperacute rejection.

Even when hyperacute rejection is prevented, xenografts are often damaged by a form of acute vascular rejection that occurs within 2 to 3 days of transplantation. This form of rejection has been called delayed xenograft rejection, accelerated acute rejection, or acute vascular rejection and is characterized by intravascular thrombosis and necrosis of vessel walls. The mechanisms of delayed xenograft rejection are incompletely understood; recent findings indicate that there may be incompatibilities between primate platelets and porcine endothelial cells that promote thrombosis independent of antibody-mediated damage.

Xenografts can also be rejected by T cell–mediated immune responses to xenoantigens. The mechanisms of cell-mediated rejection of xenografts are believed to be similar to those that we have described for allograft rejection, and T cell responses to xenoantigens can be as strong as or even stronger than responses to alloantigens.

BLOOD TRANSFUSION AND THE ABO AND Rh BLOOD GROUP ANTIGENS

Blood transfusion is a form of transplantation in which whole blood or blood cells from one or more individuals are transferred intravenously into the circulation of a host. Blood transfusions are most often performed to replace blood lost by hemorrhage or to correct defects caused by inadequate production of blood cells, which may occur in a variety of diseases. The major barrier to successful blood transfusions is the immune response to cell surface molecules that differ between individuals. The most important alloantigen system in blood transfusion is the ABO system, which we will discuss in detail below. ABO antigens are expressed on virtually all cells, including red blood cells. Individuals lacking a particular blood group antigen produce natural IgM antibodies against that antigen. If such individuals are given blood cells expressing the target antigen, the preexisting antibodies bind to the transfused cells, activate complement, and cause **transfusion reactions**, which can be life-threatening. Transfusion across an ABO barrier may trigger an immediate hemolytic reaction, resulting in both intravascular lysis of red blood cells, probably mediated by the complement system, and extensive phagocytosis of antibody- and complement-coated erythrocytes by macrophages of the liver and spleen. Hemoglobin is liberated from the lysed red cells in quantities that may be toxic for kidney cells, causing acute renal tubular cell necrosis and kidney failure. High fevers, shock, and disseminated intravascular coagulation may also develop, suggestive of massive cytokine release (e.g., of TNF or IL-1). The disseminated intravascular coagulation consumes clotting factors faster than they can be synthesized, and the patient may paradoxically die of bleeding in the presence of widespread clotting. More delayed hemolytic reactions may result from incompatibilities of minor blood group antigens. These result in progressive loss of the transfused red cells, leading to anemia, and jaundice, a consequence of overloading the liver with hemoglobin-derived pigments.

We will now discuss the ABO blood group antigens as well as other blood group antigens of clinical relevance.

ABO Blood Group Antigens

The ABO antigens are carbohydrates linked to cell surface proteins and lipids that are synthesized by polymorphic glycosyltransferase enzymes, which vary in activity depending on the inherited allele (Fig. 16-13). The ABO antigens were the first alloantigen system to be defined in mammals. All normal individuals synthesize a common core glycan, called the O antigen, that is mainly attached to plasma membrane proteins. Most individuals possess a fucosyltransferase that adds a fucose moiety to a nonterminal sugar residue of the O antigen, and the fucosylated glycan is called the H antigen. A single gene on chromosome 9 encodes a glycosyltransferase enzyme, which further modifies the H antigen. There are three allelic variants of this gene. The O allele gene product is devoid of enzymatic activity. The A allele–encoded enzyme transfers a terminal *N*-acetylgalactosamine moiety, and the B allele gene product transfers a terminal galactose moiety. Individuals who are homozygous for the O allele cannot attach terminal sugars to the H antigen and express only the H antigen. In contrast, individuals who possess an A allele (AA homozygotes, AO heterozygotes, or AB

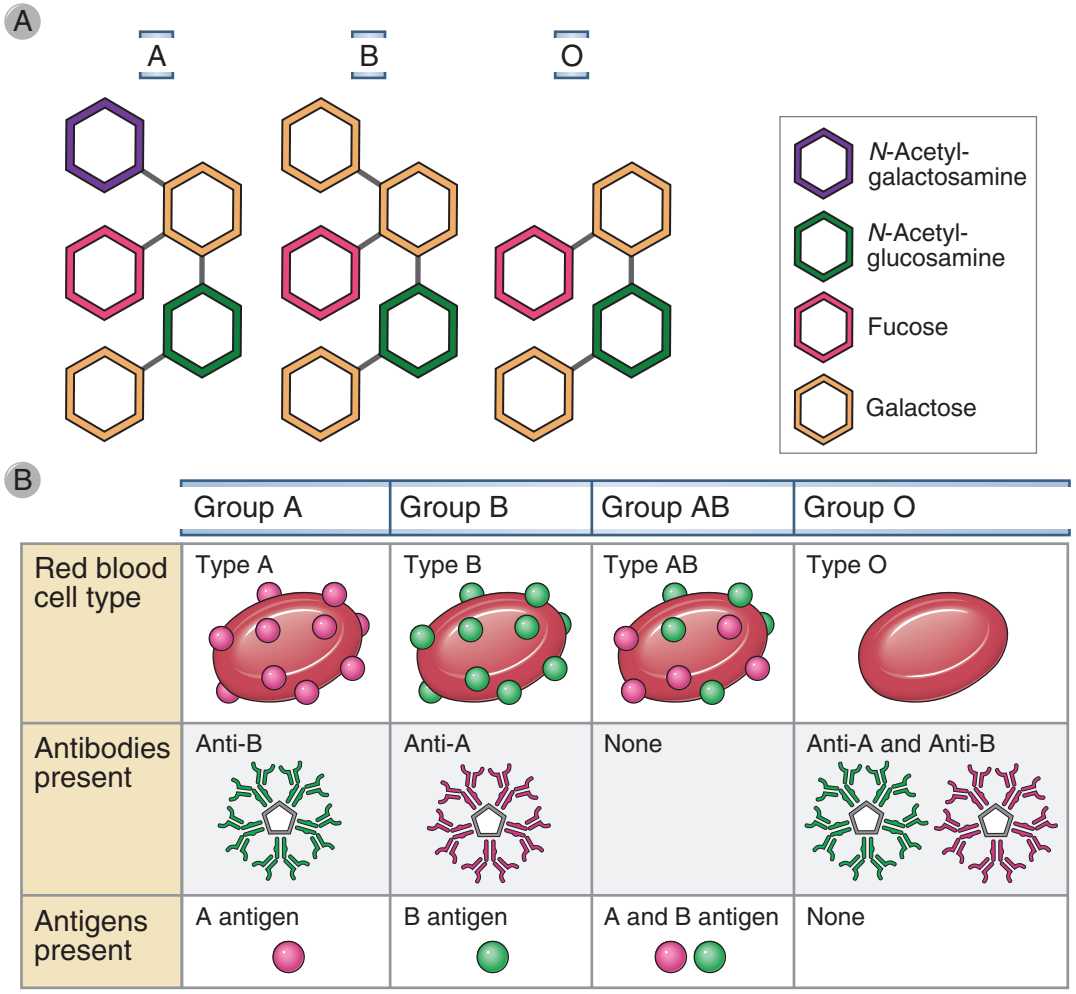


Figure 16–13 ABO blood group antigens. A, Blood group antigens are carbohydrate structures added onto cell surface proteins by the action of glycosyltransferases. Most people inherit a gene that encodes L-fucosyltransferase, which produces the H antigen. Inheritance of a gene for *N*-acetyl-D-galactosaminyl transferase, which generates the A antigen, and the gene that encodes D-galactosyltransferase, which generates the B antigen, varies between people. A person who does not inherit genes for either of these enzymes will be type O; a person who inherits only one of these glycosal transferase genes will be type A or B; and a person who inherits genes for both enzymes will be type AB.

heterozygotes) form the A antigen by adding terminal *N*-acetylgalactosamine to some of their H antigens. Similarly, individuals who express a B allele (BB homozygotes, BO heterozygotes, or AB heterozygotes) form the B antigen by adding terminal galactose to some of their H antigens. AB heterozygotes form both A and B antigens from some of their H antigens. The terminology has been simplified so that OO individuals are said to be blood type O; AA and AO individuals are blood type A; BB and BO individuals are blood type B; and AB individuals are blood type AB. Mutations in the gene encoding the fucosyltransferase that produces the H antigen are rare; people who are homozygous for such a mutation are said to have the Bombay blood group and cannot produce H, A, or B antigens. These individuals make antibodies against H, A, and B antigens and cannot receive type O, A, B, or AB blood.

Individuals who express a particular ABO antigen are tolerant to that antigen, but individuals who do not express that antigen produce natural antibodies that recognize the antigen. Virtually all individuals express the H antigen, and therefore they are tolerant to this antigen and do not produce anti-H antibodies. Individuals who express A or B antigens are tolerant to these molecules and do not produce anti-A or anti-B antibodies, respectively. However, blood group O and A individuals produce anti-B IgM antibodies, and blood group O and B individuals produce anti-A IgM antibodies. On face value, it seems paradoxical that individuals who do not express a blood group antigen make antibodies against it. The likely explanation is that the antibodies are produced against glycolipids of intestinal bacteria that happen to cross-react with the ABO antigens, unless the individual is tolerant to one or more of these.

In clinical transfusion, the choice of blood donors for a particular recipient is based on the expression of blood group antigens and the antibody responses to them. If a patient receives a transfusion of red blood cells from a donor who expresses the antigen not expressed on self red blood cells, a transfusion reaction may result (described before). It follows that AB individuals can tolerate transfusions from all potential donors and are therefore called universal recipients; similarly, O individuals can tolerate transfusions only from O donors but can provide blood to all recipients and are therefore

called universal donors. In general, differences in minor blood groups lead to red cell lysis only after repeated transfusions trigger a secondary antibody response.

ABO antigens are expressed on many other cell types in addition to blood cells, including endothelial cells. For this reason, ABO typing is critical to avoid hyperacute rejection of certain solid organ allografts, as discussed earlier in the chapter. ABO incompatibility between mother and fetus generally does not cause problems for the fetus because most of the anti-carbohydrate antibodies are IgM and do not cross the placenta.

Other Blood Group Antigens

Lewis Antigen

The same glycoproteins that carry the ABO determinants can be modified by other glycosyltransferases to generate minor blood group antigens. For example, addition of fucose moieties at other nonterminal positions can be catalyzed by different fucosyltransferases and results in epitopes of the Lewis antigen system. Lewis antigens have recently received much attention from immunologists because these carbohydrate groups serve as ligands for E-selectin and P-selectin.

Rhesus (Rh) Antigen

The Rhesus (Rh) antigens, named after the monkey species in which they were originally identified, are another clinically important group of blood group antigens. Rh antigens are nonglycosylated, hydrophobic cell surface proteins found in red blood cell membranes and are structurally related to other red cell membrane glycoproteins with transporter functions. Rh proteins are encoded by two tightly linked and highly homologous genes, but only one of them, called RhD, is commonly considered in clinical blood typing. This is because up to 15% of the population has a deletion or other alteration of the RhD allele. These people, called Rh negative, are not tolerant to the RhD antigen and will make antibodies to the antigen if they are exposed to Rh-positive blood cells.

The major clinical significance of anti-Rh antibodies is related to hemolytic reactions associated with pregnancy that are similar to transfusion reactions. Rh-negative mothers carrying an Rh-positive fetus can be sensitized by fetal red blood cells that enter the maternal circulation, usually during childbirth. Since the Rh antigen is a protein, as opposed to the carbohydrate ABO antigens, class-switched IgG antibodies are generated in Rh-negative mothers. Subsequent pregnancies in which the fetus is Rh positive are at risk because the maternal anti-Rh antibodies can cross the placenta and mediate the destruction of the fetal red blood cells. This causes **erythroblastosis fetalis** (hemolytic disease of the newborn) and can be lethal for the fetus. This disease can be prevented by administration of anti-RhD antibodies to the mother within 72 hours of birth of the first Rh-positive baby. The treatment prevents the baby's Rh-positive red blood cells that entered the mother's circulation from inducing the production of anti-Rh antibodies in the mother. The exact mechanisms of action of the administered antibodies are not clear but may include phagocytic clearance or complement-mediated lysis of the baby's red cells or Fc receptor–dependent feedback inhibition of the mother's RhD-specific B cells (see Chapter 11).

HEMATOPOIETIC STEM CELL TRANSPLANTATION

The transplantation of allogeneic pluripotent hematopoietic stem cells is done commonly using an inoculum of bone marrow cells collected by aspiration, and the procedure is often called bone marrow transplantation. Hematopoietic stem cells can also be purified from the blood of donors after treatment with colony-stimulating factors, which mobilize stem cells from the bone marrow. The recipient is treated before transplantation to deplete bone marrow cells to free up niches for the transferred stem cells. After transplantation, stem cells repopulate the recipient's bone marrow and differentiate into all the hematopoietic lineages. We consider bone marrow transplantation separately because this type of grafting has several unique features that are not encountered with solid organ transplantation.

Bone marrow transplantation is most often used clinically in the treatment of leukemias. In some forms of leukemia, the grafted cells are effective in destroying residual leukemia cells. In addition, the chemotherapeutic agents needed to destroy cancer cells also destroy normal marrow elements, and bone marrow transplantation is used to "rescue" the patient from the side effects of chemotherapy. Hematopoietic stem cell transplantation is also used clinically to treat diseases caused by inherited mutations in genes affecting only cells derived from hematopoietic stem cells, such as lymphocytes or red blood cells. Examples of such diseases that can be cured by hematopoietic stem cell transfer are adenosine deaminase (ADA) deficiency, X-linked severe combined immunodeficiency disease, and hemoglobin mutations such as beta-thalassemia major and sickle cell disease.

Allogeneic hematopoietic stem cells are rejected by even a minimally immunocompetent host, and therefore the donor and recipient must be carefully matched at all MHC loci. The mechanisms of rejection of bone marrow cells are not completely known, but in addition to adaptive immune mechanisms, hematopoietic stem cells may be rejected by NK cells. The role of NK cells in bone marrow rejection has been studied in experimental animals. Irradiated F_1 hybrid mice reject bone marrow donated by either inbred parent. This phenomenon, called hybrid resistance, appears to violate the classical laws of solid tissue transplantation (see Fig. 16-2). Hybrid resistance is seen in T cell–deficient mice, and depletion of recipient NK cells with anti–NK cell antibodies prevents the rejection of parental bone marrow. Hybrid resistance is probably due to host NK cells reacting against bone marrow precursors that lack class I MHC molecules expressed by the host. Recall that normally, recognition of self class I MHC inhibits the activation of NK cells, and if these self MHC molecules are missing, the NK cells are released from inhibition (see Chapter 4, Fig. 4-6). Donor-versus-recipient NK cell alloreactivity has been used to reduce leukemia relapses after HLA haplotype–mismatched hematopoietic stem cell transplantation.

Even after successful engraftment, two additional problems are frequently associated with bone marrow transplantation, namely, graft-versus-host disease and immunodeficiency.

Graft-Versus-Host Disease

Graft-versus-host disease (GVHD) is caused by the reaction of grafted mature T cells in the marrow inoculum with alloantigens of the host. It occurs when the host is immunocompromised and therefore unable to reject the allogeneic cells in the graft. In most cases, the reaction is directed against minor histocompatibility antigens of the host because bone marrow transplantation is not performed when the donor and recipient have differences in MHC molecules. GVHD may also develop when solid organs that contain significant numbers of T cells are transplanted, such as the small bowel, lung, or liver.

GVHD is the principal limitation to the success of bone marrow transplantation. As in solid organ transplantation, GVHD may be classified on the basis of histologic patterns into acute and chronic forms.

Acute GVHD is characterized by epithelial cell death in the skin, liver (mainly the biliary epithelium), and gastrointestinal tract (Fig. 16-14). It is manifested clinically by rash, jaundice, diarrhea, and gastrointestinal hemorrhage. When the epithelial cell death is extensive, the skin or lining of the gut may slough off. In this circumstance, acute GVHD may be fatal.

Chronic GVHD is characterized by fibrosis and atrophy of one or more of the same organs, without evidence of acute cell death. Chronic GVHD may also involve the lungs and produce obliteration of small airways. When it is severe, chronic GVHD leads to complete dysfunction of the affected organ.

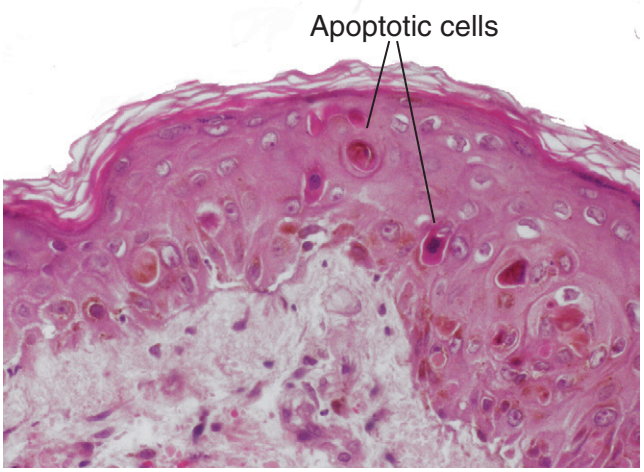


FIGURE 16–14 Histopathology of acute GVHD in the skin. A sparse lymphocytic infiltrate can be seen at the dermal-epidermal junction, and damage to the epithelial layer is indicated by spaces at the dermal-epidermal junction (vacuolization), cells with abnormal keratin staining (dyskeratosis), apoptotic keratinocytes, and disorganization of maturation of keratinocytes from the basal layer to the surface. *(Courtesy of Dr. Scott Grantor, Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts.)*

In animal models, acute GVHD is initiated by mature T cells present in the bone marrow inoculum, and elimination of mature donor T cells from the graft can prevent the development of GVHD. In clinical hematopoietic stem cell transplantation, efforts to eliminate T cells from the marrow inoculum have reduced the incidence of GVHD but also decrease the graft-versus-leukemia effect that is often critical in treating leukemias by this type of transplantation. T cell–depleted marrow also tends to engraft poorly, perhaps because mature T cells produce colony-stimulating factors that aid in stem cell repopulation. One approach that has been tried is to combine removal of T cells with supplemental colony-stimulating factor treatment to promote engraftment.

Although GVHD is initiated by grafted T cells recognizing host alloantigens, the effector cells that cause epithelial cell injury are less well defined. On histologic examination, NK cells are often attached to the dying epithelial cells, suggesting that NK cells are important effector cells of acute GVHD. $CD8^+$ CTLs and cytokines also appear to be involved in tissue injury in acute GVHD.

The relationship of chronic GVHD to acute GVHD is not known and raises issues similar to those of relating chronic allograft rejection to acute allograft rejection. For example, chronic GVHD may represent the fibrosis of wound healing secondary to loss of epithelial cells. However, chronic GVHD can arise without evidence of prior acute GVHD. An alternative explanation is that chronic GVHD represents a response to ischemia caused by vascular injury.

Both acute and chronic GVHD are commonly treated with intense immunosuppression. It is not clear that either condition responds very well. A possible explanation for this therapeutic failure is that conventional immunosuppression is targeted against T lymphocytes, which may be only one of several mediators of GVHD. Cyclosporine and the metabolic toxin methotrexate are also used for prophylaxis against GVHD. Various new therapies are being studied in clinical trials, including rapamycin, anti-TNF antibodies, and regulatory T cell transfer.

Immunodeficiency After Bone Marrow Transplantation

Bone marrow transplantation is often accompanied by clinical immunodeficiency. Several factors may contribute to defective immune responses in recipients. Bone marrow transplant recipients may be unable to regenerate a complete new lymphocyte repertoire. Radiation therapy and chemotherapy used to prepare recipients for transplantation are likely to deplete the patient's memory cells and long-lived plasma cells, and it can take a long time to regenerate these populations.

The consequence of immunodeficiency is that bone marrow transplant recipients are susceptible to viral infections, especially cytomegalovirus infection, and to many bacterial and fungal infections. They are also susceptible to Epstein-Barr virus–provoked B cell lymphomas. The immune deficiencies of bone marrow transplant recipients can be more severe than those of conventionally immunosuppressed patients. Therefore, bone marrow

transplant recipients commonly receive prophylactic antibiotics and anti-cytomegalovirus therapy and are often actively immunized against capsular bacteria such as pneumococcus before transplantation.

There is great interest in the use of pluripotent stem cells to repair tissues with little natural regenerative capacity, such as cardiac muscle, brain, or spinal cord. One approach is to use embryonic stem cells, which are pluripotent stem cells derived from the blastocyst stage of human embryos. Although embryonic stem cells have not yet been used clinically, it is highly likely that a major barrier to their usefulness will be their alloantigenicity and rejection by the recipient's immune system. A possible solution to this may be to use induced pluripotent stem (iPS) cells, which can be derived from adult somatic tissues by transduction of certain genes. The immunologic advantage of the iPS cell approach is that these cells can be derived from somatic cells harvested from the patient, and therefore they will be syngeneic to the patient.

SUMMARY

- Transplantation of tissues from one individual to a genetically nonidentical recipient leads to a specific immune response called rejection that can destroy the graft. The major molecular targets in allograft rejection are allogeneic class I and class II MHC molecules.
- Allogeneic MHC molecules may be presented on donor APCs to recipient T cells (the direct pathway), or the alloantigens may be picked up by host APCs that enter the graft or reside in draining lymphoid organs and be processed and presented to T cells as peptides associated with self MHC molecules (the indirect pathway).
- The frequency of T cells capable of recognizing allogeneic MHC molecules is very high, explaining why the response to alloantigens is much stronger than the response to conventional foreign antigens. The reasons for this high frequency are an inherent bias of T cells to recognize MHC molecules and because many different T cell clones specific for different foreign peptides plus self MHC molecules may cross-react with an individual allogeneic MHC molecule.
- Graft rejection is mediated by T cells, including CTLs that kill graft cells and helper T cells that cause cytokine-mediated inflammation resembling DTH reactions, and by antibodies.
- Several effector mechanisms cause rejection of solid organ grafts, and each mechanism may lead to a histologically characteristic reaction. Preexisting antibodies cause hyperacute rejection characterized by thrombosis of graft vessels. Alloreactive T cells and antibodies produced in response to the graft cause blood vessel wall damage and parenchymal cell death, called acute rejection. Chronic rejection is characterized by fibrosis and vascular abnormalities (graft vasculopathy), which may represent blood vessel damage and ischemic injury to parenchyma due to a T cell- and cytokine-mediated inflammatory reaction in the walls of arteries.
- Graft rejection may be prevented or treated by immunosuppression of the host and by minimizing the immunogenicity of the graft (by limiting MHC allelic differences). Most immunosuppression is directed at T cell responses and entails the use of cytotoxic drugs, specific immunosuppressive agents, or anti–T cell antibodies. A widely used immunosuppressive agent is cyclosporine, which blocks T cell antigen receptor signaling linked to cytokine synthesis. Immunosuppression is often combined with anti-inflammatory drugs such as corticosteroids that inhibit cytokine synthesis by macrophages and other cells.
- Patients receiving solid organ transplants may become immunodeficient because of their therapy and are susceptible to viral infections and malignant tumors.
- Xenogeneic transplantation of solid organs is limited by the presence of natural antibodies to carbohydrate antigens on the cells of discordant species that cause hyperacute rejection, antibody-mediated acute vascular rejection, T cell–mediated immune response to xenogeneic MHC molecules, and prothrombotic effects of xenogeneic endothelium on human platelets and coagulation proteins.
- The ABO blood group antigens are a set of polymorphic carbohydrate structures present on blood cells and endothelium that limit transfusions and some solid organ transplantations between individuals. Preexisting natural anti-A or anti-B IgM antibodies are present in individuals who do not express A or B antigens on their cells, respectively, and these antibodies can cause transfusion reactions and hyperacute allograft rejection.
- Hematopoietic stem cell transplants are susceptible to rejection, and recipients require intense preparatory immunosuppression. In addition, T lymphocytes in the bone marrow graft may respond to alloantigens of the host and cause GVHD. Acute GVHD is characterized by epithelial cell death in the skin, intestinal tract, and liver; it may be fatal. Chronic GVHD is characterized by fibrosis and atrophy of one or more of these same target organs as well as the lungs and may also be fatal. Bone marrow transplant recipients also often develop severe immunodeficiency, rendering them susceptible to infections.

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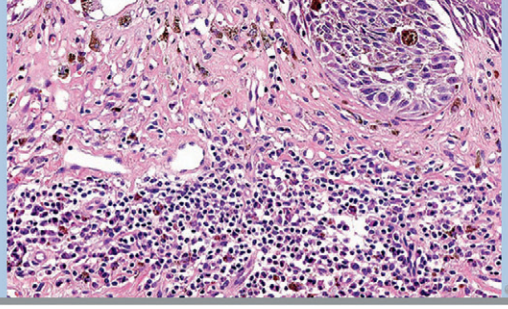
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CHAPTER

17

Immunity to Tumors

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Cancer is a major health problem worldwide and one of the most important causes of morbidity and mortality in children and adults. Cancers arise from the uncontrolled proliferation and spread of clones of malignantly transformed cells. The lethality of malignant tumors is determined in large part by their unregulated proliferative activity, the resistance of tumor cells to apoptotic death, and the ability of tumor cells to invade host tissues and metastasize to distant sites. The possibility that cancers can be eradicated by specific immune responses has been the impetus for a large body of work in the field of tumor immunology. The concept of **immune surveillance**, which was proposed by Macfarlane Burnet in the 1950s, states that a physiologic function of the immune system is to recognize and destroy clones of transformed cells before they grow into tumors and to kill tumors after they are formed. The existence of immune surveillance has been demonstrated by the increased incidence of some types of tumors in immunocompromised experimental animals and humans. Although the overall importance of immune surveillance has been controversial, it is now clear that the innate and adaptive immune systems do react against many tumors, and exploiting these reactions to specifically destroy tumors remains an important goal of tumor immunologists. In this chapter, we describe the types of antigens that are expressed by malignant tumors, how the immune system recognizes and responds to these antigens, how tumors evade the host immune system, and the application of immunologic approaches to the treatment of cancer.

GENERAL FEATURES OF TUMOR IMMUNITY

Several characteristics of tumor antigens and immune responses to tumors are fundamental to an understanding of tumor immunity and for the development of strategies for cancer immunotherapy.

- ***Tumors stimulate specific, adaptive immune responses.*** Clinical observations and animal experiments have established that although tumor cells are derived from host cells, the tumors elicit immune responses. Histopathologic studies show that many tumors are surrounded by mononuclear cell infiltrates composed of T lymphocytes, natural killer (NK) cells, and macrophages, and that activated lymphocytes and macrophages are present in lymph nodes draining the sites of tumor growth (Fig. 17-1). The presence of

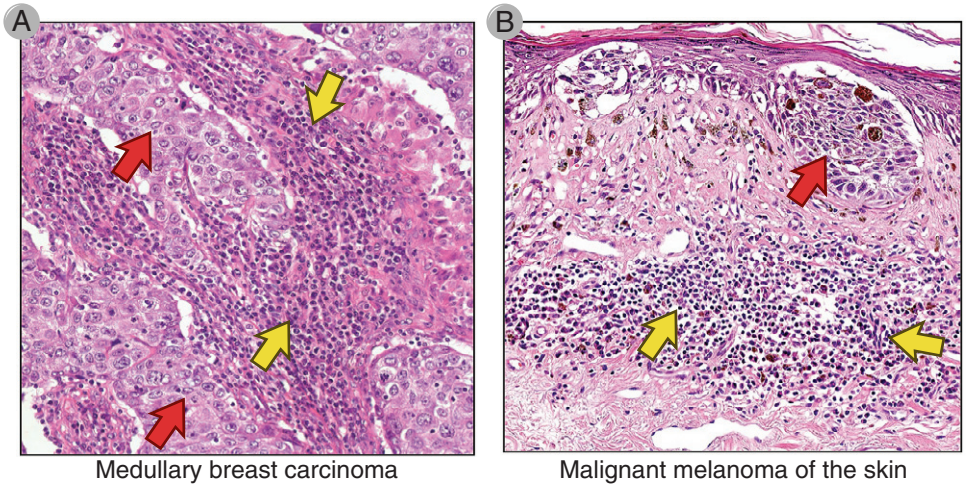


FIGURE 17–1 Lymphocytic inflammation associated with certain tumors. A, Medullary breast carcinoma. **B,** Malignant melanoma. Red arrows indicate malignant cells. Yellow arrows indicate lymphocyte-rich inflammatory infiltrates.

lymphocytic infiltrates in some types of melanoma and carcinomas of the colon and breast cancer is predictive of a better prognosis. The first experimental demonstration that tumors can induce protective immune responses came from studies of transplanted tumors performed in the 1950s (Fig. 17-2). A sarcoma may be induced in an inbred mouse by painting its skin with the chemical carcinogen methylcholanthrene (MCA). If the MCA-induced tumor is excised and transplanted into other syngeneic mice, the tumor grows. In contrast, if the tumor is transplanted back into the original host, the mouse rejects the tumor. The same mouse that had become immune to its tumor is incapable of rejecting MCA-induced tumors produced in other

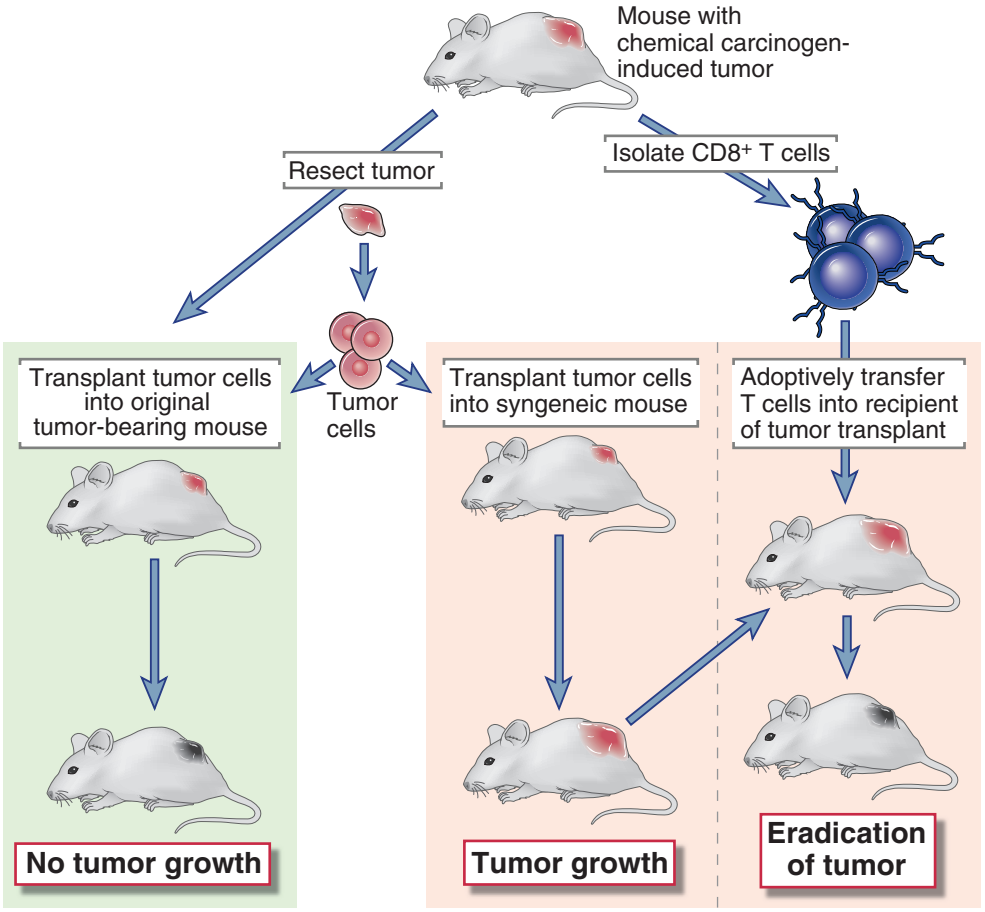


FIGURE 17–2 Experimental demonstration of tumor immunity. Mice that have been surgically cured of a chemical carcinogen (MCA)–induced tumor reject subsequent transplants of the same tumor, whereas the transplanted tumor grows in normal syngeneic mice. The tumor is also rejected in normal mice that are given adoptive transfer of T lymphocytes from the original tumor-bearing animal.

mice. Furthermore, T cells from the tumor-bearing animal can transfer protective immunity to the tumor to another tumor-free animal. Thus, immune responses to tumors exhibit the defining characteristics of adaptive immunity, namely, specificity, memory, and the key role of lymphocytes. As predicted from these transplantation experiments, the most effective response against naturally arising tumors appears to be mediated mainly by T lymphocytes.

- ***Immune responses frequently fail to prevent the growth of tumors.*** There may be several reasons that antitumor immunity is unable to eradicate transformed cells. First, tumor cells are derived from host cells and resemble normal cells in many respects. Therefore, most tumors tend to be weakly immunogenic. Tumors that elicit strong immune responses include those induced by oncogenic viruses, in which the viral proteins are foreign antigens, and tumors induced in animals by potent carcinogens (such as methylcholanthrene), which often cause mutations in normal cellular genes. Many spontaneous tumors induce weak or even undetectable immunity, and studies of such tumors led to considerable skepticism about the concept of immune surveillance. It is now clear that the importance of immune surveillance and tumor immunity varies with the type of tumor. Second, the rapid growth and spread of tumors may overwhelm the capacity of the immune system to effectively control a tumor, which requires that all the malignant cells be eliminated. Third, many tumors have specialized mechanisms for evading host immune responses. We will return to these mechanisms later in the chapter.
- ***The immune system can be activated by external stimuli to effectively kill tumor cells and eradicate tumors.*** As we shall see at the end of the chapter, this realization has spurred new directions in tumor immunotherapy in which augmentation of the host anti-tumor response is the goal of treatment.

The existence of specific antitumor immunity implies that tumors must express antigens that are recognized as foreign by the host. The nature and significance of these antigens are described next.

TUMOR ANTIGENS

A variety of tumor antigens that may be recognized by T and B lymphocytes have been identified in human and animal cancers. In the experimental situation, as in MCA-induced mouse sarcomas, it is often possible to demonstrate that these antigens elicit adaptive immune responses and are the targets of such responses. Tumor antigens have also been identified in humans, but the methods used in this case are generally not suitable for proving that these antigens can elicit protective immunity to tumors. Nevertheless, it is important to identify tumor antigens in humans because they may be used as components of tumor vaccines, and antibodies and effector T cells generated against these antigens may be used for immunotherapy.

The earliest classification of tumor antigens was based on their patterns of expression. Antigens that are expressed on tumor cells but not on normal cells are called **tumor-specific antigens**; some of these antigens are unique to individual tumors, whereas others are shared among tumors of the same type. Tumor antigens that are also expressed on normal cells are called **tumor-associated antigens**; in most cases, these antigens are normal cellular constituents whose expression is aberrant or dysregulated in tumors. The modern classification of tumor antigens is based on the molecular structure and source of antigens expressed by tumor cells that stimulate T cell or antibody responses in their hosts.

Identification of Tumor Antigens

The identification of many antigens expressed by naturally occurring human tumors represents a major advance in the field of tumor immunology. Various biochemical and molecular genetic approaches have been used to identify these antigens. For tumor antigens recognized by $CD8^+$ cytotoxic T lymphocytes (CTLs), investigators have established cloned lines of tumor-reactive CTLs from cancer patients and used these as probes to specifically identify the relevant peptide antigens or the genes encoding the peptides. For example, many cloned CTL lines specific for melanomas have been generated from the T cells of patients. Melanomas, which are malignant tumors of melanocytes, are often readily accessible, surgically resectable tumors that may be grown in tissue culture. The T cells can be isolated from peripheral blood, lymph nodes draining the tumor, or directly from tumor tissue removed from patients. These T cells can be stimulated to grow in vitro by coculture with the tumor cells, and individual clones can be isolated. Because the T cells and the tumor are from the same individual, the major histocompatibility complex (MHC) restriction of the T cells matches the MHC alleles expressed by the tumor. These tumor antigen–specific CTL clones have been used to detect responses to tumor-derived peptides or responses to proteins made by complementary DNA (cDNA) libraries of the tumor (Fig. 17-3). Such approaches were first used to identify human melanoma antigens that stimulated CTL responses in patients with melanoma. The same methods have been used to identify antigens that are recognized by $CD4^+$ helper cells, in which case the probes are helper T cell clones derived from patients' $CD4^+$ T cells.

A successful method for identification of tumor antigens that have stimulated humoral immune responses in tumor patients is called the serologic analysis of recombinant cDNA expression (SEREX). In this method, expression libraries of cDNA derived from a patient's tumor RNA are transfected into a cell line, and assays are performed to detect binding of the cancer patient's serum immunoglobulins to the transfected cells. In this way, gene sequences for targeted proteins are obtained, and the encoded proteins that have stimulated antibody responses in the patient are identified.

In the following section, we describe the main classes of tumor antigens (Table 17-1). We will include tumor antigens known to induce immune responses in humans

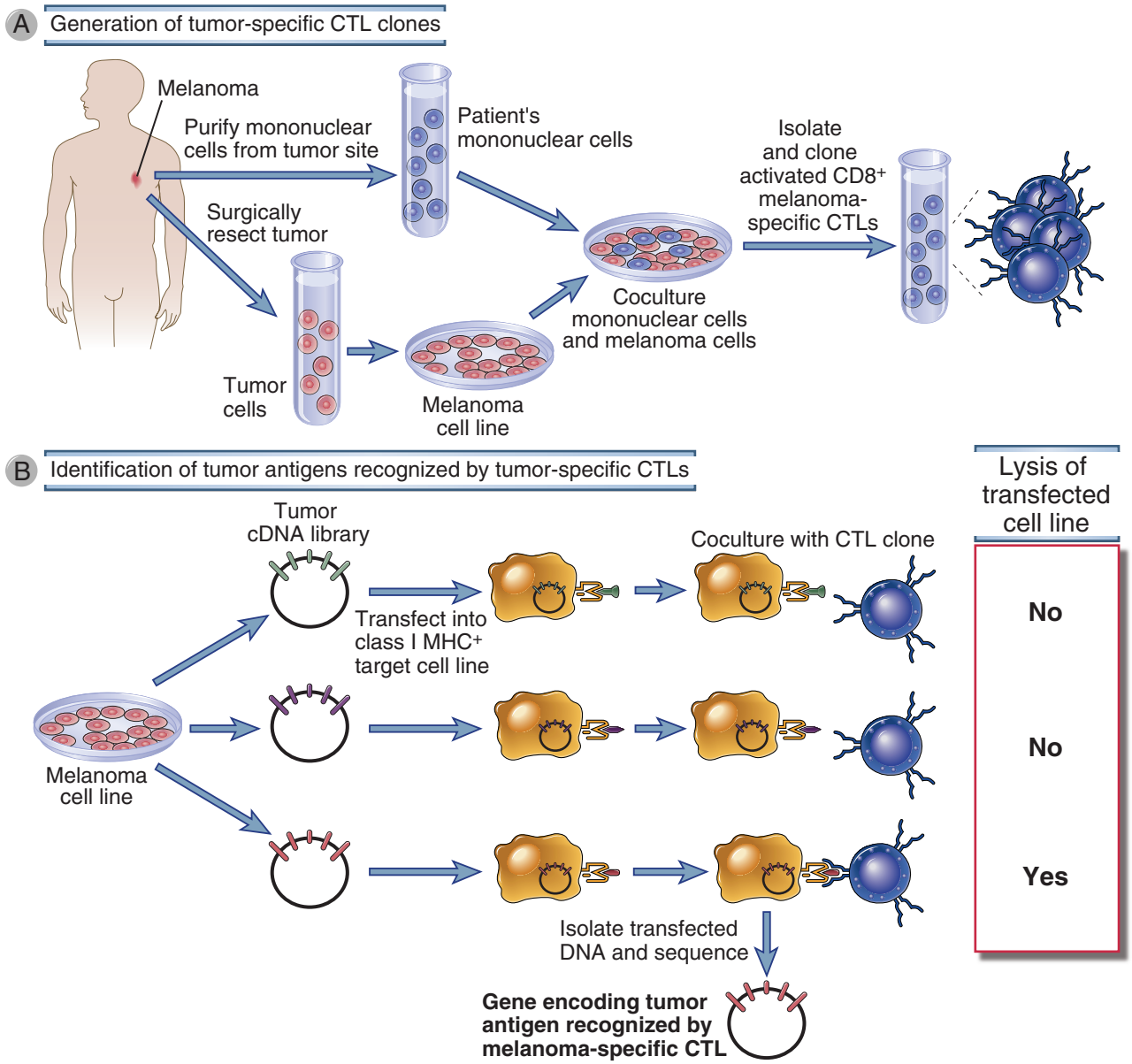


FIGURE 17–3 Cloned CTL lines specific for human tumors are used to identify specific tumor antigens. A, $CD8^+$ T cells isolated from blood, lymph nodes, or tumors of patients with melanoma are propagated in culture by stimulating them with melanoma cell lines derived from the patient's tumor. Single T cells from these cultures are expanded into clonal CTL lines. **B,** DNA from melanoma gene libraries is transfected into class I MHC–expressing target cells. Genes that sensitize the target cells for lysis by the melanoma-specific CTL clones are analyzed to identify the melanoma protein antigens recognized by the patient's CTLs.

with cancers as well as tumor-associated antigens that may not naturally induce host immune responses but are potential targets for immunotherapy or are useful markers for clinical diagnosis and for observation of patients.

Products of Mutated Genes

Oncogenes and mutated tumor suppressor genes produce proteins that differ from normal cellular proteins and are, therefore, recognized as tumor antigens. Many tumors express genes whose products are required for malignant transformation or for maintenance of the malignant phenotype. Often, these genes are produced by point mutations, deletions, chromosomal translocations, or viral gene insertions affecting cellular proto-oncogenes or tumor suppressor genes. The products of these oncogenes and altered tumor suppressor genes are synthesized in the cytoplasm of the tumor cells and may enter the class I antigen-processing pathway. In addition, these proteins may enter the class II antigen-processing pathway in antigen-presenting cells (APCs) that have phagocytosed dead tumor cells. Because these altered genes are not present in normal cells, peptides derived from them do not induce self-tolerance and may stimulate T cell responses in the host. Some patients with cancer have circulating $CD4^+$ and $CD8^+$ T cells that can

TABLE 17–1 Tumor Antigens

Type of Antigen	Examples of Human Tumor Antigens
Products of mutated oncogenes, tumor suppressor genes	Oncogene products: Ras mutations (~10% of human carcinomas), p210 product of Bcr/Abl rearrangements (CML) Tumor suppressor gene products: mutated p53 (present in ~50% of human tumors)
Unmutated but overexpressed products of oncogenes	HER2/Neu (breast and other carcinomas)
Mutated forms of cellular genes not involved in tumorigenesis	Various mutated proteins in melanomas recognized by CTLs
Products of genes that are silent in most normal tissues	Cancer/testis antigens expressed in melanomas and many carcinomas; normally expressed mainly in the testis and placenta
Normal proteins overexpressed in tumor cells	Tyrosinase, gp100, MART in melanomas (normally expressed in melanocytes)
Products of oncogenic viruses	Papillomavirus E6 and E7 proteins (cervical carcinomas) EBNA-1 protein of EBV (EBV-associated lymphomas, nasopharyngeal carcinoma)
Oncofetal antigens	Carcinoembryonic antigen on many tumors, also expressed in liver and other tissues during inflammation α-Fetoprotein
Glycolipids and glycoproteins	GM_2, GD_2 on melanomas
Differentiation antigens normally present in tissue of origin	Prostate-specific antigen in prostate carcinomas CD20 on B cell lymphomas

CML, chronic myelogenous leukemia; CTL, cytotoxic T lymphocyte; EBNA, Epstein-Barr nuclear antigen; EBV, Epstein-Barr virus; MART, melanoma antigen recognized by T cells.

respond to the products of mutated oncogenes such as Ras and Bcr/Abl proteins and mutated tumor supressor genes such as *p53*. Furthermore, in animals, immunization with mutated Ras or p53 proteins induces CTLs and rejection responses against tumors expressing these mutants. However, these proteins do not appear to be major targets of tumor-specific CTLs in most patients with a variety of tumors.

Tumor antigens may be produced by randomly mutated genes whose products are not related to the malignant phenotype. Tumor antigens that were defined by the transplantation of carcinogen-induced tumors in animals, called tumor-specific transplantation antigens, are mutants of various host cellular proteins. Studies with chemically induced rodent sarcomas, such as those illustrated in Figure 17-2, established that different rodent tumors, all induced by the same carcinogen, expressed different transplantation antigens. The tumor antigens identified by such experiments are peptides derived from mutated self proteins and presented in the form of peptide–class I MHC complexes capable of stimulating CTLs. These antigens are extremely diverse because the carcinogens that induce the tumors may randomly mutagenize any host gene, and the class I MHC antigen-presenting pathway can display peptides from any mutated cytosolic protein in each tumor. Mutated cellular proteins are found more frequently in chemical carcinogen– or radiation-induced animal tumors than in spontaneous human cancers, probably because chemical carcinogens and radiation mutagenize many cellular genes. However, because of the intrinsic genomic instability of many cancers, a wide variety of genes may be mutated in tumor cells. Even if these mutations do not contribute to the malignant phenotype, they may encode abnormal proteins that are recognized by the immune system.

Abnormally Expressed but Unmutated Cellular Proteins

Tumor antigens that elicit immune responses may be normal cellular proteins that are abnormally expressed in tumor cells. Many such antigens have been identified in human tumors, such as melanomas, by the molecular cloning of antigens that are recognized by T cells and antibodies from tumor-bearing patients (see Fig. 17-3). One of the surprises that emerged from these studies was that some tumor antigens are normal proteins that are produced at low levels in normal cells and overexpressed in tumor cells (see Table 17-1). One such antigen is tyrosinase, an enzyme involved in melanin biosynthesis that is expressed only in normal melanocytes and melanomas. Both class I MHC–restricted $CD8^+$ CTL clones and class II MHC–restricted $CD4^+$ helper T cell clones from melanoma patients recognize peptides derived from tyrosinase. On face value, it is surprising that these patients are able to respond to a normal self antigen. The likely explanation is that tyrosinase is normally produced in such small amounts and in so few cells that it is not recognized by the immune system and fails to induce tolerance. Therefore, the increased amount produced by melanoma cells is able to elicit immune responses. The finding of tyrosinase-specific T cell responses in patients raises the possibility that tyrosinase vaccines may

stimulate such responses to melanomas; clinical trials with these vaccines are ongoing.

Cancer/testis antigens are proteins expressed in gametes and trophoblasts and in many types of cancers but not in normal somatic tissues. The first cancer/testis antigens were identified by cloning genes from human melanomas that encoded cellular protein antigens recognized by melanoma-specific CTL clones derived from the melanoma-bearing patients. These were called MAGE proteins, and they were subsequently found to be expressed in other tumors in addition to melanomas, including carcinomas of the bladder, breast, skin, lung, and prostate and some sarcomas, as well as in normal testes. Subsequent to identification of the MAGE genes, several other unrelated gene families have been identified that encode melanoma antigens recognized by CTL clones derived from melanoma patients. Like the MAGE proteins, these other melanoma antigens are silent in most normal tissues, except the testes or trophoblasts in the placenta, but they are expressed in a variety of malignant tumors. There are now more than 40 different cancer/testis antigen families identified. About half are encoded by genes on the X chromosome; the rest are encoded by genes distributed throughout the genome. Although some cancer/testis antigens have been shown to regulate transcription or translation of other genes, the functions of most of these proteins are unknown. In general, they are not required for the malignant phenotype of the cells, and their sequences are identical to the corresponding genes in normal cells; that is, they are not mutated. Several X-linked cancer/testis antigens are currently being used in tumor vaccine trials.

Antigens of Oncogenic Viruses

The products of oncogenic viruses function as tumor antigens and elicit specific T cell responses that may serve to eradicate the tumors. DNA viruses are implicated in the development of a variety of tumors in humans and experimental animals. Examples in humans include the Epstein-Barr virus (EBV), which is associated with B cell lymphomas and nasopharyngeal carcinoma, and human papillomavirus (HPV), which is associated with cervical carcinoma. Papovaviruses, including polyomavirus and simian virus 40 (SV40), and adenoviruses induce malignant tumors in neonatal or immunodeficient adult rodents. In most of these DNA virus–induced tumors, virus-encoded protein antigens are found in the nucleus, cytoplasm, or plasma membrane of the tumor cells. These endogenously synthesized proteins can be processed, and complexes of processed viral peptides with class I MHC molecules may be expressed on the tumor cell surface. Because the viral peptides are foreign antigens, DNA virus–induced tumors are among the most immunogenic tumors known.

The ability of adaptive immunity to prevent the growth of DNA virus–induced tumors has been established by many observations. For instance, EBV-associated lymphomas and HPV-associated skin and cervical cancers arise more frequently in immunosuppressed individuals, such as allograft recipients receiving immunosuppressive therapy and patients with acquired immunodeficiency syndrome (AIDS), than in normal individuals. Tumor transplantation experiments of the kind illustrated in Figure 17-2 have shown that animals may be specifically immunized against DNA virus–induced tumors and will reject transplants of these tumors. Unlike MCA-induced tumor antigens, which are the products of randomly mutated cellular genes, virus-encoded tumor antigens are not unique for each tumor but are shared by all tumors induced by the same type of virus. Thus, a competent immune system plays a role in surveillance against virus-induced tumors because of its ability to recognize and kill virus-infected cells. In fact, the concept of immune surveillance against tumors is better established for DNA virus–induced tumors than for any other type of tumor.

The realization that immune responses against viruses protect individuals from virus-induced cancers has led to the development of vaccines against oncogenic viruses. For example, a vaccine against HPV is now in use, which has the potential to reduce the incidence of cervical cancer in women. The vaccine is composed of recombinant HPV capsid proteins from the most common oncogenic strains of HPV, which form virus-like particles free of viral genome. Vaccination against hepatitis B virus is also reducing the incidence of liver cancer. In this case, the virus is not oncogenic, but it promotes the development of liver cancer probably by inducing chronic inflammation, which is a risk factor for cancer development (discussed later in the chapter).

RNA tumor viruses (retroviruses) are important causes of tumors in animals. Retroviral oncogene products theoretically have the same potential antigenic properties as mutated cellular oncogenes, and humoral and cell-mediated immune responses to retroviral gene products on tumor cells can be observed experimentally. The only well-defined human retrovirus that is known to cause tumors is human T cell lymphotropic virus 1 (HTLV-1), the etiologic agent of adult T cell leukemia/lymphoma (ATL), a malignant tumor of $CD4^+$ T cells. Although immune responses specific for HTLV-1–encoded antigens have been demonstrated in individuals infected with the virus, it is not clear whether they play any role in protective immunity against the development of tumors. Furthermore, patients with ATL are often profoundly immunosuppressed, probably because the virus infects $CD4^+$ T cells and induces functional abnormalities in these cells.

Oncofetal Antigens

Oncofetal antigens are proteins that are expressed at high levels in cancer cells and in normal developing fetal but not adult tissues. It is believed that the genes encoding these proteins are silenced during development and are derepressed with malignant transformation. Oncofetal antigens are identified with antibodies raised in other species, and their main importance is that they provide markers that aid in tumor diagnosis. As techniques for detecting these antigens have improved, it has become clear that their expression in adults is not limited to tumors. The proteins are increased in tissues and in the

circulation in various inflammatory conditions and are found in small quantities even in normal tissues. There is no evidence that oncofetal antigens are important inducers or targets of anti-tumor immunity. The two most thoroughly characterized oncofetal antigens are carcinoembryonic antigen (CEA) and α-fetoprotein (AFP).

CEA (CD66) is a highly glycosylated membrane protein that is a member of the immunoglobulin (Ig) superfamily and functions as an intercellular adhesion molecule. High CEA expression is normally restricted to cells in the gut, pancreas, and liver during the first two trimesters of gestation, and low expression is seen in normal adult colonic mucosa and the lactating breast. CEA expression is increased in many carcinomas of the colon, pancreas, stomach, and breast, and serum levels are increased in these patients. The level of serum CEA is used to monitor the persistence or recurrence of the tumors after treatment. The usefulness of CEA as a diagnostic marker for cancer is limited by the fact that serum CEA can also be elevated in the setting of non-neoplastic diseases, such as chronic inflammatory conditions of the bowel or liver.

AFP is a circulating glycoprotein normally synthesized and secreted in fetal life by the yolk sac and liver. Fetal serum concentrations can be as high as 2 to 3 mg/mL, but in adult life, the protein is replaced by albumin, and only low levels are present in serum. Serum levels of AFP can be significantly elevated in patients with hepatocellular carcinoma, germ cell tumors, and, occasionally, gastric and pancreatic cancers. An elevated serum AFP level is a useful indicator of advanced liver or germ cell tumors or of recurrence of these tumors after treatment. Furthermore, the detection of AFP in tissue sections by immunohistochemical techniques can help in the pathologic identification of tumor cells. The diagnostic value of AFP as a tumor marker is limited by the fact that elevated serum levels are also found in non-neoplastic diseases, such as cirrhosis of the liver.

Altered Glycolipid and Glycoprotein Antigens

Most human and experimental tumors express higher than normal levels or abnormal forms of surface glycoproteins and glycolipids, which may be diagnostic markers and targets for therapy. These altered molecules include gangliosides, blood group antigens, and mucins. Some aspects of the malignant phenotype of tumors, including tissue invasion and metastatic behavior, may reflect altered cell surface properties that result from abnormal glycolipid and glycoprotein synthesis. Many antibodies have been raised in animals that recognize the carbohydrate groups or peptide cores of these molecules. Although most of the epitopes recognized by these antibodies are not specifically expressed on tumors, they are present at higher levels on cancer cells than on normal cells. This class of tumor-associated antigen is a target for cancer therapy with specific antibodies.

Gangliosides, including GM_2, GD_2, and GD_3, are glycolipids expressed at high levels in neuroblastomas, melanomas, and many sarcomas. Because of the tumor-selective expression of these molecules, they are an attractive target for tumor-specific therapies such as antibody therapy. Clinical trials of anti-ganglioside antibodies and immunization with ganglioside vaccines are under way in patients with melanoma. Mucins are high-molecular-weight glycoproteins containing numerous O-linked carbohydrate side chains on a core polypeptide. Tumors often have dysregulated expression of the enzymes that synthesize these carbohydrate side chains, which leads to the appearance of tumor-specific epitopes on the carbohydrate side chains or on the abnormally exposed polypeptide core. Several mucins have been the focus of diagnostic and therapeutic studies, including CA-125 and CA-19-9, expressed on ovarian carcinomas, and MUC-1, expressed on breast carcinomas. Unlike many mucins, MUC-1 is an integral membrane protein that is normally expressed only on the apical surface of breast ductal epithelium, a site that is relatively sequestered from the immune system. In ductal carcinomas of the breast, however, the molecule is expressed in an nonpolarized fashion and contains new, tumor-specific carbohydrate and peptide epitopes detectable by mouse monoclonal antibodies. The peptide epitopes induce both antibody and T cell responses in cancer patients, and efforts are under way to develop vaccines containing immunogenic forms of MUC-1 epitopes.

Tissue-Specific Differentiation Antigens

Tumors may express molecules that are present only on the normal cells of origin and not on cells from other tissues. These antigens are called differentiation antigens because they are specific for particular lineages or differentiation stages of various cell types. Their importance is as potential targets for immunotherapy and for identification of the tissue of origin of tumors. For example, several melanoma antigens that are targets of CTLs in patients are melanocyte differentiation antigens, such as tyrosinase, mentioned earlier. Lymphomas may be diagnosed as B cell–derived tumors by the detection of surface markers characteristic of this lineage, such as CD10 (previously called common acute lymphoblastic leukemia antigen, or CALLA) and CD20. Antibodies against these molecules are also used for tumor immunotherapy; the most successful immunotherapy for non-Hodgkin's B cell lymphomas is an anti-CD20 antibody (rituximab). The idiotypic determinants of the surface Ig of a clonal B cell population are markers for that B cell clone because all other B cells express different idiotypes. Therefore, the Ig idiotype is a highly specific tumor antigen for B cell lymphomas and leukemias. These differentiation antigens are normal self molecules, and therefore they do not usually induce strong immune responses in tumor-bearing hosts.

IMMUNE RESPONSES TO TUMORS

The effector mechanisms of both innate and adaptive immunity have been shown to kill tumor cells. The challenge for tumor immunologists is to determine which of these mechanisms may contribute to protection against tumors and to enhance these effector mechanisms in

ways that are tumor specific. In this section, we review the evidence for tumor killing by various immune effector mechanisms and discuss which are the most likely to be relevant to human tumors.

Innate Immune Responses to Tumors

Some of the early research on functions of effector cells of the innate immune system, including NK cells and macrophages, focused on the ability of these cells to kill cultured tumor cells.

NK Cells

NK cells kill many types of tumor cells, especially cells that have reduced class I MHC expression and express ligands for NK cell activating receptors. In vitro, NK cells can kill virally infected cells and certain tumor cell lines, especially hematopoietic tumors. NK cells also respond to the absence of class I MHC molecules because the recognition of class I MHC molecules delivers inhibitory signals to NK cells (see Chapter 4, Fig. 4-6). As we shall see later, some tumors lose expression of class I MHC molecules, perhaps as a result of selection against class I MHC–expressing cells by CTLs. This loss of class I MHC molecules makes the tumors particularly good targets for NK cells. Some tumors also express MIC-A, MIC-B, and ULB, which are ligands for the NKG2D activating receptor on NK cells. In addition, NK cells can be targeted to IgG antibody–coated tumor cells by Fc receptors (FcγRIII or CD16). The tumoricidal capacity of NK cells is increased by cytokines, including interferon-γ (IL-γ), IL-15, and IL-12, and the anti-tumor effects of these cytokines are partly attributable to stimulation of NK cell activity. IL-2–activated NK cells, called lymphokine-activated killer (LAK) cells, are derived by culture of peripheral blood cells or tumor-infiltrating lymphocytes from tumor patients with high doses of IL-2. These cells are more potent killers of tumors than are unactivated NK cells. The use of LAK cells in adoptive immunotherapy for tumors is discussed later.

The importance of NK cells in tumor immunity in vivo is unclear. In some studies, T cell–deficient mice do not have a high incidence of spontaneous tumors, and this is attributed to the presence of normal numbers of NK cells that serve an immune surveillance function. A few patients have been described with deficiencies of NK cells and an increased incidence of EBV-associated lymphomas.

Macrophages

Macrophages are capable of both inhibiting and promoting the growth and spread of cancers, depending on their activation state. Classically activated M1 macrophages, discussed in Chapter 10, display various anti-tumor functions. These cells can kill many tumor cells more efficiently than they can kill normal cells. How macrophages are activated by tumors is not known. Possible mechanisms include direct recognition of some surface antigens of tumor cells and activation of macrophages by IFN-γ produced by tumor-specific T cells. M1 macrophages can kill tumor cells by several mechanisms, probably the same as the mechanisms of macrophage killing of infectious organisms. These include the release of lysosomal enzymes, reactive oxygen species, and nitric oxide. M1 macrophages also produce the cytokine tumor necrosis factor (TNF), which was first characterized, as its name implies, as an agent that can kill tumors. We now know it does so mainly by inducing thrombosis in tumor blood vessels. In contrast, M2 macrophages may contribute to tumor progression. These cells secrete vascular endothelial growth factor (VEGF), transforming growth factor-β (TGF-β), and other soluble factors that promote tumor angiogenesis. The role of these cells and other components of the host response in enhancing tumor growth is discussed at the end of the chapter.

Adaptive Immune Responses to Tumors

Tumors elicit both T cell–mediated and humoral immune responses. T cells are the principal mediators of anti-tumor immunity, and this realization has led to considerable efforts to enhance T cell responses for the immunotherapy of cancers.

T Lymphocytes

The principal mechanism of adaptive tumor immunity is killing of tumor cells by $CD8^+$ CTLs. The ability of CTLs to provide effective anti-tumor immunity in vivo is most clearly seen in animal experiments using carcinogen-induced and DNA virus–induced tumors. As discussed previously, CTLs may perform a surveillance function by recognizing and killing potentially malignant cells that express peptides that are derived from tumor antigens and are presented in association with class I MHC molecules. The role of immune surveillance in preventing common, nonvirally induced tumors remains controversial because the frequency of such tumors in T cell–deficient people is not clearly greater than the frequency in immunocompetent individuals. However, tumor-specific CTLs can be isolated from animals and humans with established tumors, and there is evidence that the prognosis of some types of human tumors is better when more CTLs are present. Furthermore, mononuclear cells derived from the inflammatory infiltrate in human solid tumors, called tumor-infiltrating lymphocytes (TILs), contain CTLs with the capacity to kill the tumor from which they were derived.

$CD8^+$ T cell responses specific for tumor antigens may require cross-presentation of the tumor antigens by dendritic cells. Most tumor cells are not derived from APCs and therefore do not express the costimulators needed to initiate T cell responses or the class II MHC molecules needed to stimulate helper T cells that promote the differentiation of $CD8^+$ T cells. A likely explanation for how T cell responses to tumors are initiated is that tumor cells or their antigens are ingested by host APCs, particularly dendritic cells, and tumor antigens are processed inside the APCs. Peptides derived from these antigens are then displayed bound to class I MHC molecules for recognition by $CD8^+$ T cells. The APCs express costimulators that provide the signals needed for differentiation of $CD8^+$ T cells into anti-tumor CTLs, and the APCs express class II MHC molecules that may present internalized tumor antigens and activate $CD4^+$ helper T cells as well (Fig. 17-4). This process of cross-presentation, or

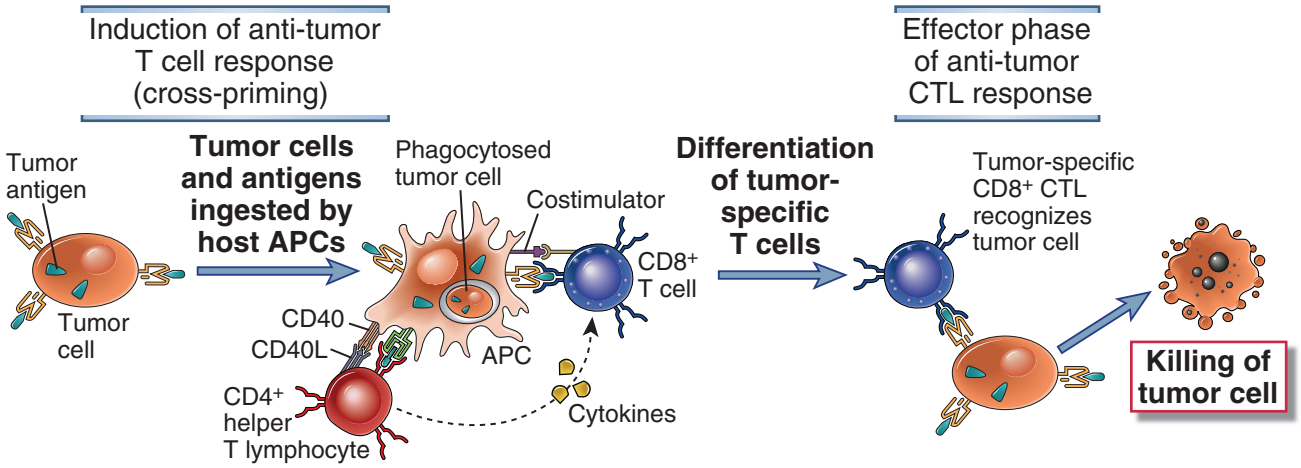


FIGURE 17–4 Induction of T cell responses to tumors. $CD8^+$ T cell responses to tumors may be induced by cross-priming (cross-presentation), in which the tumor cells or tumor antigens are taken up, processed, and presented to T cells by professional antigen-presenting cells (APCs). In some cases, B7 costimulators expressed by the APCs provide the second signals for differentiation of $CD8^+$ T cells. The APCs may also stimulate $CD4^+$ helper T cells, which provide the second signals for CTL development. Differentiated CTLs kill tumor cells without a requirement for costimulation or T cell help. (The roles of cross-presentation and $CD4^+$ helper T cells in CTL responses are discussed in Chapters 6 and 9.)

cross-priming, has been described in earlier chapters (see Chapter 6, Fig. 6-20). Once effector CTLs are generated, they are able to recognize and kill the tumor cells without a requirement for costimulation. A practical application of the concept of cross-priming is to grow dendritic cells from a patient with cancer, incubate the APCs with the cells or antigens from that patient's tumor, and use these antigen-pulsed APCs as vaccines to stimulate anti-tumor T cell responses.

The importance of $CD4^+$ helper T cells in tumor immunity is less clear. $CD4^+$ cells may play a role in anti-tumor immune responses by providing cytokines for effective CTL development (see Chapter 9). In addition, helper T cells specific for tumor antigens may secrete cytokines, such as TNF and IFN-γ, that can increase tumor cell class I MHC expression and sensitivity to lysis by CTLs. IFN-γ may also activate macrophages to kill tumor cells. The importance of IFN-γ in tumor immunity is demonstrated by the finding of increased incidence of tumors in knockout mice lacking this cytokine, the IFN-γ receptor, or components of the IFN-γ receptor signaling cascade.

Antibodies

Tumor-bearing hosts may produce antibodies against various tumor antigens. For example, patients with EBV-associated lymphomas have serum antibodies against EBV-encoded antigens expressed on the surface of the lymphoma cells. Antibodies may kill tumor cells by activating complement or by antibody-dependent cell-mediated cytotoxicity, in which Fc receptor–bearing macrophages or NK cells mediate the killing. However, the ability of antibodies to eliminate tumor cells has been demonstrated largely in vitro, and there is little evidence for effective humoral immune responses against tumors. Some effective therapeutic anti-tumor antibodies that are passively administered to patients likely work by antibody-dependent cell mediated cytotoxicity, as discussed later.

EVASION OF IMMUNE RESPONSES BY TUMORS

Many cancers develop mechanisms that allow them to evade anti-tumor immune responses. These mechanisms can broadly be divided into those that are intrinsic to the tumor cells and those that are mediated by other cells (Fig. 17-5). A major focus of tumor immunology is to understand the immune evasion mechanisms of tumors, with the hope that interventions to prevent immune evasion will increase the immunogenicity of tumors and maximize the responses of the host. Experimental evidence in mouse models indicates that immune responses to tumor cells impart selective pressures that result in the survival and outgrowth of variant tumor cells with reduced immunogenicity, a process that has been called tumor editing. For example, when tumors are induced by carcinogen treatment in either immunodeficient or immunocompetent mice and the tumors are then transplanted into new immunocompetent mice, the tumors that were derived from the immunodeficient mice are more frequently rejected by the recipient animal's immune system than are the tumors derived from the immunocompetent mice. This result indicates that tumors developing in the setting of a normal immune system become less immunogenic over time, which is consistent with selection of less immunogenic variant cells. Tumor immunoediting is thought to underlie the emergence of tumors that "escape" immune surveillance. We will now discuss the intrinsic and extrinsic tumor mechanisms that may underlie editing and escape.

Intrinsic Mechanisms of Immune Evasion by Tumor Cells

Several properties of tumor cells enable them to escape host defenses.

- ***Tumors may lose expression of antigens that elicit immune responses.*** Such "antigen loss variants" are

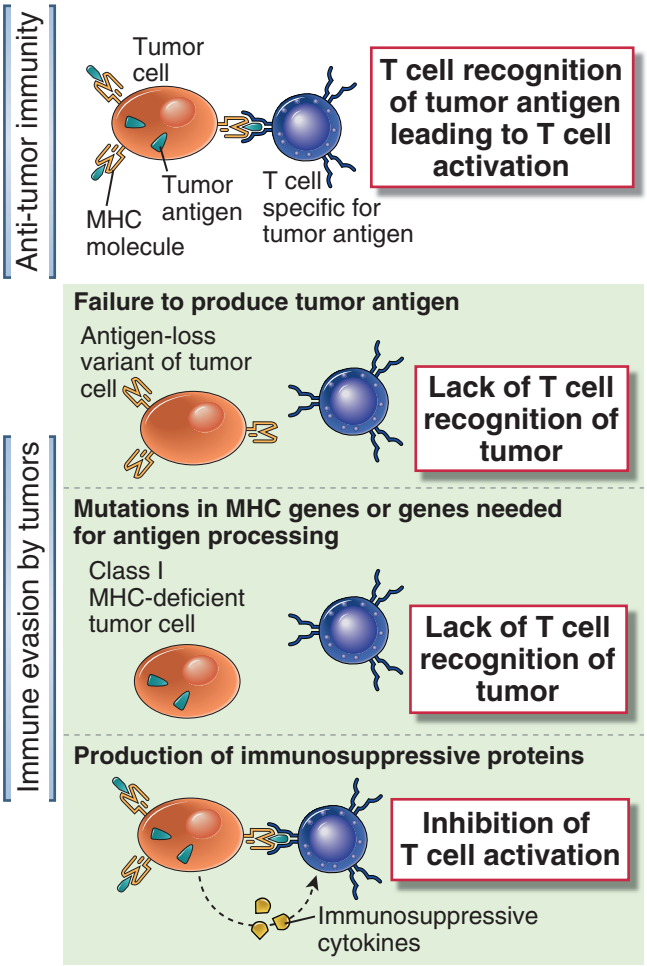


FIGURE 17–5 Mechanisms by which tumors escape immune defenses. Anti-tumor immunity develops when T cells recognize tumor antigens and are activated. Tumor cells may evade immune responses by losing expression of antigens or MHC molecules or by producing immunosuppressive cytokines.

common in rapidly growing tumors and can readily be induced in tumor cell lines by culture with tumor-specific antibodies or CTLs. Given the high mitotic rate of tumor cells and their genetic instability, mutations or deletions in genes encoding tumor antigens are common. If these antigens are not required for growth of the tumors or maintenance of the transformed phenotype, the antigen-negative tumor cells have a growth advantage in the host. Analysis of tumors that are serially transplanted from one animal to another has shown that the loss of antigens recognized by tumor-specific CTLs correlates with increased growth and metastatic potential. Apart from tumor-specific antigens, class I MHC expression may be downregulated on tumor cells so that they cannot be recognized by CTLs. Various tumors show decreased synthesis of class I MHC molecules, β_2-microglobulin, or components of the antigen-processing machinery, including the transporter associated with antigen processing and some subunits of the proteasome. These mechanisms are presumably adaptations of the tumors that arise in response to the selection pressures of host immunity, and they may allow tumor cells to evade T cell–mediated immune responses. However, there is not a clear correlation between the level of MHC expression on a broad range of experimental or human tumor cells and the in vivo growth of these cells.

- ***Tumor antigens may be inaccessible to the immune system.*** The cell surface antigens of tumors may be hidden from the immune system by glycocalyx molecules, such as sialic acid–containing mucopolysaccharides. This process is called antigen masking and may be a consequence of the fact that tumor cells often express more of these glycocalyx molecules than normal cells do.
- ***Tumors may fail to induce strong effector T cell responses because most tumor cells do not express costimulators or class II MHC molecules.*** Costimulators are required for initiation of T cell responses, and class II molecules are needed for the activation of helper T cells, which in some circumstances are required for the differentiation of CTLs. Therefore, the induction of tumor-specific T cell responses often requires cross-priming by dendritic cells, which do express costimulators and class II molecules. If such APCs do not adequately take up and present tumor antigens and activate helper T cells, CTLs specific for the tumor cells may not develop. Tumor cells transfected with genes encoding the costimulators B7-1 (CD80) and B7-2 (CD86) are able to elicit strong cell-mediated immune responses. Predictably, CTLs induced by B7-transfected tumors are effective against the parent (B7-negative) tumor as well because the effector phase of CTL-mediated killing does not require costimulation (see Fig. 17-4). As we shall see later, these experimental results are being extended to the clinical situation as immunotherapy for tumors.
- ***Tumors may engage molecules that inhibit immune responses.*** There is good experimental evidence that T cell responses to some tumors are inhibited by the involvement of CTLA-4 or PD-1, two of the best-defined inhibitory pathways in T cells (see Chapter 14). A possible reason for this role of CTLA-4 is that tumor antigens are presented by APCs in the absence of strong innate immunity and thus with low levels of B7 costimulators. These low levels may be enough to engage the high-affinity receptor CTLA-4. PD-L1, a B7 family protein that binds to the T cell inhibitory receptor PD-1 (see Chapter 14), is expressed on many human tumors, and animal studies indicate that anti-tumor T cell responses are compromised by PD-L1 expression. PD-L1 on APCs may also be involved in inhibiting tumor-specific T cell activation. As we will discuss later, there are ongoing clinical trials of blockade of the CTLA-4 and PD-L1/PD-1 pathways to enhance tumor immunity. Some tumors express Fas ligand (FasL), which recognizes the death receptor Fas on leukocytes that attempt to attack the tumor; engagement of Fas by FasL may result in apoptotic death of the leukocytes. The importance of this mechanism of tumor escape is not established because FasL has been detected on only a few spontaneous tumors, and when it is expressed in tumors by gene transfection, it is not always protective.

- ***Secreted products of tumor cells may suppress anti-tumor immune responses.*** An example of an immunosuppressive tumor product is TGF-β, which is secreted in large quantities by many tumors and inhibits the proliferation and effector functions of lymphocytes and macrophages (see Chapter 10).

Extrinsic Cellular Suppression of Anti-Tumor Immunity

Several cell populations have been described in tumor-bearing patients and animals that suppress anti-tumor immunity.

- ***Tumor-associated macrophages may promote tumor growth and invasiveness by altering the tissue microenvironment and by suppressing T cell responses.*** These macrophages have an M2 phenotype, as we discussed briefly earlier in the chapter, and they secrete mediators, such as IL-10, prostaglandin E_2, and arginase, that impair T cell activation and effector functions. Tumor-associated macrophages also secrete factors that promote angiogenesis, such as TGF-β and VEGF, which enhances tumor growth.
- ***Regulatory T cells may suppress T cell responses to tumors.*** Evidence from mouse model systems and cancer patients indicates that the numbers of regulatory T cells are increased in tumor-bearing individuals, and these cells can be found in the cellular infiltrates in certain tumors. Depletion of regulatory T cells in tumor-bearing mice enhances anti-tumor immunity and reduces tumor growth.
- ***Myeloid-derived suppressor cells (MDSCs) are immature myeloid precursors that are recruited from the bone marrow and accumulate in lymphoid tissues, blood, or tumors of tumor-bearing animals and cancer patients and suppress anti-tumor innate and T cell responses.*** MDSCs are a heterogeneous group of cell types, including precursors of dendritic cells, monocytes, and neutrophils. They share some common surface markers, including Ly6C or Ly6G and CD11b in mice and CD33, CD11b, and CD15 in humans. Recruitment of MDSCs from the bone marrow into lymph nodes and other tissues is induced by various proinflammatory mediators produced by tumors. These mediators, which include prostaglandin E_2, IL-6, VEGF, and complement fragment C5a, are not specific to tumors, and in fact, MDSCs accumulate at sites of chronic inflammation unrelated to tumors. MDSCs suppress innate immune responses by secreting IL-10, which inhibits various macrophage inflammatory functions. MDSCs suppress T cell responses by a variety of mechanisms. They express arginase and inducible nitric oxide synthase, which work together in generating reactive oxygen species, such as peroxynitrite, that inhibit T cell activation. MDSCs also produce indolamine 2,3-dioxygenase, which catabolizes tryptophan needed for T cell proliferation. MDSCs indirectly impair anti-tumor T cell responses by inducing the development of regulatory T lymphocytes (Tregs) and skewing helper T cell differentiation toward T_H2 cells.

Other host cells and mechanisms that may inhibit anti-tumor immunity are described later.

IMMUNOTHERAPY FOR TUMORS

The potential for treatment of cancer patients by immunologic approaches has held great promise for oncologists and immunologists for many years. The main reason for interest in an immunologic approach is that most current therapies for cancer rely on drugs that kill dividing cells or block cell division, and these treatments have severe effects on normal proliferating cells. As a result, the treatment of cancers causes significant morbidity and mortality. Immune responses to tumors may be specific for tumor antigens and will not injure most normal cells. Therefore, immunotherapy has the potential of being the most tumor-specific treatment that can be devised. Advances in our understanding of the immune system and in defining antigens on tumor cells have encouraged many new strategies. Immunotherapy for tumors aims to augment the weak host immune response to the tumors (active immunity) or to administer tumor-specific antibodies or T cells, a form of passive immunity. In this section, we describe some of the modes of tumor immunotherapy that have been tried in the past or are currently being investigated.

Stimulation of Active Host Immune Responses to Tumors

The earliest attempts to boost anti-tumor immunity relied on nonspecific immune stimulation. More recently, vaccines composed of killed tumor cells, tumor antigens, or dendritic cells incubated with tumor antigens have been administered to patients, and strategies to enhance immune responses against the tumor are being developed.

Vaccination with Tumor Antigens

Immunization of tumor-bearing individuals with tumor antigens may result in enhanced immune responses against the tumor (Table 17-2 and Fig. 17-6). The identification of peptides recognized by tumor-specific CTLs and the cloning of genes that encode tumor-specific antigens recognized by CTLs have provided many candidates for tumor vaccines; we have mentioned several examples earlier in the chapter. One of the earliest vaccine approaches, immunization with purified tumor antigens plus adjuvants, is still being tried. More recently, therapeutic dendritic cell vaccines have been used to immunize cancer patients against their own tumors. In this approach, dendritic cells that are purified from patients are either incubated with tumor antigens or transfected with genes encoding these antigens and then injected back into the patient. For example, a cell-based vaccine is now approved to treat advanced prostate cancer. This vaccine is composed of a preparation of a patient's peripheral blood leukocytes that is enriched for dendritic cells, which is exposed to a recombinant fusion protein consisting of granulocyte-macrophage colony-stimulating factor (GM-CSF) and the tumor-associated antigen prostatic acid

TABLE 17–2 Tumor Vaccines

Type of Vaccine	Vaccine Preparation	Animal Models	Clinical Trials
Killed tumor vaccine	Killed tumor cells + adjuvants Tumor cell lysates + adjuvants	Melanoma, colon cancer, others Sarcoma	Melanoma, colon cancer Melanoma
Purified tumor antigens	Melanoma antigens Heat shock proteins	Melanoma Various	Melanoma Melanoma, renal cancer, sarcoma
Dendritic cell–based vaccines	Dendritic cells pulsed with tumor antigens Dendritic cells transfected with genes encoding tumor antigens	Melanoma, B cell lymphoma, sarcoma Melanoma, colon cancer	Prostate carcinomas (approved), melanoma, non-Hodgkin's lymphoma, others Various carcinomas
Cytokine- and costimulator-enhanced vaccines	Tumor cells transfected with cytokine or B7 genes APCs transfected with cytokine genes and pulsed with tumor antigens	Renal cancer, sarcoma, B cell leukemia, lung cancer	Melanoma, sarcoma, others Melanoma, renal cancer, others
DNA vaccines	Immunization with plasmids encoding tumor antigens	Melanoma	Melanoma
Viral vectors	Adenovirus, vaccinia encoding tumor antigen ± cytokines	Melanoma, sarcoma	Melanoma, prostate carcinoma

phosphatase. An alternative approach in clinical trials is the use of DNA vaccines composed of plasmids of viral vectors encoding tumor antigens. The cell-based and DNA vaccines may be the best ways to induce CTL responses because the encoded antigens are synthesized in the cytoplasm and enter the class I MHC pathway of antigen presentation. For antigens that are unique to individual tumors, such as antigens produced by random point mutations in cellular genes, these vaccination methods are impractical because they would require identification of the antigens from every tumor. On the other hand, tumor antigens shared by many tumors, such as the MAGE, tyrosinase, and gp100 antigens on melanomas and mutated Ras and p53 proteins in various tumors, are potentially useful immunogens for all patients with certain types of cancer. A limitation of treating established tumors with vaccines is that these vaccines need to be therapeutic and not simply preventive, and it is often difficult to induce a strong enough immune response to eradicate all the cells of growing tumors.

The development of virally induced tumors can be blocked by preventive vaccination with viral antigens or attenuated live viruses. As mentioned earlier, newly developed HPV vaccines promise to reduce the incidence of HPV-induced tumors, including uterine cervical carcinoma. This approach has been extremely successful in reducing the incidence of feline leukemia virus–induced hematologic malignant tumors in cats and in preventing

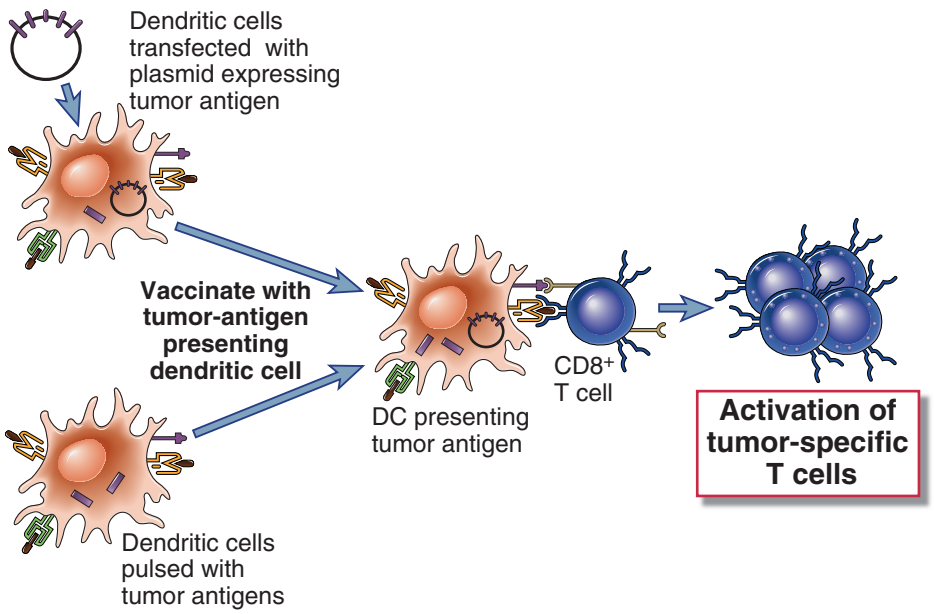


FIGURE 17–6 Tumor vaccines. Two types of tumor vaccines that have shown efficacy in clinical trials and animal models are illustrated. Autologous dendritic cells are prepared from patients' own peripheral blood cells. The dendritic cells are either pulsed with recombinant protein or transfected with a gene construct that expresses the protein. The construct may also express costimulatory molecules (not shown).

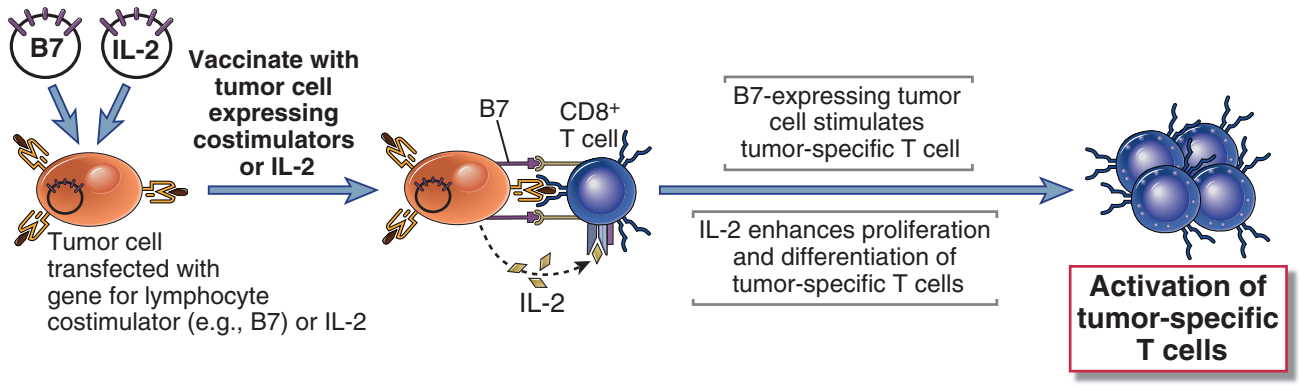


FIGURE 17–7 Enhancement of tumor cell immunogenicity by transfection of costimulator and cytokine genes. Tumor cells that do not adequately stimulate T cells on transplantation into an animal will not be rejected and will therefore grow into tumors. Vaccination with tumor cells transfected with genes encoding costimulators or cytokines, such as IL-2, can lead to enhanced activation of T cells. This approach of using transfected tumor cells as vaccines has worked in mouse models, but clinical trials have not yet been successful.

the herpesvirus-induced lymphoma called Marek's disease in chickens.

Augmentation of Host Immunity to Tumors with Costimulators and Cytokines

Cell-mediated immunity to tumors may be enhanced by expressing costimulators and cytokines in tumor cells and by treating tumor-bearing individuals with cytokines that stimulate the proliferation and differentiation of T lymphocytes and NK cells. As discussed earlier in this chapter, tumor cells may induce weak immune responses because they lack costimulators and usually do not express class II MHC molecules, so they do not activate helper T cells. Two potential approaches for boosting host responses to tumors are to artificially provide costimulation for tumor-specific T cells and to provide cytokines that can enhance the activation of tumor-specific T cells, particularly $CD8^+$ CTLs (Fig. 17-7). Many cytokines also have the potential to induce nonspecific inflammatory responses, which by themselves may have anti-tumor activity.

The efficacy of enhancing T cell costimulation for anti-tumor immunotherapy has been demonstrated by animal experiments in which tumor cells were transfected with genes that encode B7 costimulatory molecules and used to vaccinate animals. These B7-expressing tumor cells induce protective immunity against unmodified tumor cells injected at a distant site. The successes with experimental tumor models have led to therapeutic trials in which a sample of a patient's tumor is propagated in vitro, transfected with costimulator genes, irradiated, and reintroduced into the patient. Such approaches may succeed even if the immunogenic antigens expressed on tumors are not known.

It may be possible to use cytokines to enhance adaptive and innate immune responses against tumors. In mouse experiments, injection of live tumor cells transfected with cytokine genes (e.g., IL-2 GM-CSF) caused the rejection of established tumors. This approach has been attempted in cancer patients without success.

Cytokines may also be administered systemically for the treatment of various human tumors (Table 17-3). The largest clinical experience is with high-dose IL-2, which stimulates the production of other cytokines by T cells, such as TNF and IFN-γ, and these cytokines act on vascular endothelium and other cell types. IL-2 has been effective in inducing measurable tumor regression responses in about 10% of patients with advanced melanoma and renal cell carcinoma and is currently an approved therapy for these cancers. IFN-α is approved for treatment of malignant melanoma, in combination with chemotherapy, and carcinoid tumors. It is also used to treat certain lymphomas and leukemias. The mechanisms of the antineoplastic effects of IFN-α probably include inhibition of tumor cell proliferation, increased cytotoxic activity of NK cells, and increased class I MHC

TABLE 17–3 Systemic Cytokine Therapy for Tumors

Cytokine	Tumor Rejection in Animals	Clinical Trials	Toxicity
Interleukin-2	Yes	Melanoma, renal cancer, colon cancer; limited success (<15% response rate)	Vascular leak, shock, pulmonary edema
Interferon-α	No	Approved for melanoma, carcinoid tumors	Fever, fatigue
TNF	Only with local administration	Sarcoma, melanoma (isolated limb perfusion)	Septic shock syndrome
GM-CSF	No	In routine use to promote bone marrow recovery	Bone pain

GM-CSF, granulocyte-macrophage colony-stimulating factor; TNF, tumor necrosis factor.

expression on tumor cells, which makes them more susceptible to killing by CTLs (see Chapter 10). Other cytokines, such as TNF and IFN-γ, are effective anti-tumor agents in animal models, but their use in patients is limited by serious toxic side effects. Hematopoietic growth factors, including GM-CSF and G-CSF are used in cancer treatment protocols to shorten periods of neutropenia and thrombocytopenia after chemotherapy or autologous bone marrow transplantation.

Blocking Inhibitory Pathways to Promote Tumor Immunity

Another immunotherapeutic strategy is based on the idea we discussed earlier that tumor cells exploit various normal pathways of immune regulation or tolerance to evade the host immune response. One series of studies in mice and humans has targeted the inhibitory receptor for B7, CTLA-4, which functions normally to shut off responses against self antigens (see Chapter 14). Combination therapy with a tumor vaccine and an antibody that blocks CTLA-4 induces a strong anti-tumor T-cell response that destroys the tumor. Anti–CTLA-4 antibody has been used with some success in clinical trials with patients with advanced tumors. A common complication of this treatment has been the development of autoimmune reactions, which is predictable in light of the known role of CTLA-4 in maintaining self-tolerance. As we discussed earlier, T cell responses against tumors may also be inhibited by the PD-L1/PD-1 pathway. Antibody blockade of PD-1 is effective in enhancing T cell killing of tumors in mice, and human clinical trials using this approach are under way. The depletion of regulatory T cells may also enhance anti-tumor immunity, and this approach is being tested in animal models.

Nonspecific Stimulation of the Immune System

Immune responses to tumors may be stimulated by the local administration of inflammatory substances or by systemic treatment with agents that function as polyclonal activators of lymphocytes. Nonspecific immune stimulation of patients with tumors by injection of inflammatory substances such as bacillus Calmette-Guérin (BCG) at the sites of tumor growth has been tried for many years. The BCG mycobacteria activate macrophages and thereby promote macrophage-mediated killing of the tumor cells. In addition, the bacteria function as adjuvants and may stimulate T cell responses to tumor antigens. Intra-vesicular BCG is currently used to treat bladder cancer. Cytokine therapies, discussed before, represent another method of enhancing immune responses in a nonspecific manner.

Passive Immunotherapy for Tumors with T Cells and Antibodies

Passive immunotherapy involves the transfer of immune effectors, including tumor-specific T cells and antibodies, into patients. Passive immunization against tumors is rapid but does not lead to long-lived immunity. Some anti-tumor antibodies are now approved for the treatment of certain cancers. Several other approaches to passive immunotherapy are being tried, with variable success.

Adoptive Cellular Therapy

Adoptive cellular immunotherapy is the transfer of cultured immune cells that have anti-tumor reactivity into a tumor-bearing host. The cells to be transferred are expanded from the lymphocytes of patients with the tumor. One protocol for adoptive cellular immunotherapy is to generate LAK cells by culturing peripheral blood leukocytes from patients with the tumor in high concentrations of IL-2, and injecting the LAK cells back into the patients (Fig. 17-8). As discussed previously, LAK cells are derived mainly from NK cells. Adoptive therapy with autologous LAK cells, in conjunction with in vivo administration of IL-2 or chemotherapeutic drugs, has yielded impressive results in mice, with regression of solid tumors. Human LAK cell therapy trials have thus far been largely restricted to advanced cases of metastatic tumors, and the efficacy of this approach appears to vary from patient to patient. A variation of this approach is to isolate TILs from the inflammatory infiltrate present in

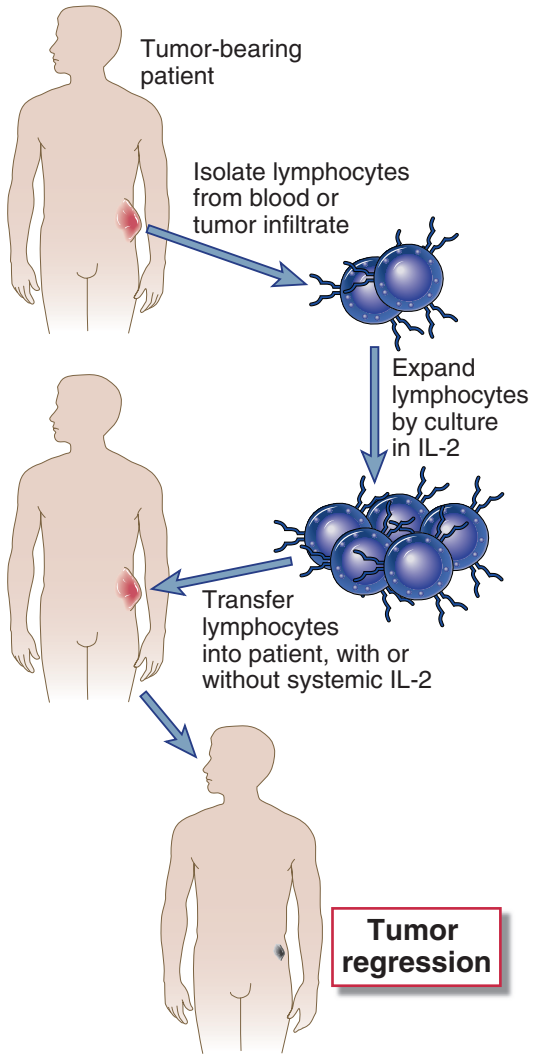


FIGURE 17–8 Adoptive cellular therapy. In a commonly used approach for adoptive cellular therapy, lymphocytes isolated from the blood or tumor infiltrate of a patient are expanded by culture in IL-2 and are infused back into the patient. This treatment, often combined with systemic IL-2 administration, leads to tumor regression in some patients.

and around solid tumors, obtained from surgical resection specimens, and to expand the TILs by culture in IL-2. The rationale for this approach is that TILs may be enriched for tumor-specific CTLs and for activated NK cells. TIL therapy for metastatic melanoma is now being used in many centers. Another approach for adoptive therapy now in development is T cell receptor (TCR) gene therapy in which a tumor patient's T cells are transduced in vitro with genes encoding a TCR specific for a tumor antigen, expanded, and then infused back into the patient.

Graft-Versus-Leukemia Effect

In leukemia patients, administration of alloreactive T cells together with hematopoietic stem cell transplants can contribute to eradication of the tumor. This graft-versus-leukemia effect is directed at the allogeneic MHC molecules present on the recipient's hematopoietic cells, including the leukemia cells. The challenge in use of this treatment to improve clinical outcome is to minimize the dangerous graft-versus-host disease that may be mediated by the same donor T cells (see Chapter 16).

Therapy with Anti-Tumor Antibodies

Tumor-specific monoclonal antibodies may be useful for specific immunotherapy for tumors. The potential of using antibodies as "magic bullets" has been alluring to investigators for many years and is still an active area of research. Currently, there are more than 100 different monoclonal antibodies being considered, either in experimental animal studies or in human trials, as therapeutic agents for cancer, and a few have been approved for clinical use (Table 17-4). Anti-tumor antibodies may eradicate tumors by the same effector mechanisms that are used to eliminate microbes, including opsonization and phagocytosis, activation of the complement system, and antibody-dependent cellular cytotoxicity (see Chapter 12). These mechanisms are likely at work in B cell lymphoma patients treated with anti-CD20, one of the most successful anti-tumor antibody treatments to date. In addition, some antibodies may directly activate intrinsic apoptosis pathways in tumor cells; this is the proposed mechanism for the use of anti-CD30 to treat lymphomas, currently in clinical trials. A monoclonal antibody (Herceptin) specific for the oncogene product HER2/Neu, is an approved treatment for breast cancer patients whose tumors express high levels of HER2/Neu. In addition to eliciting immune effector mechanisms, the anti–HER2/Neu antibody interferes with growth-signaling functions of the HER2/Neu molecule.

TABLE 17–4 Anti-Tumor Monoclonal Antibodies in Trials or Approved for Clinical Use

Specificity of Antibody	Form of Antibody Used	Clinical Use
HER2/Neu	Humanized mouse monoclonal	Breast cancer (approved)
CD20 (B cell marker)	Humanized mouse monoclonal	B cell lymphoma (approved)
CD25	Humanized mouse monoclonal, immunotoxin	T cell lymphomas/ leukemias (trials)
Carcinoembryonic antigen	Humanized mouse monoclonal	Gastrointestinal cancers, lung cancer (trials)
CA-125	Mouse monoclonal	Ovarian cancer
GD_3 ganglioside	Humanized mouse monoclonal	Melanoma (trials)

Because the anti-tumor antibodies used in the early human trials were mouse monoclonal antibodies, an immune response frequently occurred against the mouse Ig, resulting in anti–mouse Ig antibodies that caused increased clearance of the anti-tumor antibodies or blocked binding of the therapeutic agent to its target. This problem has been diminished by use of "humanized" antibodies consisting of the variable regions of a mouse monoclonal antibody specific for the tumor antigen combined with human Fc portions. One of the most difficult problems with the use of anti-tumor antibodies is the outgrowth of antigen loss variants of the tumor cells that no longer express the antigens that the antibodies recognize. One way to avoid this problem may be to use cocktails of antibodies specific for different antigens expressed on the same tumor.

Many variations on anti-tumor antibodies have been tried in attempts to improve their effectiveness. Tumor-specific antibodies may be coupled to toxic molecules, radioisotopes, and anti-tumor drugs to promote the delivery of these cytotoxic agents specifically to the tumor. Toxins such as ricin and diphtheria toxin are potent inhibitors of protein synthesis and can be effective at extremely low doses if they are carried to tumors attached to anti-tumor antibodies; such conjugates are called immunotoxins. This approach requires covalent coupling of the toxin (lacking its cell-binding component) to an anti-tumor antibody molecule without loss of toxicity or antibody specificity. The systemically injected immunotoxin is endocytosed by tumor cells, and the toxin part is delivered to its intracellular site of action. Several practical difficulties must be overcome for this technique to be successful. The specificity of the antibody must be such that it does not bind to non-tumor cells. A sufficient amount of antibody must reach the appropriate tumor target before it is cleared from the blood by Fc receptor–bearing phagocytic cells. The toxins, drugs, or radioisotopes attached to the antibody may have systemic effects as a result of circulation through normal tissues. For example, hepatotoxicity and vascular leak syndromes are common problems with immunotoxin therapy. Administration of immunotoxins may result in antibody responses against the toxins and the injected antibodies. Because of these practical difficulties, clinical trials of immunotoxins have had variable and modest success.

Anti-idiotypic antibodies have been used to treat B cell lymphomas that express surface Ig with particular idiotypes. The idiotype is a highly specific tumor antigen because it is expressed only on the neoplastic clone of B cells, and it was once hoped that anti-idiotypic antibodies would be effective therapeutic reagents with absolute tumor specificity. (Anti-idiotypic antibodies are raised by

immunizing animals with a patient's B cell tumor and depleting the serum of reactivity against all other human immunoglobulins.) The approach has not proved generally successful, largely because of the selective outgrowth of tumor cells with altered idiotypes that are not recognized by the anti-idiotypic antibody. In part, this result may reflect the high rate of somatic mutation in Ig genes and the fact that the surface Ig is dispensable for tumor growth.

Tumor growth is usually dependent on growth factors, which are potential targets for therapy. Antibodies that block the epidermal growth factor receptor are approved for the treatment of colorectal tumors. Tumors depend on the formation of new blood vessels that supply the tumor with oxygen and nutrients. This process, called tumor angiogenesis, is dependent on other specialized growth factors, including VEGF. Various inhibitors of these angiogenic factors can block tumor growth. Anti-VEGF antibodies are now approved for clinical use, in combination with chemotherapeutic agents, to treat some metastatic tumors, although their efficacy is modest.

Anti-tumor antibodies are also used to remove cancer cells from the bone marrow before autologous marrow transplantation. In this protocol, some of the patient's bone marrow is removed, and the patient is given doses of radiation and chemotherapy lethal enough to destroy tumor cells as well as the remaining normal marrow cells. The bone marrow cells removed from the patient are treated with antibodies or immunotoxins specific for tumor antigens in order to kill most or all tumor cells. The treated marrow, having been purged of tumor cells, is transplanted back into the patient to reconstitute the hematopoietic system destroyed by irradiation and chemotherapy.

THE ROLE OF THE IMMUNE SYSTEM IN PROMOTING TUMOR GROWTH

Although much of the emphasis in tumor immunology has been on the role of the immune system in eradicating tumors, it is clear that the immune system may also contribute to the development of some solid tumors. In fact, chronic inflammation has long been recognized as a risk factor for development of tumors in many different tissues, especially those affected by chronic inflammatory diseases such as Barrett's esophagus, Crohn's disease, pancreatitis, and prostatitis, for example. Some cancers associated with infections are also considered to be an indirect result of the carcinogenic effects of the chronic inflammatory states that are induced by the infectious organisms. These include gastric cancer in the setting of chronic *Helicobacter pylori* infection and hepatocellular carcinomas associated with chronic hepatitis B and C virus infections. Although the mechanisms by which chronic inflammation can promote tumor development are not well understood, there are several possibilities, supported by data in rodent models. Cells of the innate immune system are considered the most direct tumor-promoting culprits among immune cells. Tumor-associated macrophages of the M2 phenotype as well as other cells are sources of VEGF, which promotes angiogenesis, and matrix metalloproteinases, which modify the extracellular tissue. Therefore, chronic activation of some innate immune cells is characterized by angiogenesis and tissue remodeling, which favor tumor formation and spread. Innate immune cells may also contribute to malignant transformation of cells by generating free radicals that cause DNA damage and lead to mutations in tumor suppressor genes and oncogenes. Some data suggest that cells of the innate immune system, including mast cells, neutrophils, and macrophages, secrete soluble factors that promote cell cycle progression and survival of tumor cells. The transcription factor NF-κB, which is a key mediator of innate immune responses, may play an important role in inflammation-associated cancer progression. The adaptive immune system can promote chronic activation of innate immune cells in several ways, including T cell–mediated activation of macrophages in the setting of persistent intracellular microbial infections as well as during early malignant disease even when infectious agents are not present. There is also experimental evidence that B lymphocytes may contribute to tumor progression by their secretion of factors that directly regulate proliferation programs in tumor cells as well as by their ability to chronically activate innate immune cells present in early tumors. Thus, the adaptive immune system may indirectly enhance the tumor-promoting activities of the innate immune system. The tumor-promoting effects of the immune system are paradoxical and a topic of active investigation at present. These effects of chronic inflammation are theoretically also excellent targets for pharmacologic intervention because there are a large variety of effective anti-inflammatory drugs already available. The challenge for oncologists is to achieve a beneficial balance in which protective anti-tumor adaptive immune responses are not compromised while potentially harmful chronic inflammatory reactions are controlled.

SUMMARY

- Tumors express antigens that are recognized by the immune system, but most tumors are weakly immunogenic, and immune responses often fail to prevent the growth of tumors. The immune system can be stimulated to effectively kill tumors.
- Tumor antigens recognized by CTLs are the principal inducers of and targets for anti-tumor immunity. These antigens include mutants of oncogenes and other cellular proteins, normal proteins whose expression is dysregulated or increased in tumors, and products of oncogenic viruses.
- Antibodies specific for tumor cells recognize antigens that are used for diagnosis and are potential targets for antibody therapy. These antigens include oncofetal antigens, which are expressed normally during fetal life and whose expression is dysregulated in some tumors; altered surface glycoproteins and glycolipids; and molecules that are normally expressed on the cells from which the tumors arise and are thus differentiation antigens for particular cell types.

- Immune responses that are capable of killing tumor cells consist of CTLs, NK cells, and activated macrophages. The role of these immune effector mechanisms in protecting individuals from tumors is not well defined.
- Tumors evade immune responses by several mechanisms, including downregulation of expression of MHC molecules, outgrowth of cells that do not express tumor antigens, production of immunosuppressive substances, and induction of tolerance to tumor antigens. Tumor-associated macrophages and myeloid-derived suppressor cells, found in most solid tumors, suppress antitumor immunity.
- Immunotherapy for tumors is designed to augment active immune responses against these tumors or to administer tumor-specific immune effectors to patients. Immune responses may be actively enhanced by vaccination with tumor cells or antigens, administration of tumors modified to express high levels of costimulators or cytokines that stimulate T cell proliferation and differentiation, and systemic administration of cytokines. Antitumor immunity may also be enhanced by blocking inhibitory pathways of immunoregulation. Approaches for passive immunotherapy include the administration of anti-tumor antibodies, antibodies conjugated with toxic drugs (immunotoxins), and tumor-reactive T cells and NK cells isolated from patients and expanded by culture with growth factors.

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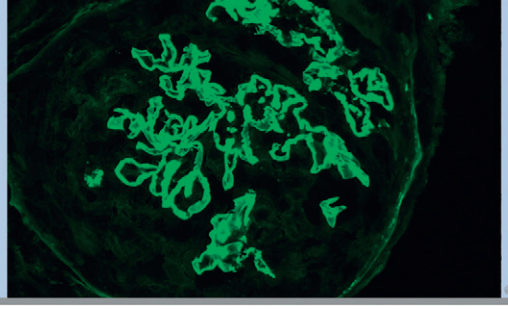
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CHAPTER

18

Hypersensitivity Disorders

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Adaptive immunity serves the important function of host defense against microbial infections, but immune responses are also capable of causing tissue injury and disease. Disorders caused by immune responses are called **hypersensitivity diseases**. This term arose from the clinical definition of immunity as "sensitivity," which is based on the observation that an individual who has been exposed to an antigen exhibits a detectable reaction or is "sensitive" to subsequent encounters with that antigen. Normally, immune responses eradicate infecting organisms without serious injury to host tissues. However, these responses are sometimes inadequately controlled, inappropriately targeted to host tissues, or triggered by commensal microorganisms or environmental antigens that are usually harmless. In these situations, the normally beneficial immune response is the cause of disease.

In this chapter, we describe the pathogenesis of different types of hypersensitivity diseases, with an emphasis on the effector mechanisms that cause tissue injury. We conclude with a brief consideration of the treatment of immunologic diseases and examples of diseases that illustrate important principles.

CAUSES OF HYPERSENSITIVITY DISEASES

Immune responses against antigens from different sources can be the underlying cause of hypersensitivity disorders.

- ***Autoimmunity.*** Failure of the normal mechanisms of self-tolerance results in reactions against one's own cells and tissues that are called **autoimmunity** (see Chapter 14). The diseases caused by autoimmunity are referred to as **autoimmune diseases**. Autoimmune diseases are estimated to affect 2% to 5% of the population in developed countries, and the incidence of these disorders is rising. Many of these diseases are common in individuals in the 20- to 40-year age group. They are also more common in women than in men, for reasons that remain obscure. Autoimmune diseases are chronic and debilitating and an enormous medical and economic burden. In the early part of the 21st century, many new treatments for these disorders have been developed based on scientific principles; these are among the most impressive successes in medicine. The mechanisms of autoimmunity were described in Chapter 14; in this chapter, we will refer to various autoimmune diseases to illustrate how autoimmunity can cause disease.
- ***Reactions against microbes.*** Immune responses against microbial antigens may cause disease if the reactions are excessive or the microbes are unusually persistent. T cell responses against persistent microbes may give

rise to severe inflammation, sometimes with the formation of granulomas; this is the cause of tissue injury in tuberculosis and some other chronic infections. If antibodies are produced against microbial antigens, the antibodies may bind to the antigens to produce immune complexes, which deposit in tissues and trigger inflammation. Rarely, antibodies or T cells against a microbe may cross-react with a host tissue. In some diseases involving the intestinal tract, called inflammatory bowel disease, the immune response is directed against commensal bacteria that normally reside in the gut and cause no harm. Sometimes the disease-causing immune response may be entirely normal, but in the process of eradicating the infection, host tissues are injured. In viral hepatitis, the virus that infects liver cells is not cytopathic, but it is recognized as foreign by the immune system. Cytotoxic T lymphocytes (CTLs) try to eliminate infected cells, and this normal immune response damages liver cells. This type of normal reaction is not considered hypersensitivity.

- ***Reactions against environmental antigens.*** Most healthy individuals do not react against common, generally harmless environmental substances, but almost 20% of the population is abnormally responsive to one or more of these substances. These individuals produce immunoglobulin E (IgE) antibodies that cause allergic diseases (see Chapter 19). Some individuals become sensitized to environmental antigens and chemicals that contact the skin and develop T cell reactions that lead to cytokine-mediated inflammation, resulting in contact sensitivity.

In all these conditions, the mechanisms of tissue injury are the same as those that normally function to eliminate infectious pathogens. These mechanisms include innate immune responses, T lymphocytes, various other effector cells, and mediators of inflammation. The problem in hypersensitivity diseases is that the response is triggered and maintained inappropriately. Because the stimuli for these abnormal immune responses are difficult or impossible to eliminate (e.g., self antigens, commensal microbes, and environmental antigens) and the immune system has many built-in positive feedback loops (amplification mechanisms), once a pathologic immune response starts, it is difficult to control or to terminate it. Therefore, these hypersensitivity diseases tend to be chronic and progressive and are major therapeutic challenges in clinical medicine.

MECHANISMS AND CLASSIFICATION OF HYPERSENSITIVITY REACTIONS

Hypersensitivity diseases are commonly classified according to the type of immune response and the effector mechanism responsible for cell and tissue injury (Table 18-1). This classification was originally developed by two British immunologists, Philip Gell and Robin Coombs.

- Immediate hypersensitivity (type I hypersensitivity) caused by IgE antibodies specific for environmental antigens and mast cells is the most prevalent type of hypersensitivity disease and will be described separately in Chapter 19. Immediate hypersensitivity diseases, commonly called allergic or atopic disorders, are the prototypes of diseases caused by activation of the T_H2 subset of helper T cells, in which the T cells stimulate the production of IgE antibodies and inflammation.
- IgG and IgM antibodies can cause tissue injury by activating the complement system, by recruiting inflammatory cells, and by interfering with normal cellular functions. Some of these antibodies are specific for antigens of particular cells or the extracellular matrix and are found either attached to these cells or tissues or as unbound antibodies in the circulation; the diseases induced by such antibodies are called type II hypersensitivity disorders. Other antibodies may form immune complexes in the circulation, and the complexes are subsequently deposited in tissues, particularly in blood vessels, and cause injury. Immune complex diseases are also called type III hypersensitivity disorders.
- Tissue injury may be due to T lymphocytes that induce inflammation or directly kill target cells; such conditions are called type IV hypersensitivity disorders. We

TABLE 18–1 Classification of Immunologic Diseases

Type of Hypersensitivity	Pathologic Immune Mechanisms	Mechanisms of Tissue Injury and Disease
Immediate hypersensitivity: type I	IgE antibody	Mast cells and their mediators (vasoactive amines, lipid mediators, cytokines)
Antibody mediated: type II	IgM, IgG antibodies against cell surface or extracellular matrix antigens	Opsonization and phagocytosis of cells Complement- and Fc receptor–mediated recruitment and activation of leukocytes (neutrophils, macrophages) Abnormalities in cellular functions, e.g., hormone receptor signaling
Immune complex mediated: type III	Immune complexes of circulating antigens and IgM or IgG antibodies	Complement- and Fc receptor–mediated recruitment and activation of leukocytes
T cell mediated: type IV	$CD4^+$ T cells (cytokine-mediated inflammation) $CD8^+$ CTLs (T cell–mediated cytolysis)	Recruitment and activation of leukocytes Direct target cell killing, cytokine-mediated inflammation

now realize that many hypersensitivity diseases are caused by the activation of T_H1 or T_H17 subsets of helper T cells, which secrete cytokines that promote inflammation, and the tissue injury is caused by the recruited leukocytes, mainly neutrophils and macrophages. Helper T cells also stimulate the production of antibodies that damage tissues and induce inflammation. CTLs may also contribute to tissue injury in some diseases.

This classification is useful because distinct types of pathologic immune responses show different patterns of tissue injury and may vary in their tissue specificity. As a result, the different immunologic mechanisms cause disorders with distinct clinical and pathologic features. However, immunologic diseases in the clinical situation are often complex and caused by combinations of humoral and cell-mediated immune responses and multiple effector mechanisms. This complexity is not surprising given that a single antigen may normally stimulate both humoral and cell-mediated immune responses, in which several types of antibodies and effector T cells are produced. Because multiple mechanisms may be involved and inflammation, typically chronic inflammation, is a major component of the pathology and clinical manifestations of these disorders, they are sometimes grouped under the rubric **immune-mediated inflammatory diseases**. Considering these diseases together also has some clinical value because, as we shall discuss later in the chapter, many of them are treated with the same or related biologic agents. In the discussion that follows, we use descriptions that identify the pathogenic mechanisms rather than the less informative numerical designations for types of hypersensitivity.

With this background, we proceed to a discussion of antibody- and T cell-mediated diseases.

DISEASES CAUSED BY ANTIBODIES

Antibody-mediated diseases are produced either by antibodies that bind to antigens on particular cells or in extracellular tissues or by antigen-antibody complexes that form in the circulation and are deposited in vessel walls (Fig. 18-1). To prove that a disease is caused by antibodies, one would need to demonstrate that the lesions can be induced in a normal animal by the adoptive transfer of immunoglobulin purified from the blood or affected tissues of individuals with the disease. An experiment of nature is occasionally seen in children of mothers suffering from antibody-mediated diseases.

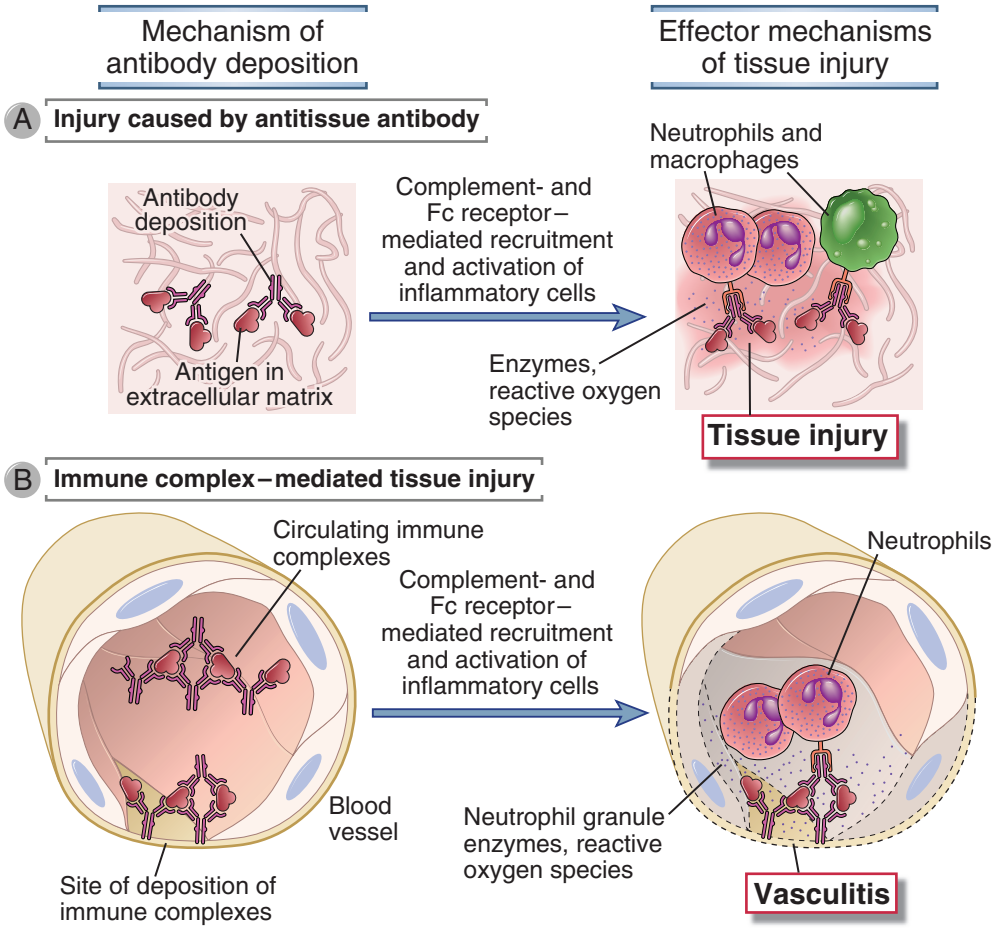


FIGURE 18–1 Types of antibody-mediated diseases. Antibodies may bind specifically to tissue antigens **(A)**, or they may be deposited as immune complexes that are formed in the circulation **(B)**. In both cases, the deposited antibodies induce inflammation, leading to tissue injury.

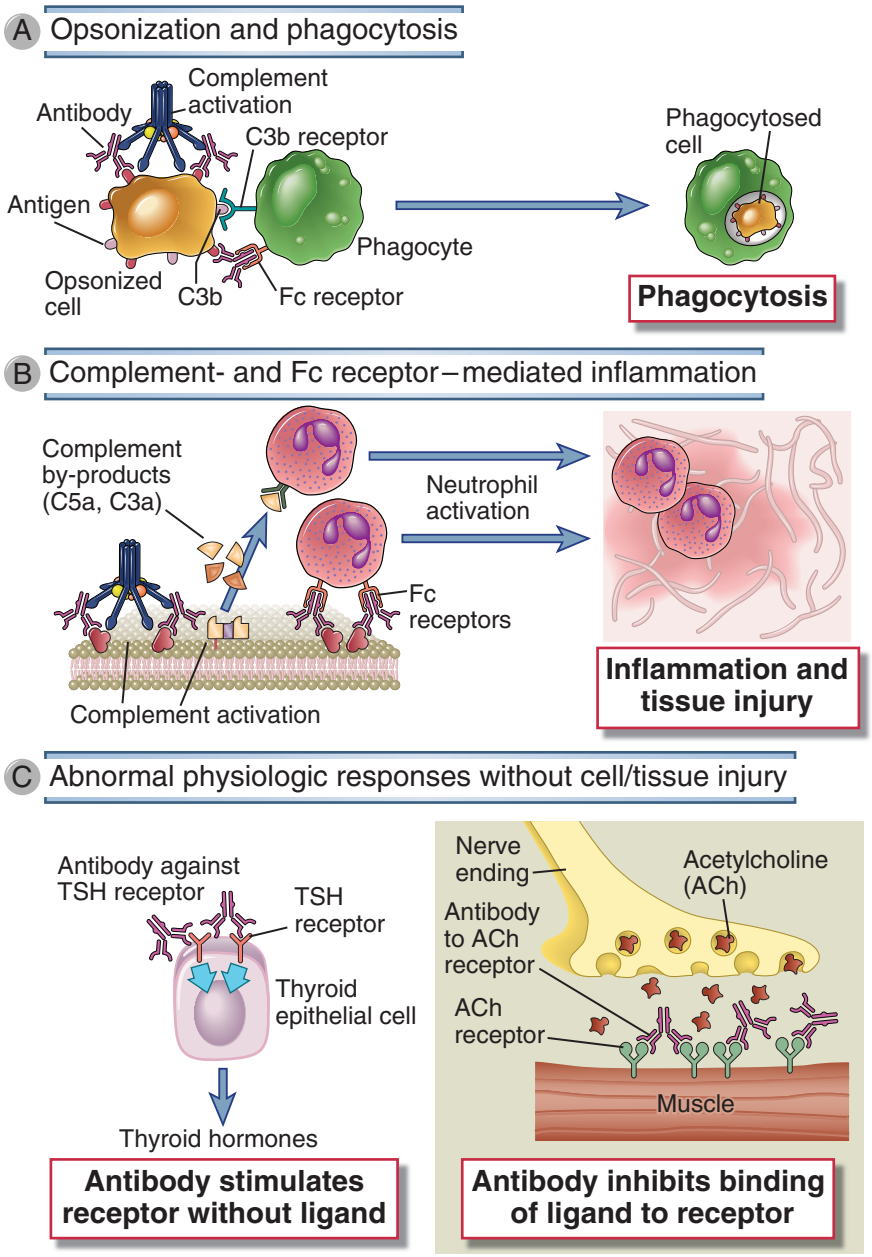


FIGURE 18–2 Effector mechanisms of antibody-mediated disease. A, Antibodies opsonize cells and may activate complement, generating complement products that also opsonize cells, leading to phagocytosis of the cells through phagocyte Fc receptors or C3 receptors. **B,** Antibodies recruit leukocytes by binding to Fc receptors or by activating complement and thereby releasing byproducts that are chemotactic for leukocytes. **C,** Antibodies specific for cell surface receptors for hormones or neurotransmitters may stimulate the activity of the receptors even in the absence of the hormone *(left panel)* or may inhibit binding of the neurotransmitter to its receptor *(right panel)*. TSH, thyroid-stimulating hormone.

These infants may be born with transient manifestations of such diseases because of transplacental passage of antibodies. However, in clinical situations, the diagnosis of diseases caused by antibodies or immune complexes is usually based on the demonstration of antibodies or immune complexes in the circulation or deposited in tissues as well as clinicopathologic similarities with experimental diseases that are proved to be antibody mediated by adoptive transfer.

Diseases Caused by Antibodies Against Fixed Cell and Tissue Antigens

Antibodies against cellular or matrix antigens cause diseases that specifically affect the cells or tissues where these antigens are present, and these diseases are often not systemic. Antibodies against tissue antigens cause disease by three main mechanisms (Fig. 18-2).

- Antibodies that bind to cell surface antigens may directly opsonize cells, or they may activate the complement system, resulting in the production of complement proteins that opsonize cells. These opsonized cells are phagocytosed and destroyed by phagocytes that express receptors for the Fc portions of IgG antibodies and receptors for complement proteins. This is the principal mechanism of cell destruction in autoimmune hemolytic anemia and autoimmune thrombocytopenic purpura. The same mechanism is responsible for hemolysis in transfusion reactions (see Chapter 16).

- Antibodies deposited in tissues recruit neutrophils and macrophages, which bind to the antibodies or to attached complement proteins by IgG Fc and complement receptors. These leukocytes are activated by signaling from the receptors, particularly Fc receptors, and leukocyte products, including lysosomal enzymes and reactive oxygen species, are secreted and cause tissue injury. The mechanism of injury in antibody-mediated glomerulonephritis and many other diseases is inflammation and leukocyte activation.
- Antibodies that bind to normal cellular receptors or other proteins may interfere with the functions of these receptors or proteins and cause disease without inflammation or tissue damage. Antibody-mediated functional abnormalities are the cause of Graves' disease and myasthenia gravis.

Antibodies that cause cell- or tissue-specific diseases are usually autoantibodies produced as part of an autoimmune reaction against antigens in these cells or tissues. Examples of these autoantibodies are listed in Table 18-2. Less commonly, the antibodies may be produced against a foreign (e.g., microbial) antigen that is immunologically cross-reactive with a component of self tissues. In a rare sequel of streptococcal infection called rheumatic fever, antibodies produced against the bacteria cross-react with antigens in the heart, deposit in this organ, and cause inflammation and tissue damage. Tissue deposits of antibodies may be detected by morphologic examination in some of these diseases, and the deposition of antibody is often associated with local complement activation, inflammation, and tissue injury (Fig. 18-3A).

Immune Complex–Mediated Diseases

Immune complexes that cause disease may be composed of antibodies bound to either self antigens or foreign antigens. The pathologic features of diseases caused by immune complexes reflect the site of immune complex deposition and are not determined by the cellular source of the antigen. Therefore, immune complex–mediated diseases tend to affect multiple tissues and organs, although some are particularly susceptible, such as kidneys and joints.

The occurrence of diseases caused by immune complexes was suspected as early as 1911 by an astute physician named Clemens von Pirquet. At that time, diphtheria infections were being treated with serum from horses immunized with the diphtheria toxin, which is an example of passive immunization against the toxin by the transfer of serum containing antitoxin antibodies. von Pirquet noted that joint inflammation (arthritis), rash, and fever developed in patients injected with the antitoxin-containing horse serum. Two clinical features of this reaction suggested that it was not due to the infection or a toxic component of the serum itself. First, these symptoms appeared even after the injection of horse

TABLE 18–2 Examples of Diseases Caused by Cell- or Tissue-Specific Antibodies

Disease	Target Antigen	Mechanisms of Disease	Clinicopathologic Manifestations
Autoimmune hemolytic anemia	Erythrocyte membrane proteins (Rh blood group antigens, I antigen)	Opsonization and phagocytosis of erythrocytes, complement-mediated lysis	Hemolysis, anemia
Autoimmune thrombocytopenic purpura	Platelet membrane proteins (gpIIb-IIIa integrin)	Opsonization and phagocytosis of platelets	Bleeding
Pemphigus vulgaris	Proteins in intercellular junctions of epidermal cells (desmoglein)	Antibody-mediated activation of proteases, disruption of intercellular adhesions	Skin vesicles (bullae)
Vasculitis caused by ANCA	Neutrophil granule proteins, presumably released from activated neutrophils	Neutrophil degranulation and inflammation	Vasculitis
Goodpasture's syndrome	Non-collagenous NC1 protein of basement membrane in glomeruli and lung	Complement- and Fc receptor–mediated inflammation	Nephritis, lung hemorrhage
Acute rheumatic fever	Streptococcal cell wall antigen; antibody cross-reacts with myocardial antigen	Inflammation, macrophage activation	Myocarditis, arthritis
Myasthenia gravis	Acetylcholine receptor	Antibody inhibits acetylcholine binding, downmodulates receptors	Muscle weakness, paralysis
Graves' disease (hyperthyroidism)	TSH receptor	Antibody-mediated stimulation of TSH receptors	Hyperthyroidism
Insulin-resistant diabetes	Insulin receptor	Antibody inhibits binding of insulin	Hyperglycemia, ketoacidosis
Pernicious anemia	Intrinsic factor of gastric parietal cells	Neutralization of intrinsic factor; decreased absorption of vitamin B_{12}	Abnormal erythropoiesis, anemia

ANCA, antineutrophil cytoplasmic antibodies; TSH, thyroid-stimulating hormone.

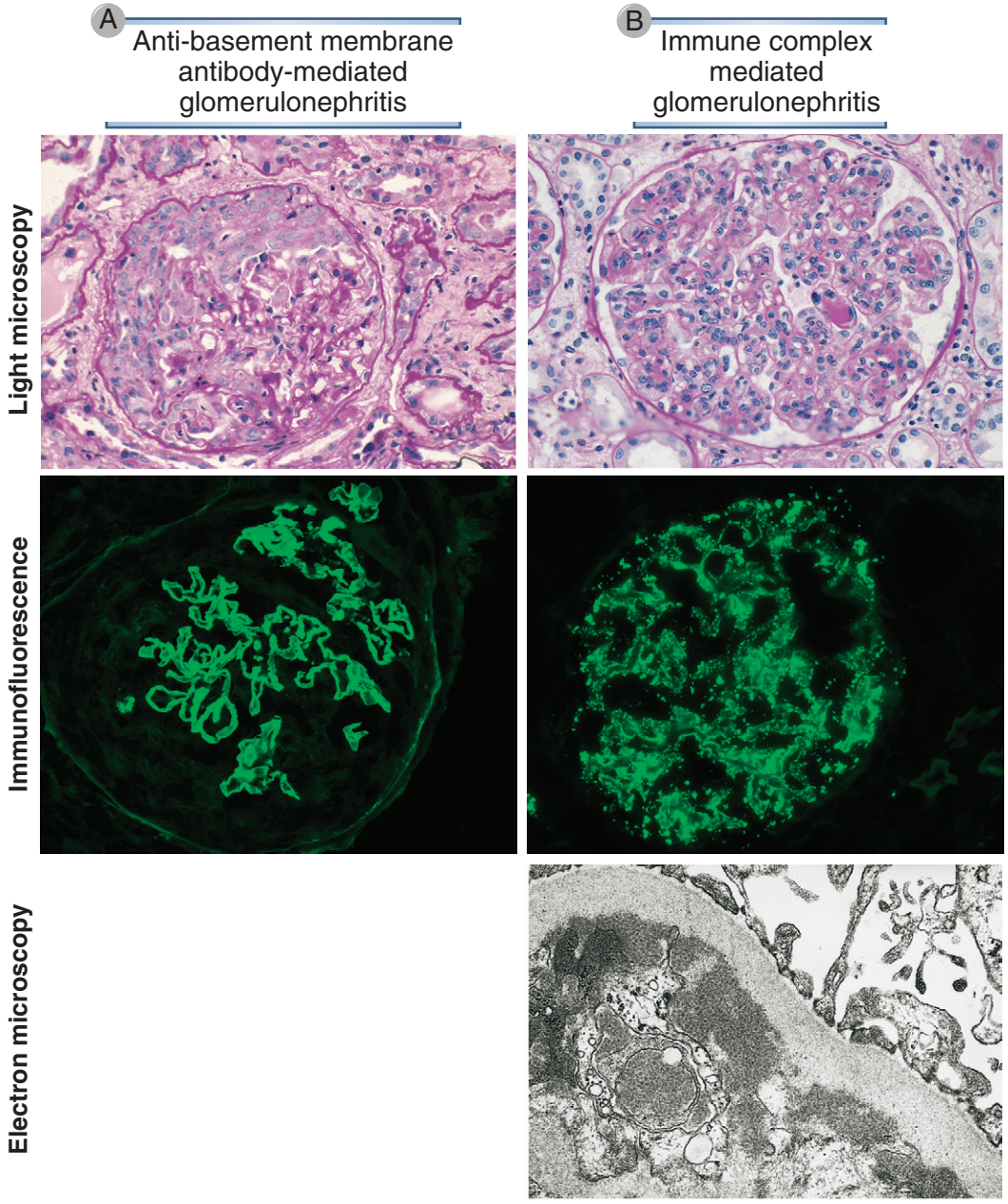


FIGURE 18–3 Pathologic features of antibody-mediated glomerulonephritis. A, Glomerulonephritis induced by an antibody against the glomerular basement membrane (Goodpasture's syndrome): the light micrograph shows glomerular inflammation and severe damage, and immunofluorescence shows smooth (linear) deposits of antibody along the basement membrane. **B,** Glomerulonephritis induced by the deposition of immune complexes (systemic lupus erythematosus): the light micrograph shows neutrophilic inflammation, and the immunofluorescence and electron micrograph show coarse (granular) deposits of antigen-antibody complexes along the basement membrane. *(Immunofluorescence micrographs are courtesy of Dr. Jean Olson, Department of Pathology, University of California, San Francisco, and the electron micrograph is courtesy of Dr. Helmut Rennke, Department of Pathology, Brigham and Women's Hospital, Boston Massachusetts.)*

serum not containing the antitoxin, so the lesions could not be attributed to the anti-diphtheria antibody. Second, the symptoms appeared at least a week after the first injection of horse serum and more rapidly with each repeated injection. von Pirquet concluded that this disease was due to a host response to some component of the serum. He suggested that the host made antibodies to horse serum proteins, these antibodies formed complexes with the injected proteins, and the disease was due to the antibodies or immune complexes. We now know that his conclusions were entirely accurate. He called this disease serum disease; it is now more commonly known as serum sickness and is the prototype for systemic immune complex–mediated disorders.

Experimental Models of Immune Complex–Mediated Diseases

Serum Sickness

Much of our current knowledge of immune complex diseases is based on analyses of experimental models of

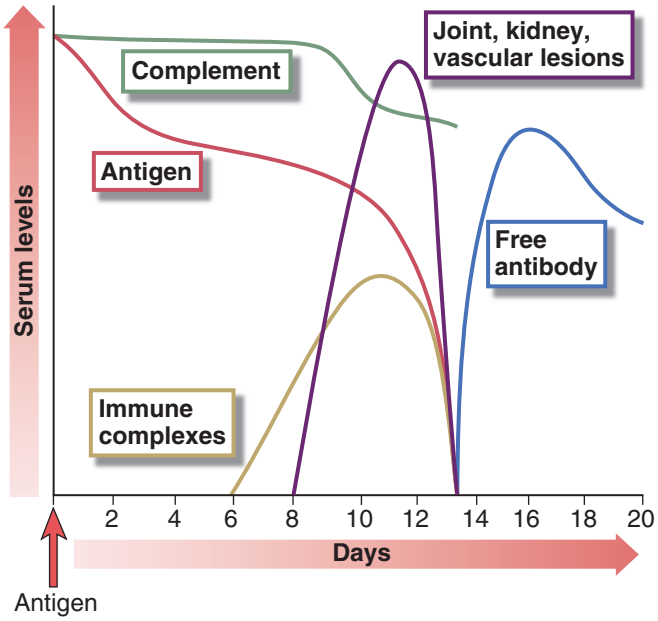


FIGURE 18–4 Sequence of immunologic responses in experimental acute serum sickness. Injection of bovine serum albumin into a rabbit leads to the production of specific antibody and the formation of immune complexes. These complexes are deposited in multiple tissues, activate complement (leading to a fall in serum complement levels), and cause inflammatory lesions, which resolve as the complexes and the remaining antigen are removed and free antibody (not bound to antigen) appears in the circulation *(Adapted from Cochrane CG. Immune complex–mediated tissue injury. In Cohen S, PA Ward, and RT McCluskey [eds]. Mechanisms of Immunopathology. Werbel & Peck, New York, 1979, pp 29-48.* ©1979, Wiley-Liss, Inc.)

serum sickness. Immunization of an animal such as a rabbit with a large dose of a foreign protein antigen leads to the formation of antibodies against the antigen (Fig. 18-4). These antibodies bind to and form complexes with circulating antigen, which are initially cleared by macrophages in the liver and spleen. As more and more antigen-antibody complexes are formed, some of them are deposited in vascular beds. In these tissues, the antibodies in the complexes may activate complement, with a concomitant fall in serum complement levels. Complement activation leads to recruitment and activation of inflammatory cells, predominantly neutrophils, at the sites of immune complex deposition, and the neutrophils cause tissue injury. Neutrophils also bind to the immune complexes by their Fcγ receptors, and Fc receptor signaling activates the leukocytes to produce substances that damage tissues, as in diseases caused by antibodies against fixed tissues. Because the complexes are deposited mainly in small arteries, renal glomeruli, and the synovia of joints, the clinical and pathologic manifestations are vasculitis, nephritis, and arthritis. The clinical symptoms are usually short-lived, and the lesions heal unless the antigen is injected again. This type of disease is an example of acute serum sickness. A more indolent and prolonged disease, called chronic serum sickness, is produced by multiple injections of antigen, which lead to the formation of smaller complexes that are deposited most often in the kidneys, arteries, and lungs.

Arthus Reaction

A localized form of experimental immune complex–mediated vasculitis is called the Arthus reaction. It is induced by injection of an antigen subcutaneously into a previously immunized animal or an animal that has been given intravenous antibody specific for the antigen. Circulating antibodies rapidly bind to the injected antigen and form immune complexes that are deposited in the walls of small arteries at the injection site. This deposition gives rise to a local cutaneous vasculitis with tissue necrosis. This model has been used to study the cells and molecules involved in immune complex diseases.

Pathogenesis of Immune Complex–Mediated Diseases

Antigen-antibody complexes are produced during normal immune responses, but they cause disease only when they are produced in excessive amounts, are not efficiently cleared, and become deposited in tissues. The amount of immune complex deposition in tissues is determined by the nature of the complexes and the characteristics of the blood vessels. Small complexes are often not phagocytosed and tend to be deposited in vessels more than large complexes, which are usually cleared by phagocytes. Complexes containing cationic antigens bind avidly to negatively charged components of the basement membranes of blood vessels and kidney glomeruli. Such complexes typically produce severe and long-lasting tissue injury. Capillaries in the renal glomeruli and synovia are vessels in which plasma is ultrafiltered (to form urine and synovial fluid, respectively) by passing through the capillary wall at high hydrostatic pressure, and these locations are among the most common sites of immune complex deposition. However, immune complexes may be deposited in small vessels in virtually any tissue. Immune complexes may also bind to Fc receptors of mast cells and leukocytes and activate these cells to secrete cytokines and vasoactive mediators. These mediators may cause more immune complex deposition in vessel walls by increasing vascular permeability and blood flow.

The deposition of immune complexes in vessel walls leads to complement- and Fc receptor–mediated inflammation and injury to the vessels and adjacent tissues. Deposits of antibody and complement may be detected in the vessels, and if the antigen is known, it is possible to identify antigen molecules in the deposits as well (see Fig. 18-3B).

Many systemic immunologic diseases in humans are caused by the deposition of immune complexes in blood vessels. Some common examples of autoimmune immune complex diseases are systemic lupus erythematosus (SLE), in which the complexes consist of nuclear antigens and antibodies, and several forms of nephritis and vasculitis (Table 18-3). In almost 50% of cases of one type of immune complex–mediated vasculitis involving medium-size muscular arteries, called polyarteritis nodosa, the complexes are made up of viral antigen and antibodies, and the disease is a late complication of viral infection, most often with hepatitis B virus. This also is the mechanism of a disease called poststreptococcal glomerulonephritis that develops in rare cases after streptococcal infection and is caused by complexes of streptococcal antigen and antibodies depositing in the glomeruli of the kidney.

TABLE 18–3 Examples of Human Immune Complex–Mediated Diseases

Disease	Antigen Involved	Clinicopathologic Manifestations
Systemic lupus erythematosus	DNA, nucleoproteins, others	Nephritis, arthritis, vasculitis
Polyarteritis nodosa	Hepatitis B virus surface antigen	Vasculitis
Poststreptococcal glomerulonephritis	Streptococcal cell wall antigens; may be "planted" in glomerular basement membrane	Nephritis
Serum sickness	Various proteins	Arthritis, vasculitis, nephritis

DISEASES CAUSED BY T LYMPHOCYTES

T lymphocytes injure tissues either by triggering inflammation or by directly killing target cells (Fig. 18-5). Inflammatory reactions are elicited mainly by $CD4^+$ T cells of the T_H1 and T_H17 subsets, which secrete cytokines that recruit leukocytes. In some T cell–mediated disorders, $CD8^+$ CTLs kill target cells bearing class I major histocompatibility complex (MHC)–associated antigens. The T cells that cause tissue injury may be autoreactive, or they may be specific for foreign protein antigens that are present in or bound to cells or tissues. T lymphocyte–mediated tissue injury may also accompany strong protective immune responses against persistent microbes, especially intracellular microbes that resist eradication by phagocytes and antibodies.

A role for T cells in causing a particular immunologic disease is suspected largely on the basis of the demonstration of T cells in lesions and the isolation of T cells specific for self or microbial antigens from the tissues or blood of patients. Animal models have been very useful for elucidating the pathogenesis of these disorders.

Diseases Caused by Cytokine-Mediated Inflammation

In immune-mediated inflammation, T_H1 and T_H17 cells secrete cytokines that recruit and activate leukocytes. IL-17, produced by T_H17 cells, promotes neutrophil recruitment; interferon-γ (IFN-γ), produced by T_H1 cells, activates macrophages; and tumor necrosis factor (TNF) and chemokines, produced by T lymphocytes and other cells, are involved in the recruitment and activation of many types of leukocytes. Tissue injury results from the products of activated neutrophils and macrophages, such as lysosomal enzymes, reactive oxygen species, nitric oxide, and proinflammatory cytokines (see Chapter 10).

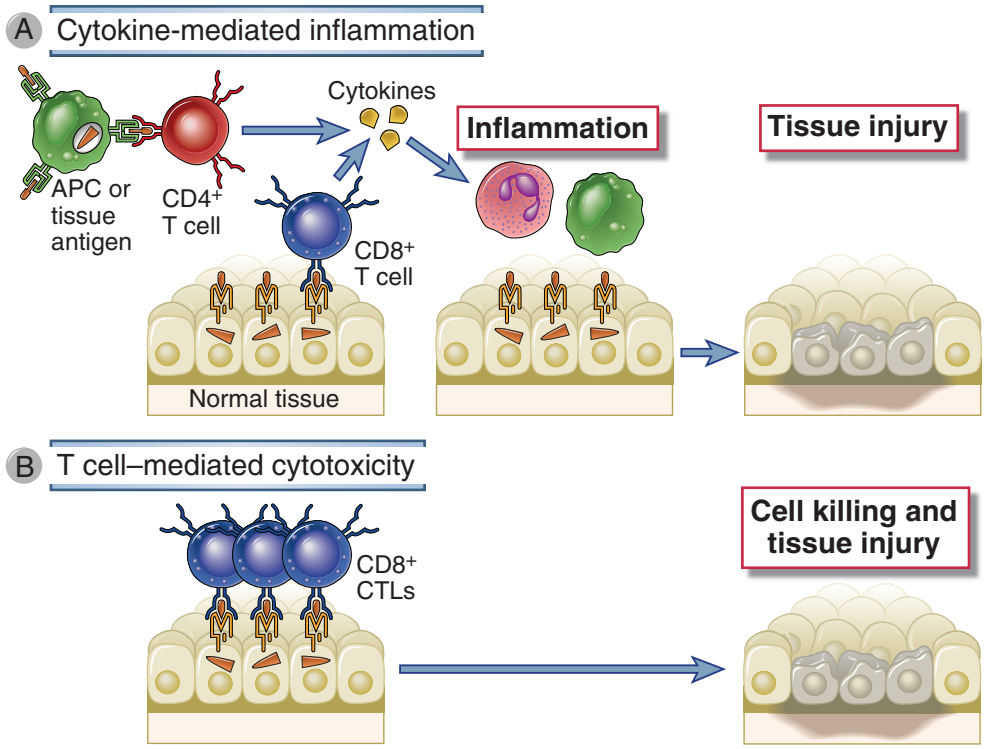


FIGURE 18–5 Mechanisms of T cell–mediated diseases. A, In cytokine-mediated inflammatory reactions, $CD4^+$ T cells (and sometimes $CD8^+$ cells) respond to tissue antigens by secreting cytokines that stimulate inflammation and activate phagocytes, leading to tissue injury. APC, antigen-presenting cell. **B,** In some diseases, $CD8^+$ CTLs directly kill tissue cells.

TABLE 18–4 T Cell–Mediated Diseases

Disease	Specificity of Pathogenic T Cells	Principal Mechanisms of Tissue Injury
Rheumatoid arthritis	Collagen? Citrullinated self proteins?	Inflammation mediated by T_H17 (and T_H1?) cytokines Role of antibodies and immune complexes?
Multiple sclerosis	Protein antigens in myelin (e.g., myelin basic protein)	Inflammation mediated by T_H1 and T_H17 cytokines Myelin destruction by activated macrophages
Type 1 diabetes mellitus	Antigens of pancreatic islet β cells (insulin, glutamic acid decarboxylase, others)	T cell–mediated inflammation Destruction of islet cells by CTLs
Inflammatory bowel disease	Enteric bacteria Self antigens?	Inflammation mediated by T_H17 and T_H1 cytokines
Autoimmune myocarditis	Myosin heavy chain protein	CTL-mediated killing of myocardial cells Inflammation mediated by T_H1 cytokines

Examples of human T cell–mediated diseases are listed. In many cases, the specificity of the T cells and the mechanisms of tissue injury are inferred on the basis of the similarity with experimental animal models of the diseases.

Vascular endothelial cells in the lesions may express increased levels of cytokine-regulated surface proteins such as adhesion molecules and class II MHC molecules. The inflammation associated with T cell–mediated diseases is typically chronic, but bouts of acute inflammation may be superimposed on a background of chronic inflammation. Delayed-type hypersensitivity (DTH) is an example of such inflammatory reactions and is described later. Chronic inflammatory reactions often produce fibrosis as a result of the secretion of cytokines and growth factors by the macrophages.

Many organ-specific autoimmune diseases are caused by interaction of autoreactive T cells with self antigens, leading to cytokine release and inflammation. This is believed to be the major mechanism underlying rheumatoid arthritis, multiple sclerosis, type 1 diabetes, psoriasis, and other autoimmune diseases (Table 18-4). Some of these are described in more detail at the end of the chapter.

T cell reactions against microbes and other foreign antigens may also lead to inflammation and tissue injury at the sites of infection or antigen exposure. Intracellular bacteria such as *Mycobacterium tuberculosis* induce strong T cell and macrophage responses that result in granulomatous inflammation and fibrosis (described below); the inflammation and fibrosis may cause extensive tissue destruction and functional impairment, in this case in the lungs. Tuberculosis is a good example of an infectious disease in which tissue injury is mainly due to the host immune response (see Chapter 15). A variety of skin diseases that result from topical exposure to chemicals and environmental antigens, called **contact sensitivity**, are due to inflammatory reactions, presumably triggered by neoantigens formed by the binding of the chemicals to self proteins. Both $CD4^+$ and $CD8^+$ T cells may be the source of cytokines in contact sensitivity reactions. Examples of contact sensitivity include the rashes of poison ivy and poison oak (in which T cells react against chemicals made by the plants called urushiols) and rashes induced by contact with a variety of chemicals, such as thiuram, which is used in the manufacture of latex gloves. Some of these reactions become chronic and clinically are called eczema. T cell responses against intestinal bacteria are believed to underlie some forms of inflammatory bowel disease.

The classical T cell-mediated inflammatory reaction is called delayed type hypersensitivity, and is described next.

Delayed-Type Hypersensitivity

Delayed-type hypersensitivity (DTH) is an injurious cytokine-mediated inflammatory reaction resulting from the activation of T cells, particularly $CD4^+$ T cells. The reaction is called hypersensitivity because it reflects excessive (i.e., injurious) immune responses (which are reflections of sensitivity to an antigen) and delayed because it typically develops during 24 to 48 hours after antigen challenge.

In the classic animal model of DTH, a guinea pig is first immunized by the administration of a protein antigen in adjuvant; this step is called *sensitization*. About 2 weeks later, the animal is challenged subcutaneously with the same antigen, and the subsequent reaction is analyzed; this step is called the elicitation phase. Humans may be sensitized for DTH reactions by microbial infection, by contact sensitization with chemicals and environmental antigens, or by intradermal or subcutaneous injection of protein antigens (Fig. 18-6). Subsequent exposure to the same antigen (called *challenge*) elicits the reaction. For example, purified protein derivative (PPD), a protein antigen of *Mycobacterium tuberculosis,* elicits a DTH reaction, called the *tuberculin reaction,* when it is injected into individuals who have been exposed to *M. tuberculosis*. A positive tuberculin skin test response is a widely used clinical indicator for evidence of previous or active tuberculosis infection.

The characteristic response of DTH evolves during 24 to 48 hours. About 4 hours after the injection of antigen, neutrophils accumulate around the postcapillary venules at the injection site. By about 12 hours, the injection site becomes infiltrated by T cells and blood monocytes, also organized in a perivenular distribution (Fig. 18-7). The

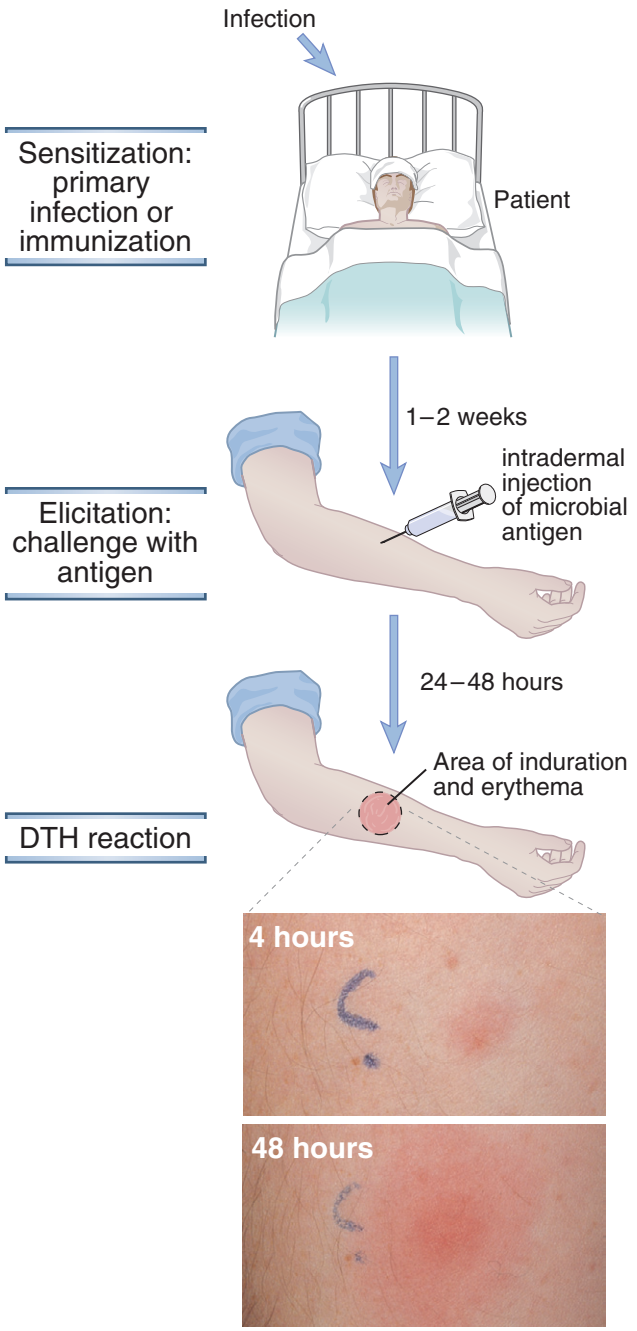


FIGURE 18–6 Delayed-type hypersensitivity reaction. Infection or immunization (vaccination) sensitizes an individual, and subsequent challenge with an antigen from the infectious agent elicits a DTH reaction. The reaction is manifested by induration with redness and swelling at the site of the challenge, which is undetectable at ~4 hours and peaks at ~48 hours. *(Courtesy of Dr. J. Faix, Department of Pathology, Stanford University School of Medicine, Palo Alto, California.)*

endothelial cells lining these venules become plump, show increased biosynthetic organelles, and become leaky to plasma macromolecules. Fibrinogen escapes from the blood vessels into the surrounding tissues, where it is converted into fibrin. The deposition of fibrin and, to a lesser extent, the accumulation of T cells and monocytes within the extravascular tissue space around the injection site cause the tissue to swell and become firm (indurated). Induration, a diagnostic feature of DTH, is detectable by about 18 hours after the injection of antigen and is maximal by 24 to 48 hours. In clinical practice, loss of DTH responses to universally encountered antigens (e.g., *Candida* antigens) is an indication of deficient T cell function, a condition known as anergy. (This general loss of immune responsiveness is different from lymphocyte anergy, a mechanism for maintaining tolerance to specific antigens, discussed in Chapter 14.)

Although DTH has traditionally been considered a T_H1-mediated injurious reaction, other T cells may contribute to the inflammation. In some DTH lesions, neutrophils are prominent, suggesting the involvement of T_H17 cells. In infections by some helminthic parasites, reactions against the parasite eggs elicit DTH with a strong component of eosinophils. In these cases, a role for T_H2 cytokines has been demonstrated. $CD8^+$ T cells also produce IFN-γ and may contribute to some DTH reactions, especially in the skin.

Chronic DTH reactions can develop if a T_H1 response to an infection activates macrophages but fails to eliminate phagocytosed microbes. If the microbes are localized in a small area, the reaction produces nodules of inflammatory tissue called *granulomas* (Fig. 18-8A). Chronic DTH, as exemplified by granulomatous inflammation, is caused by prolonged cytokine signals (Fig. 18-8B). In such reactions, the activated T cells and macrophages continue to produce cytokines and growth factors, which amplify the reactions of both cell types and progressively modify the local tissue environment. The result is a cycle of tissue injury and chronic inflammation followed by replacement with connective tissue (fibrosis). In chronic DTH reactions, activated macrophages also undergo changes in response to persistent cytokine signals. These macrophages develop increased cytoplasm and cytoplasmic organelles and histologically may resemble skin epithelial cells, because of which they are sometimes called epithelioid cells. Activated macrophages may fuse to form multinucleate giant cells. Granulomatous inflammation is an attempt to contain the infection but is also the cause of significant tissue injury and functional impairment. This type of inflammation is a characteristic response to some persistent microbes, such as *M. tuberculosis* and some fungi, and represents a form of chronic DTH with fibrosis. Much of the respiratory difficulty associated with tuberculosis or chronic fungal infection of the lung is caused by replacement of normal lung with fibrotic tissue and not directly attributable to the microbes.

Diseases Caused by Cytotoxic T Lymphocytes

CTL responses to viral infection can lead to tissue injury by killing infected cells, even if the virus itself has no cytopathic effects. The principal physiologic function of CTLs is to eliminate intracellular microbes, primarily viruses, by killing infected cells. Some viruses directly injure infected cells and are said to be cytopathic, whereas others are not. Because CTLs may not be able to distinguish between cytopathic and noncytopathic viruses, they kill virally infected cells regardless of whether the infection itself is harmful to the host. Examples of viral infections in which the lesions are due to the host CTL

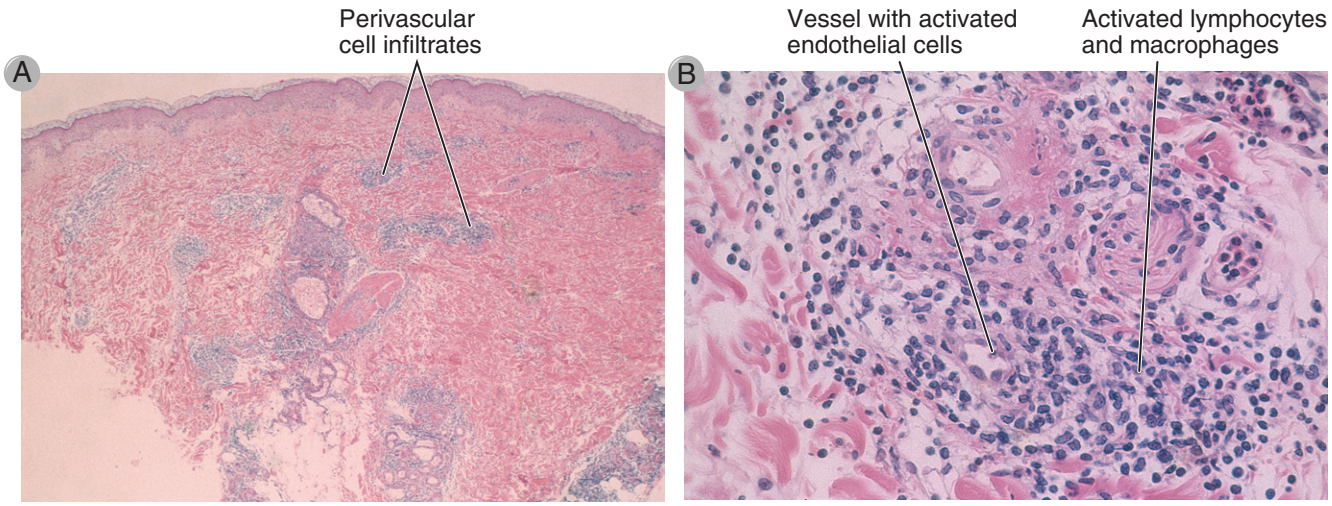


FIGURE 18–7 Morphology of a DTH reaction. A, Histopathologic examination of the reaction in skin illustrated in Figure 18-6 shows perivascular mononuclear cell infiltrates in the dermis. **B,** At higher magnification, the infiltrate is seen to consist of activated lymphocytes and macrophages surrounding small blood vessels in which the endothelial cells are also activated. *(Courtesy of Dr. J. Faix, Department of Pathology, Stanford University School of Medicine, Palo Alto, California.)*

response and not the virus itself include lymphocytic choriomeningitis in mice and certain forms of viral hepatitis in humans (see Chapter 15).

CTLs may contribute to tissue injury in autoimmune disorders that are caused primarily by $CD4^+$ T cells, such as type 1 diabetes. Few examples of autoimmune diseases mediated only by CTLs have been documented. Myocarditis with infiltration of the heart by $CD8^+$ T cells develops in mice and sometimes in humans after infection with coxsackievirus B, resulting in a form of dilated cardiomyopathy. Patients and experimental animals contain CTLs specific for myocyte proteins. It is postulated that the heart lesions are initiated by the virus infection and virus-specific CTLs, and myocardial injury leads to the exposure or alteration of self antigens and the subsequent development of autoreactive CTLs. However, in these forms of myocarditis, $CD4^+$ T cells and antibodies are also likely involved in the pathogenesis.

THERAPEUTIC APPROACHES FOR IMMUNOLOGIC DISEASES

One of the most impressive accomplishments of immunology has been the development of novel therapies

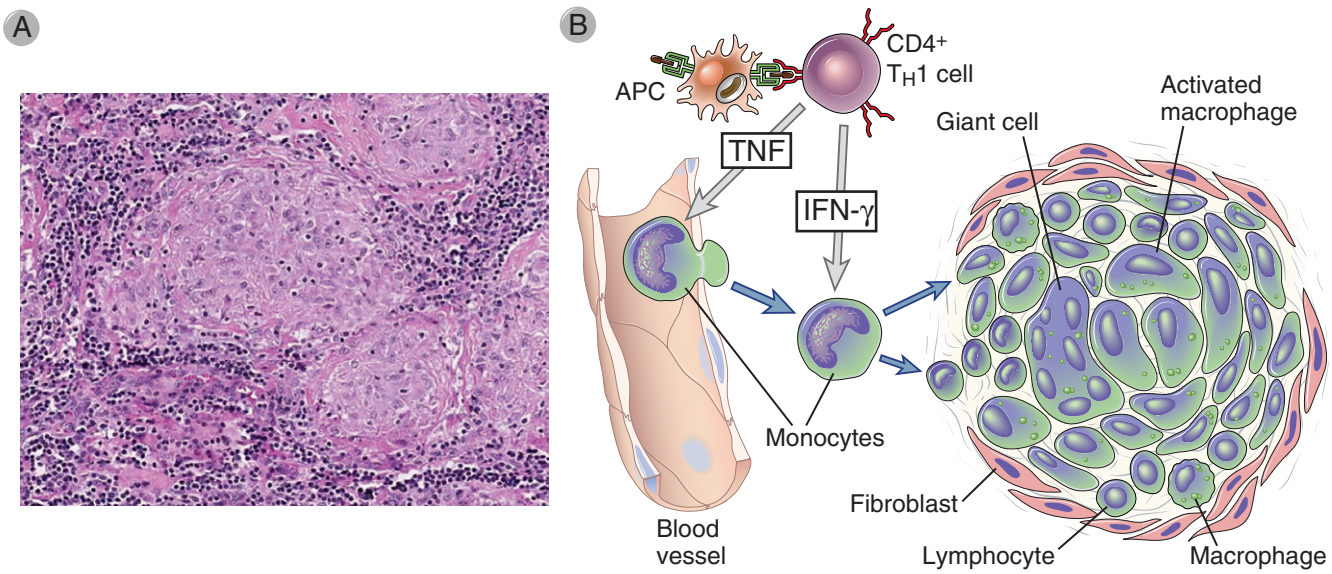


FIGURE 18–8 Granulomatous inflammation. A, Lymph node from a patient with tuberculosis containing granulomas with activated macrophages, multinucleate giant cells, and lymphocytes. In some granulomas, there may be a central area of necrosis. Immunohistochemical studies would identify the lymphocytes as T cells. **B,** Mechanisms of granuloma formation. Cytokines are involved in the generation of T_H1 cells, activation of macrophages, and recruitment of leukocytes. Prolonged reactions of this type lead to the formation of granulomas.

based on the understanding of basic science and its application to human disease. The therapies can be divided into several broad groups.

Anti-inflammatory Agents

The mainstay of therapy for hypersensitivity diseases for many years has been anti-inflammatory drugs, particularly corticosteroids. Such drugs are targeted at reducing tissue injury, specifically the inflammatory component of the pathologic immune responses.

Depletion of Cells and Antibodies

Monoclonal antibodies are given that deplete all lymphoid cells, only B cells, or only T cells. A recent and somewhat surprising development is the successful use of anti-CD20 antibody (rituximab), which depletes only B cells, to treat diseases that were thought to be caused primarily by T cell–mediated inflammation. This treatment has shown efficacy in some patients with rheumatoid arthritis and multiple sclerosis. Plasmapheresis has been used to eliminate circulating autoantibodies and immune complexes.

Anti-Cytokine Therapies

A large number of cytokines involved in inflammation are being targeted by specific antagonists for the treatment of chronic, T cell–mediated inflammatory diseases (Table 18-5). The first success with this class of biologic agents came with a soluble form of the TNF receptor and anti-TNF antibodies, which bind to and neutralize TNF. These agents are of great benefit in many patients with rheumatoid arthritis, Crohn's disease, and the skin disease psoriasis. Antagonists of other proinflammatory cytokines, such as IL-1, the p40 chain that is present in both IL-12 and IL-23, IL-6, IL-17A, and many others, are in use or in clinical trials for inflammatory diseases.

Agents That Inhibit Cell-Cell Interactions in Immune Responses

Agents that block B7 costimulators are approved for treatment of rheumatoid arthritis and psoriasis and are being tested in SLE and other diseases. Antibodies against CD40 ligand block T cell–mediated activation of B cells and macrophages and have been beneficial in patients with inflammatory bowel disease, but a small number of treated patients have developed thrombotic episodes, apparently because this molecule is expressed on human platelets (where its function is unknown). Antibodies against integrins have been used to inhibit leukocyte migration into tissues, particularly the central nervous system (CNS) in multiple sclerosis.

Intravenous IgG

Large doses of intravenous IgG (IVIG) have beneficial effects in some hypersensitivity diseases. It is not clear how this agent suppresses immune inflammation; one possibility is that the IgG binds to the inhibitory Fc receptor (FcγRIIB) on macrophages and B lymphocytes and thus attenuates inflammatory responses (see Chapter 12). IVIG may also compete with pathogenic antibodies for binding to the neonatal Fc receptor (FcRn), which functions in adults to protect antibodies from catabolism (see Chapter 5), resulting in reduced half-lives of the pathogenic antibodies.

There are ongoing attempts at more specific treatment, such as inducing tolerance in disease-producing T cells or inducing regulatory T cells specific for self antigens. Multiple sclerosis and type 1 diabetes are two immune diseases in which the target antigens have been defined; in both, clinical trials are starting in which the antigens (peptides of myelin basic protein and insulin, respectively) will be administered to patients in ways predicted

TABLE 18–5 Examples of Cytokine Antagonists in Clinical Use or Trials

Cytokine or Receptor Targeted	Predicted Biologic Effects Of Antagonist	Clinical Indications
TNF	Inhibits leukocyte migration into sites of inflammation	Rheumatoid arthritis, psoriasis, inflammatory bowel disease
IL-1	Inhibits leukocyte migration into sites of inflammation	Rare autoinflammatory syndromes, severe gout, rheumatoid arthritis
IL-6 and IL-6 receptor	Inhibits synthesis of acute-phase proteins, antibody responses?	Juvenile idiopathic arthritis, rheumatoid arthritis
IL-17	Inhibits leukocyte recruitment into sites of inflammation	Rheumatoid arthritis, psoriasis
p40 chain of IL-12 and IL-23	Inhibits T_H1 and T_H17 responses	Inflammatory bowel disease, psoriasis
IL-2 receptor (CD25)	Inhibits IL-2–mediated T cell proliferation	Acute graft rejection
IFN-α	May be multiple effects on T_H1 differentiation, antibody production	Systemic lupus erythematosus
IL-4	Inhibits T_H2 differentiation, IgE production	Asthma
IL-5	Inhibits eosinophil activation	Asthma

The table lists examples of antagonists against cytokines (antibodies or soluble receptors) that are approved for clinical use or in trials. IFN, interferon; IL, interleukin; TNF, tumor necrosis factor.

to shut off specific immune responses. A risk of many treatments that block various components of the immune system is that these will interfere with the normal function of the immune system in combating microbes and thus make individuals susceptible to infections. Antigen-specific tolerance avoids this problem by selectively targeting the disease-causing lymphocytes. These general principles are similar to those on which treatment of transplant rejection is based (see Chapter 16).

SELECTED IMMUNOLOGIC DISEASES: PATHOGENESIS AND THERAPEUTIC STRATEGIES

In the following section, we describe the pathogenesis of some selected diseases caused by antibodies and T cells and the application of novel therapies in these diseases to illustrate the principles that have been discussed before.

Systemic Lupus Erythematosus: The Prototypic Immune Complex–Mediated Disease

SLE is a chronic, remitting and relapsing, multisystem autoimmune disease that affects predominantly women, with an incidence of 1 in 700 among women between the ages of 20 and 60 years (about 1 in 250 among black women) and a female-to-male ratio of 10:1. The principal clinical manifestations are rashes, arthritis, and glomerulonephritis, but hemolytic anemia, thrombocytopenia, and CNS involvement are also common. Many different autoantibodies are found in patients with SLE. The most frequent are antinuclear, particularly anti-DNA, antibodies; others include antibodies against ribonucleoproteins, histones, and nucleolar antigens. Immune complexes formed from these autoantibodies and their specific antigens are responsible for glomerulonephritis, arthritis, and vasculitis involving small arteries throughout the body. Hemolytic anemia and thrombocytopenia are due to autoantibodies against erythrocytes and platelets, respectively. The principal diagnostic test for the disease is the presence of antinuclear antibodies; antibodies against double-stranded native DNA are specific for SLE.

Pathogenesis of Systemic Lupus Erythematosus

SLE is a complex disease in which genetic and environmental factors contribute to a breakdown of tolerance in self-reactive B and T lymphocytes. Among the genetic factors is the inheritance of particular HLA alleles. The odds ratio (relative risk) for individuals with HLA-DR2 or HLA-DR3 is 2 to 3, and if both haplotypes are present, the odds ratio is about 5. Genetic deficiencies of classical pathway complement proteins, especially C1q, C2, or C4, are seen in about 10% of patients with SLE. The complement deficiencies may result in defective clearance of immune complexes and apoptotic cells and the failure of B cell tolerance. A polymorphism in the inhibitory Fc receptor FcγRIIB has been described in some patients; this may contribute to inadequate control of B cell activation or a failure to attenuate inflammatory responses in innate immune cells. Many other genes have been detected by genome-wide association studies, but the role of each of these is not established, and their contribution to the development of the disease remains unclear. Environmental factors include exposure to ultraviolet (UV) light. It is postulated that this leads to apoptotic death of cells and release of nuclear antigens.

Two recent observations are leading to new hypotheses of the pathogenesis of SLE. First, studies in patients have revealed that blood cells show a striking molecular signature (pattern of gene expression) that indicates exposure to IFN-α, a type I interferon that is produced mainly by plasmacytoid dendritic cells. Some studies have shown that plasmacytoid dendritic cells from SLE patients also produce abnormally large amounts of IFN-α. Second, studies in animal models have shown that Toll-like receptors (TLRs) that recognize DNA and RNA, notably the DNA-recognizing TLR9, play an important role in the activation of B cells specific for self nuclear antigens. On the basis of these studies, a model for the pathogenesis of SLE has been proposed (Fig. 18-9). According to this model, UV irradiation and other environmental insults lead to the apoptosis of cells. Inadequate clearance of the nuclei of these cells, in part because of defects in clearance mechanisms such as complement proteins and receptors, results in a large burden of nuclear antigens. Polymorphisms in various susceptibility genes for lupus lead to defective ability to maintain self-tolerance in B and T lymphocytes, because of which self-reactive lymphocytes remain functional. Failure of B cell tolerance may be due to receptor editing or defective deletion of immature B cells in the bone marrow or defective peripheral tolerance. Self-reactive B cells that are not rendered tolerant are stimulated by the self nuclear antigens, and antibodies are produced against the antigens. Complexes of the antigens and antibodies bind to Fc receptors on dendritic cells and the antigen receptor on B cells and may be internalized. The nucleic acid components engage TLRs and stimulate B cells to produce autoantibodies and activate dendritic cells, particularly plasmacytoid dendritic cells, to produce IFN-α, which further enhance the immune response and cause more apoptosis. The net result is a cycle of antigen release and immune activation that leads to the production of high-affinity autoantibodies.

New Therapies for Systemic Lupus Erythematosus

The recent advances in our understanding of SLE are leading to novel therapeutic approaches. Clinical trials are under way to test the efficacy of anti–IFN-α antibodies in the disease, and attempts to inhibit TLR signals are being considered. There has been great interest in depleting B cells by use of an antibody against the B cell surface protein CD20. An antibody that blocks the B cell growth factor BAFF is now approved for the treatment of SLE.

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is an inflammatory disease involving small and large joints of the extremities, including fingers, shoulders, elbows, knees, and ankles. The

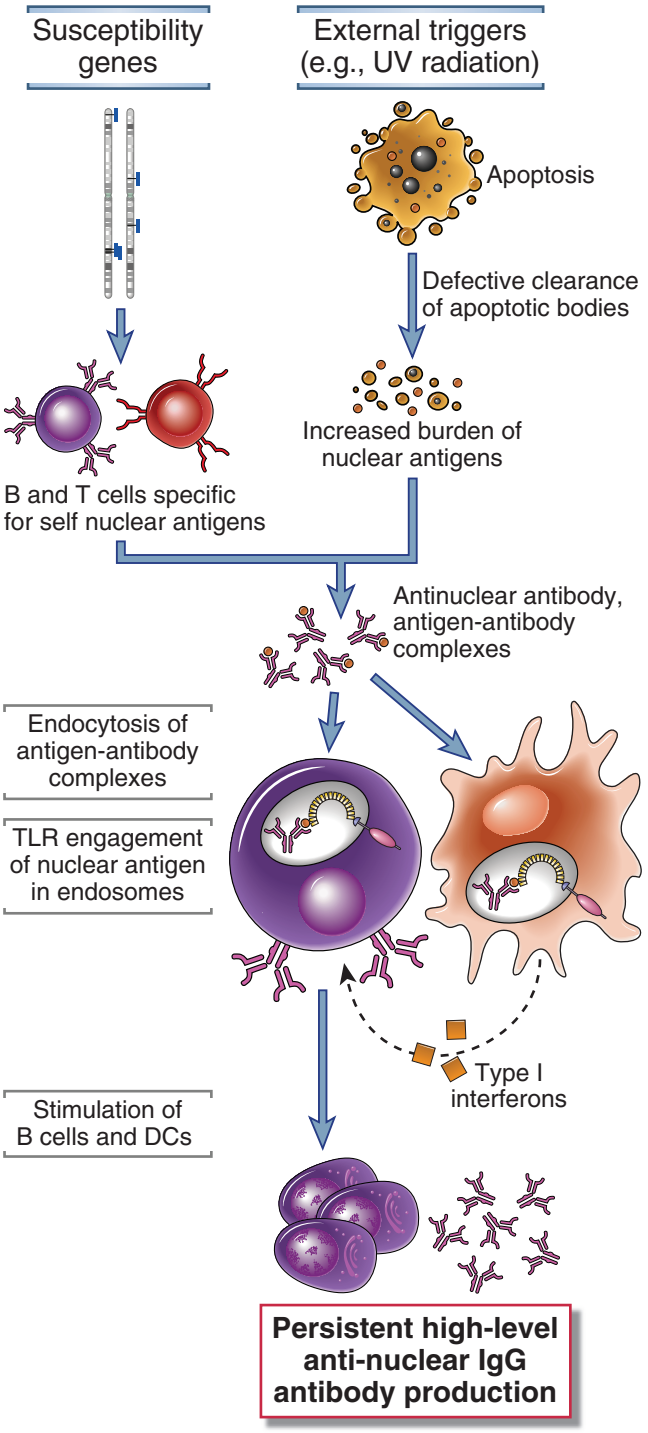


FIGURE 18–9 A model for the pathogenesis of systemic lupus erythematosus (SLE). In this hypothetical model, various susceptibility genes interfere with the maintenance of self-tolerance, and external triggers lead to persistence of nuclear antigens. The result is an antibody response against self nuclear antigens, which is amplified by the TLR-dependent activation of dendritic cells and B cells by nucleic acids, and the production of type I interferons.

disease is characterized by inflammation of the synovium associated with destruction of the joint cartilage and bone, with a morphologic picture indicative of a local immune response. Both cell-mediated and humoral immune responses may contribute to development of synovitis. $CD4^+$ T_H1 and T_H17 cells, activated B lymphocytes, plasma cells, and macrophages as well as other inflammatory cells are found in the inflamed synovium, and in severe cases, well-formed lymphoid follicles with germinal centers may be present. Numerous cytokines, including IL-1, IL-8, TNF, IL-6, IL-17, and IFN-γ, have been detected in the synovial (joint) fluid. Cytokines are believed to recruit leukocytes whose products cause tissue injury and also to activate resident synovial cells to produce proteolytic enzymes, such as collagenase, that mediate destruction of the cartilage, ligaments, and tendons of the joints. Increased osteoclast activity in the joints contributes to the bone destruction in RA, and this may be caused by the production of the TNF family cytokine RANK (receptor activator of nuclear factor κB) ligand by activated T cells. RANK ligand binds to RANK, a member of the TNF receptor family that is expressed on osteoclast precursors, and induces their differentiation and activation. Systemic complications of RA include vasculitis, presumably caused by immune complexes, and lung injury.

Although much of the emphasis in studies of RA has been on the role of T cells, antibodies may also contribute to the joint destruction. Activated B cells and plasma cells are often present in the synovia of affected joints. Patients frequently have circulating IgM or IgG antibodies that react with the Fc (and rarely Fab) portions of their own IgG molecules. These autoantibodies are called rheumatoid factors, and their presence is used as a diagnostic test for RA. Rheumatoid factors may participate in the formation of injurious immune complexes, but their pathogenic role is not established. Another type of antibody that has been detected in at least 70% of patients is specific for cyclic citrullinated peptides (CCP), which are derived from certain proteins that are modified in an inflammatory environment by the enzymatic conversion of arginine residues to citrulline. These so-called anti-CCP antibodies are a diagnostic marker for the disease and may be involved in tissue injury.

Pathogenesis of Rheumatoid Arthritis

Like other autoimmune diseases, RA is a complex disorder in which genetic and environmental factors contribute to the breakdown of tolerance to self antigens. The specificity of the pathogenic T and B cells remains unknown, because of which the understanding of the pathogenesis is incomplete. Susceptibility to RA is linked to the HLA-DR4 haplotype. Recent linkage and genome-wide association studies have revealed a large number of genes in which polymorphisms are associated with RA. There is an association with the gene encoding a tyrosine phosphatase, PTPN22, but the role of this enzyme in lymphocyte regulation is poorly understood (see Chapter 14).

The identification of anti-CCP immune responses has led to new ideas about the pathogenesis of RA (Fig. 18-10). According to one model, environmental insults, such as smoking and some infections, induce the citrullination of self proteins, leading to the creation of new antigenic epitopes. In genetically susceptible individuals, tolerance to these epitopes fails, resulting in T cell and antibody responses against the proteins. If these modified

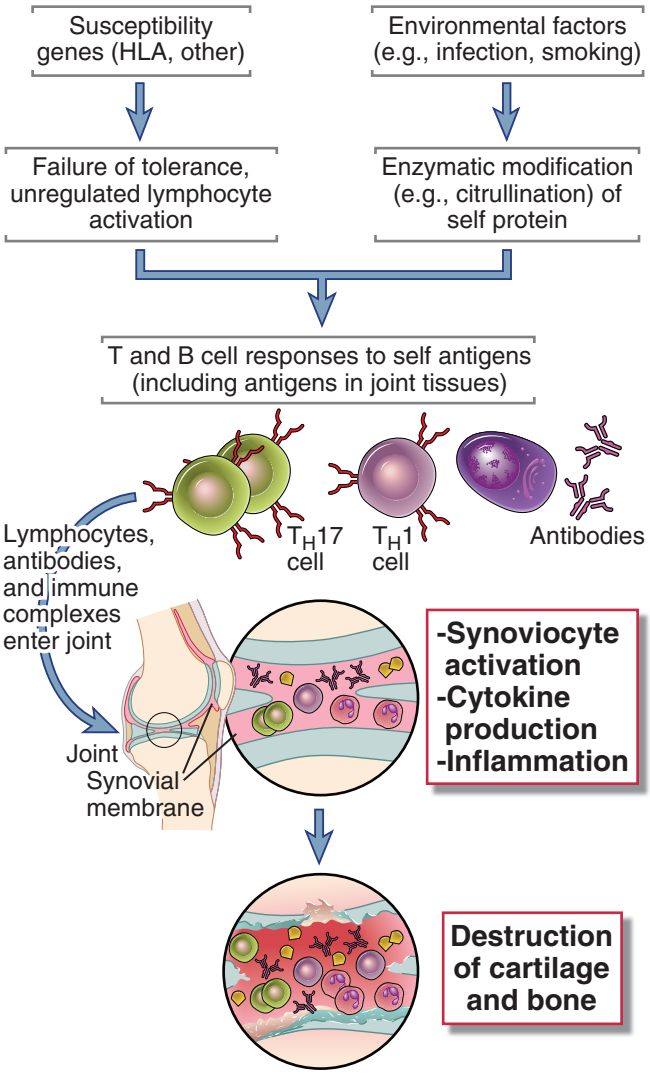


FIGURE 18–10 A model for the pathogenesis of rheumatoid arthritis. According to this hypothesis, citrullinated proteins induced by environmental stimuli elicit T cell and antibody responses in genetically susceptible individuals. The T cells and antibodies enter joints, respond to the self proteins, and cause tissue injury mainly by cytokine secretion and perhaps also by antibody-dependent effector mechanisms. Protein modifications other than citrullination may lead to the same result.

self proteins are also present in joints, the T cells and antibodies attack the joints. T_H17 and perhaps T_H1 cells secrete cytokines that recruit leukocytes into the joint and activate synovial cells to produce collagenases and other enzymes. The net result is the progressive destruction of cartilage and bone. The immune response in the joint may be strong enough that tertiary lymphoid tissues form in the synovium, and these may maintain and propagate the local inflammatory reaction.

New Therapies for Rheumatoid Arthritis

The realization of the central role of T cells and cytokines in the disease has led to a remarkable advance in treatment, in which specific molecules have been targeted on the basis of scientific understanding. Chief among these new therapies are antagonists against TNF, which have transformed the course of the disease in many patients from one of progressive and inexorable joint destruction to one of smoldering but manageable chronic inflammation. An IL-1 antagonist and an antibody against the IL-6 receptor are approved treatments, as is a fusion protein of the extracellular domain of CTLA-4 and the Fc portion of IgG, which binds to B7 molecules and blocks B7:CD28 interactions. Antibodies that block IL-17 are in clinical trials. The B cell–depleting anti-CD20 antibody is of benefit in some patients. The beneficial effect of B cell depletion does not seem to be attributable entirely to reduced production of autoantibodies, suggesting that B cells may play other roles in the disease, such as presenting antigens to pathogenic T cells.

Multiple Sclerosis and Experimental Autoimmune Encephalomyelitis

Multiple sclerosis (MS) is an autoimmune disease of the CNS in which $CD4^+$ T cells of the T_H1 and T_H17 subsets react against self myelin antigens, resulting in inflammation in the CNS with activation of macrophages around nerves in the brain and spinal cord, destruction of the myelin, abnormalities in nerve conduction, and neurologic deficits. It is the most common neurologic disease of young adults. On pathologic examination, there is inflammation in the CNS white matter with secondary demyelination. Multiple sclerosis is characterized clinically by weakness, paralysis, and ocular symptoms with exacerbations and remissions; CNS imaging suggests that in patients with active disease, there is frequent new lesion formation. The disease is modeled by experimental autoimmune encephalomyelitis (EAE) in mice, rats, guinea pigs, and nonhuman primates, and this is one of the best characterized experimental models of an organ-specific autoimmune disease mediated mainly by T lymphocytes. EAE is induced by immunizing animals with antigens normally present in CNS myelin, such as myelin basic protein, proteolipid protein, and myelin oligodendrocyte glycoprotein, with an adjuvant containing heat-killed mycobacterium, which is necessary to elicit a strong T cell response. About 1 to 2 weeks after immunization, animals develop encephalomyelitis, characterized by perivascular infiltrates composed of lymphocytes and macrophages in the CNS white matter, followed by demyelination. The neurologic lesions can be mild and self-limited or chronic and relapsing. These lesions result in progressive or remitting and relapsing paralysis. The disease can also be transferred to naive animals with T cells from diseased animals. Although antibodies against myelin antigens have been detected in patients as well as in the animal models, the pathogenic significance of these antibodies is not established.

Pathogenesis of Multiple Sclerosis

There is abundant evidence that in mice, EAE is caused by activated, $CD4^+$ T_H1 and T_H17 cells specific for protein antigens in myelin. By analogy with the experimental disease, MS is also thought to be caused by myelin-specific T_H1 and T_H17 cells, and these cells have been detected in patients and isolated from the blood and CNS. How these cells are activated in patients remains an

enigma. It has been suggested that an infection, most likely a viral infection, activates self myelin–reactive T cells by the phenomenon of molecular mimicry (see Chapter 14). Self-tolerance may fail because of the inheritance of susceptibility genes. Identical twins have a 25% to 40% concordance rate for development of MS, whereas nonidentical twins have a 1% concordance rate, implicating genetic factors in the development of the disease. Genetic polymorphisms associated with MS include the HLA locus, with HLA-DR2 being the strongest linkage. Genome-wide association studies have revealed an association with a polymorphism in the noncoding region of the gene encoding the IL-2 receptor α chain, CD25. Expression of CD25 on effector and memory T cells may be different in patients compared with healthy individuals, but how this results in disease is unclear. Some studies have suggested that regulatory T cells are defective in MS patients, but how this contributes to a failure of self-tolerance is also not known. Once myelin-specific T cells are activated, they migrate into the CNS, where they encounter myelin proteins and release cytokines that recruit and activate macrophages and more T cells, leading to myelin destruction. Studies of EAE suggest that the disease is propagated by the process known as epitope spreading (see Chapter 14). The tissue breakdown results in release of new protein antigens and expression of new, previously sequestered epitopes that activate more autoreactive T cells.

New Therapies for Multiple Sclerosis

Immunotherapy for MS has largely relied on approaches whose scientific basis is still not well understood. These include administration of β-interferon, which may alter cytokine responses, and treatment with a random polymer of four amino acids, which is postulated to bind to HLA molecules and block antigen presentation. More recently, an antibody against the VLA-4 integrin (see Chapter 3) has been used to block leukocyte migration into the CNS and has shown benefit in patients. However, in a small number of patients this treatment resulted in reactivation of a latent JC virus infection that causes a severe and sometime fatal CNS disease. Another recently approved drug to treat MS also interferes with leukocyte migration. The drug, called fingolimod (FTY720), blocks the sphingosine 1-phosphate–mediated pathway of T cell egress from lymphoid tissues (see Chapter 3). In a large subset of patients B cell depletion has been found to be therapeutically useful. An important role for B cells in the activation of pathogenic T cells is suggested by these results. Because myelin basic protein is known to be an important self antigen that is the target of the immune response in MS, attempts have been initiated to inject peptides derived from this antigen into patients, with the hope of inducing tolerance or generating regulatory T cells specific for the relevant antigen.

Type 1 Diabetes Mellitus

Type 1 diabetes mellitus, previously called insulin-dependent diabetes mellitus, is a multisystem metabolic disease resulting from impaired insulin production that affects about 0.2% of the U.S. population, with a peak age at onset of 11 to 12 years, and the incidence is increasing. The disease is characterized by hyperglycemia and ketoacidosis. Chronic complications of type 1 diabetes include progressive atherosclerosis of arteries, which can lead to ischemic necrosis of limbs and internal organs, and microvascular obstruction causing damage to the retina, renal glomeruli, and peripheral nerves. These patients have a deficiency of insulin resulting from immune-mediated destruction of the insulin-producing β cells of the islets of Langerhans in the pancreas, and continuous hormone replacement therapy is needed.

Pathogenesis of Type 1 Diabetes

Several mechanisms may contribute to β cell destruction, including inflammation mediated by $CD4^+$ T_H1 cells reactive with islet antigens (including insulin), CTL-mediated lysis of islet cells, local production of cytokines (TNF and IL-1) that damage islet cells, and autoantibodies against islet cells. In the rare cases in which the pancreatic lesions have been examined at the early active stages of the disease, the islets show cellular necrosis and lymphocytic infiltration consisting of both $CD4^+$ and $CD8^+$ T cells. This lesion is called insulitis. Autoantibodies against islet cells and insulin are also detected in the blood of these patients. In susceptible children who have not developed diabetes (such as relatives of patients), the presence of antibodies against islet cells is predictive of the development of type 1 diabetes, suggesting that the anti–islet cell antibodies are pathogenic. An informative animal model of the disease is the non-obese diabetic (NOD) mouse that develops spontaneous diabetes. In this model, there is evidence for defective survival and function of regulatory T cells as well as resistance of effector T cells to suppression.

Multiple genes are associated with type 1 diabetes. A great deal of attention has been devoted to the role of HLA genes. Between 90% and 95% of Caucasians with type 1 diabetes have HLA-DR3, or DR4, or both, in contrast to about 40% of normal subjects, and 40% to 50% of patients are DR3/DR4 heterozygotes, in contrast to 5% of normal subjects. Interestingly, susceptibility to type 1 diabetes is actually associated with alleles of DQ2 and DQ8 that are often in linkage disequilibrium with DR3 and DR4. Several non-HLA genes also contribute to the disease. The first of these to be identified is insulin, with tandem repeats in the promoter region being associated with disease susceptibility. The mechanism of this association is unknown; it may be related to the level of expression of insulin in the thymus, which determines whether insulin-specific T cells will be deleted (negatively selected) during maturation. Several other polymorphisms have been identified in patients and in NOD mice, including in the IL-2 and CD25 genes. The functional consequences of these polymorphisms are not known. Some studies have suggested that viral infections (e.g., with coxsackievirus B4) may precede the onset of type 1 diabetes, perhaps by initiating cell injury, inducing inflammation and the expression of costimulators, and triggering an autoimmune response. However, epidemiologic data suggest that repeated infections protect against type 1 diabetes, and this is similar to the NOD model. In fact, it has been postulated that one reason for the

increased incidence of type 1 diabetes in developed countries is the control of infectious diseases.

New Therapies for Type 1 Diabetes

The most interesting new therapeutic strategies for type 1 diabetes are focused on inducing tolerance with diabetogenic peptides from islet antigens (such as insulin) or generating or giving regulatory T cells to patients. These clinical trials are in their infancy.

Inflammatory Bowel Disease

Inflammatory bowel disease consists of two disorders, Crohn's disease and ulcerative colitis, in which T cell–mediated inflammation causes intestinal injury. Crohn's disease is characterized by chronic inflammation and destruction of the intestinal wall, with frequent formation of fistulas. In ulcerative colitis, the lesions are largely confined to the mucosa and consist of ulcers with underlying foci of inflammation. The pathogenesis of inflammatory bowel disease was described in Chapter 13. New therapies for these diseases include antibodies against TNF, IL-17, and the p40 chain of IL-12 and IL-23.

SUMMARY

- Disorders caused by abnormal immune responses are called hypersensitivity diseases. Pathologic immune responses may be autoimmune responses directed against self antigens or uncontrolled and excessive responses to foreign (e.g., microbial) antigens.
- Hypersensitivity diseases may result from antibodies that bind to cells or tissues, circulating immune complexes that are deposited in tissues, or T lymphocytes reactive with antigens in tissues.
- The effector mechanisms of antibody-mediated tissue injury are complement activation and Fc receptor–mediated inflammation. Some antibodies cause disease by interfering with normal cellular functions without producing tissue injury.
- The effector mechanisms of T cell–mediated tissue injury are inflammatory reactions induced by cytokines secreted mainly by $CD4^+$ T_H1 and T_H17 cells and cell lysis by CTLs. The classical T cell–mediated reaction is delayed-type hypersensitivity, induced by activation of previously primed T cells and the production of cytokines that recruit and activate various leukocytes, predominantly macrophages.
- The current treatment of autoimmune diseases is targeted at reducing immune activation and the injurious consequences of the autoimmune reaction. A future goal of therapy is to inhibit the responses of lymphocytes specific for self antigens and to induce tolerance in these cells.
- Autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis, and type 1 diabetes illustrate many of the effector mechanisms that cause tissue injury in hypersensitivity reactions and the roles of susceptibility genes and environmental factors in the development of autoimmunity.

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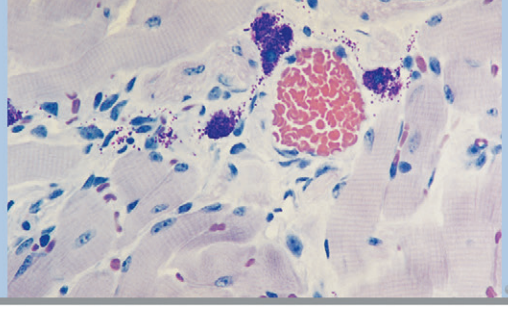
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CHAPTER

19

IgE-Dependent Immune Responses and Allergic Disease

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A variety of human diseases are caused by immune responses to nonmicrobial environmental antigens that involve T_H2 cells, immunoglobulin E (IgE), mast cells, and eosinophils. In these responses, the antigens induce $CD4^+$ T_H2 cells, which then help B cells to produce IgE antibodies that are specific for the antigens and bind to Fc receptors on mast cells and basophils. When these cell-associated IgE antibodies are cross-linked by antigen, the cells are activated to rapidly release a variety of mediators. These mediators collectively cause increased vascular permeability, vasodilation, and bronchial and visceral smooth muscle contraction. This reaction is called **immediate hypersensitivity** because it begins rapidly, within minutes of antigen challenge (immediate), and has major pathologic consequences (hypersensitivity). Following the immediate response, there is a more slowly developing inflammatory component called the **late-phase reaction** characterized by the accumulation of neutrophils, eosinophils, macrophages, and $CD4^+$ T_H2 cells. This later reaction is triggered by cytokines produced by the T_H2 cells and by mast cells as well as by lipid mediators secreted by mast cells. The term *immediate hypersensitivity* is commonly used to describe the combined immediate and late-phase reactions. In clinical medicine, these reactions are called **allergy** or **atopy**, and the associated diseases are called allergic, atopic, or immediate hypersensitivity diseases. Repeated bouts of these reactions can lead to chronic allergic diseases, with tissue damage and remodeling. Although atopy originally meant "unusual," we now realize that allergy is the most common disorder of immunity, affecting 20% of all individuals in the United States. This chapter focuses on immune reactions mediated by T_H2 cells, IgE, and mast cells. We begin by summarizing some important general features of IgE- and mast cell–dependent reactions and proceed to describe the production of IgE, the structure and functions of IgE-specific Fc receptors, and the cellular mediators of immediate hypersensitivity, including mast cells, basophils, and eosinophils. We then describe selected clinical syndromes associated with IgE- and mast cell–dependent reactions and the principles of therapy for these diseases. We conclude with a discussion of the physiologic role of IgE-mediated immune reactions in host defense.

GENERAL FEATURES OF IgE-DEPENDENT IMMUNE REACTIONS

All allergic reactions share common features, although they differ greatly in the types of antigens that elicit these reactions and their clinical and pathologic manifestations.

- ***The hallmarks of allergic diseases are the activation of T_H2 cells and the production of IgE antibody.*** Whereas healthy individuals either do not respond or have harmless T cell and antibody responses to common environmental antigens, atopic individuals develop strong T_H2 responses and produce IgE on exposure to these potentially allergenic substances.
- ***The typical sequence of events in immediate hypersensitivity consists of exposure to an antigen, activation of T_H2 cells and B cells specific for the antigen, production of IgE antibody, binding of the antibody to Fc receptors of mast cells, and triggering of the mast cells by re-exposure to the antigen, resulting in the release of mediators from the mast cells and the subsequent pathologic reaction*** (Fig. 19-1). Binding of IgE to mast cells is also called **sensitization** because IgE-coated mast cells are ready to be activated on antigen encounter (i.e., they are sensitive to the antigen). We describe each of these steps in the following sections.
- ***There is a strong genetic predisposition for the development of atopy.*** Many susceptibility genes are associated with these disorders. These genes are thought to influence different steps in the development and reactions of immediate hypersensitivity. We will discuss some of the major known susceptibility genes and their likely roles later in the chapter.
- ***The antigens that elicit immediate hypersensitivity, also called allergens, are usually common environmental proteins and chemicals that can modify proteins.*** Allergens include a wide variety of structurally distinct molecules.
- ***The cytokines produced by T_H2 cells are responsible for many of the features of immediate hypersensitivity.*** Immediate hypersensitivity is the prototypic T_H2-mediated disorder, in contrast to delayed-type hypersensitivity, which is the classical T_H1-mediated immune reaction.
- ***The clinical and pathologic manifestations of immediate hypersensitivity consist of the vascular and smooth muscle reaction that develops rapidly after repeated exposure to the allergen (the immediate reaction) and a delayed inflammatory reaction.*** All these reactions may be triggered by IgE-mediated mast cell activation, but different mediators are responsible for different components of the immediate and late-phase reactions. Because mast cells are present in connective tissues and under epithelia, these tissues are the most common sites of immediate hypersensitivity reactions. Some immediate hypersensitivity reactions may be triggered by nonimmunologic stimuli, such as exercise and exposure to cold. Such stimuli induce mast cell degranulation and the release of mediators without

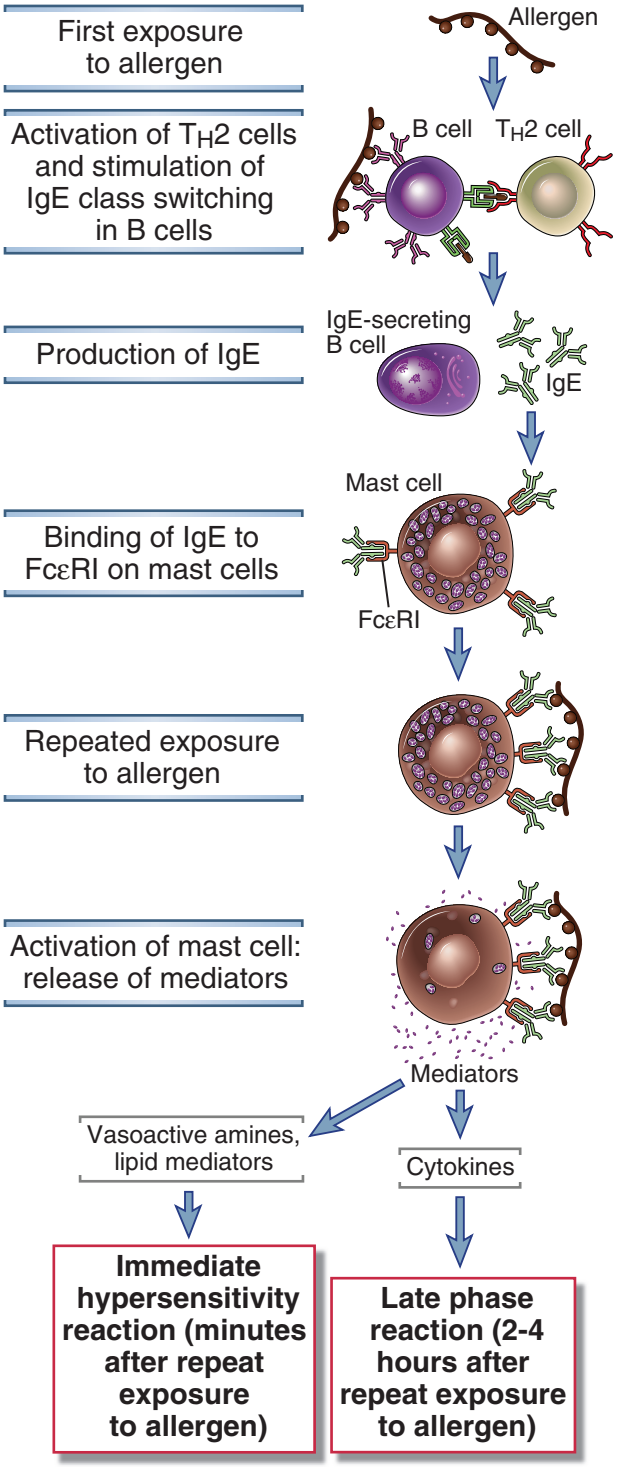


FIGURE 19–1 Sequence of events in immediate hypersensitivity reactions. Immediate hypersensitivity diseases are initiated by the introduction of an allergen, which stimulates T_H2 reactions and IgE production. IgE sensitizes mast cells by binding to FcεRI, and subsequent exposure to the allergen activates the mast cells to secrete the mediators that are responsible for the pathologic reactions of immediate hypersensitivity.

antigen exposure or IgE production. Such reactions are said to be nonatopic.

- ***Immediate hypersensitivity reactions are manifested in different ways, depending on the tissues affected, including rashes, sinus congestion, bronchial constriction, abdominal pain, diarrhea, and systemic shock.*** In the most extreme systemic form, called **anaphylaxis,** mast cell–derived mediators can restrict airways to the point of asphyxiation and produce cardiovascular collapse leading to death. (The term *anaphylaxis* was coined to indicate that antibodies, especially IgE antibodies, could confer the opposite of protection [prophylaxis] on an unfortunate individual.) We will return to the pathogenesis of these reactions later in the chapter.

With this introduction, we proceed to a description of the steps in the development and reactions of immediate hypersensitivity.

PRODUCTION OF IgE

Atopic individuals produce high levels of IgE in response to environmental allergens, whereas normal individuals generally synthesize other Ig isotypes, such as IgM and IgG, and only small amounts of IgE. Regulation of IgE synthesis depends on the propensity of an individual to mount a T_H2 response to allergens because T_H2 cell–derived cytokines stimulate heavy chain isotype switching to the IgE class in B cells. This propensity toward T_H2 responses against particular antigens may be influenced by a variety of factors, including inherited genes, the nature of the antigens, and the history of antigen exposure.

IgE antibody is responsible for sensitizing mast cells and provides recognition of antigen for immediate hypersensitivity reactions. IgE is the antibody isotype that contains the ε heavy chain (see Chapter 5), and of all the Ig isotypes, IgE is the most efficient at binding to Fc receptors on mast cells and activating these cells.

The Nature of Allergens

Antigens that elicit immediate hypersensitivity reactions (allergens) are proteins or chemicals bound to proteins to which the atopic individual is chronically exposed. Typical allergens include proteins in pollen, house dust mites, animal dander, foods, and chemicals like the antibiotic penicillin. It is not known why some antigens induce strong T_H2 responses and allergic reactions whereas others do not. Two important characteristics of allergens are that individuals are exposed to them repeatedly and, unlike microbes, they do not generally stimulate the innate immune responses that are associated with macrophage and dendritic cell secretion of T_H1- and T_H17-inducing cytokines. Chronic or repeated T cell activation in the absence of strong innate immunity may drive $CD4^+$ T cells toward the T_H2 pathway, as the T cells themselves make IL-4, the major T_H2-inducing cytokine (see Chapter 9).

The property of being allergenic may also reside in the chemical nature of the antigen itself. Although no structural characteristics of proteins can definitively predict whether they will be allergenic, some features are typical of many common allergens. These features include low to medium molecular weight (5 to 70 kD), stability, glycosylation, and high solubility in body fluids. Anaphylactic responses to foods are typically induced by highly glycosylated small proteins. These structural features probably protect the antigens from denaturation and degradation in the gastrointestinal tract and allow them to be absorbed intact. Curiously, many allergens, such as the cysteine protease of the house dust mite *Dermatophagoides pteronyssinus* and phospholipase A_2 in bee venom, are enzymes, but the importance of the enzymatic activity in triggering immediate hypersensitivity reactions is not known.

Because immediate hypersensitivity reactions are dependent on $CD4^+$ T cells, T cell–independent antigens such as polysaccharides cannot elicit these reactions unless they become attached to proteins. Some drugs, such as penicillin, often do elicit strong IgE responses. These drugs react chemically with amino acid residues in self proteins to form hapten-carrier conjugates, which stimulate T_H2 responses and IgE production.

The natural history of antigen exposure is an important determinant of the amount of specific IgE antibodies produced. Repeat exposure to a particular antigen is necessary for development of an allergic reaction to that antigen because switching to the IgE isotype and sensitization of mast cells with IgE must happen before a hypersensitivity reaction to an antigen can occur. Individuals with allergic rhinitis or asthma often benefit from a geographic change of residence with a change in indigenous plant pollens, although environmental antigens in the new residence may trigger an eventual return of the symptoms. A dramatic example of the importance of repeated exposure to antigen in allergic disease is seen in cases of bee stings. The proteins in the insect venoms are not usually of concern on the first encounter because an atopic individual has no preexisting specific IgE antibodies. However, an IgE response may occur after a single encounter with antigen, and a second sting by an insect of the same species may induce fatal anaphylaxis! Similarly, exposures to small amounts of peanuts can trigger fatal reactions in previously sensitized individuals.

Activation of T_H2 Cells

IgE synthesis is dependent on the activation of $CD4^+$ helper T cells of the T_H2 subset and their secretion of IL-4 and IL-13. It is likely that dendritic cells in epithelia through which allergens enter capture the antigens, transport them to draining lymph nodes, process them, and present peptides to naive $CD4^+$ T cells. The T cells then differentiate into T_H2 cells and into follicular helper T (T_{FH}) cells that secrete T_H2 cytokines. The major factors that drive differentiation of T_H2 cells are cytokines, especially IL-4, which may be produced by various cell types (see Chapter 9). In addition, the cytokine thymic stromal lymphopoietin, secreted by epithelial cells in the skin, gut, and lungs, enhances the ability of tissue dendritic

cells to promote T_H2 differentiation. T_H2 and T_{FH} cells then induce B cell switching to IgE through the actions of CD40 ligand and the cytokines IL-4 and IL-13.

T_H2 cells are involved in other components of the immediate hypersensitivity reaction in addition to promotion of switching to IgE. IL-5 secreted by T_H2 cells activates eosinophils, a cell type that is abundant in many immediate hypersensitivity reactions. IL-13 stimulates epithelial cells (e.g., in the airways) to secrete increased amounts of mucus, and excessive mucus production is also a common feature of these reactions. T_H2 cells also contribute to the inflammation of the late-phase reaction, described later.

Consistent with a central role of T_H2 cells in immediate hypersensitivity, larger numbers of allergen-specific IL-4–secreting T cells are found in the blood of atopic individuals than of nonatopic persons. In atopic patients, the allergen-specific T cells also produce more IL-4 per cell than in normal individuals. In animal models, a disease resembling human asthma can be induced by generation of T_H2 cells specific for an inhaled antigen or by adoptive transfer of these cells into naive mice. Accumulations of T_H2 cells are found at sites of immediate hypersensitivity reactions in the skin and bronchial mucosa.

Activation of B Cells and Switching to IgE

B cells specific for allergens are activated by T_H2 and T_{FH} cells, as in other T cell–dependent B cell responses (see Chapter 11). Under the influence of CD40 ligand and cytokines, mainly IL-4, produced by these helper T cells, the B cells undergo heavy chain isotype switching and produce IgE. IgE circulates as a bivalent antibody and is normally present in plasma at a concentration of less than 1 μg/mL. In pathologic conditions such as helminthic infections and severe atopy, this level can rise to more than 1000 μg/mL. Allergen-specific IgE produced by plasmablasts and plasma cells enters the circulation and binds to Fc receptors on tissue mast cells, so that these cells are sensitized and poised to react to a subsequent encounter with the allergen. Circulating basophils are also capable of binding IgE.

ROLE OF MAST CELLS, BASOPHILS, AND EOSINOPHILS IN IMMEDIATE HYPERSENSITIVITY

Mast cells, basophils, and eosinophils are the effector cells of immediate hypersensitivity reactions and allergic disease. Although each of these cell types has unique characteristics, all three contain cytoplasmic granules whose contents are the major mediators of allergic reactions, and all three cell types produce lipid mediators and cytokines that induce inflammation. T_H2 cells also function as effector cells of immediate hypersensitivity; their role has been discussed earlier. In this section of the chapter, we discuss the properties and functions of mast cells, basophils, and eosinophils (Table 19-1). Because mast cells are the major cell type responsible for immediate hypersensitivity reactions in tissues, much of our subsequent discussion focuses on mast cells.

Properties of Mast Cells and Basophils

All mast cells are derived from progenitors in the bone marrow. Normally, mature mast cells are not found in the circulation. Progenitors migrate to the peripheral tissues as immature cells and undergo differentiation in situ. Mature mast cells are found throughout the body, predominantly near blood vessels (Fig. 19-2A) and nerves and beneath epithelia. They are also present in lymphoid organs. Human mast cells vary in shape and have round nuclei, and the cytoplasm contains membrane-bound granules and lipid bodies. The granules contain acidic proteoglycans that bind basic dyes.

TABLE 19–1 Properties of Mast Cells, Basophils, and Eosinophils

Characteristic	Mast Cells	Basophils	Eosinophils
Major site of maturation	Connective tissue	Bone marrow	Bone marrow
Major cells in circulation	No	Yes (0.5% of blood leukocytes)	Yes (~2% of blood leukocytes)
Mature cells recruited into tissues from circulation	No	Yes	Yes
Mature cells residing in connective tissue	Yes	No	Yes
Proliferative ability of mature cells	Yes	No	No
Life span	Weeks to months	Days	Days to weeks
Major development factor (cytokine)	Stem cell factor, IL-3	IL-3	IL-5
Expression of FcεRI	High levels	High levels	Low levels (function unclear)
Major granule contents	Histamine, heparin and/or chondroitin sulfate, proteases	Histamine, chondroitin sulfate, protease	Major basic protein, eosinophil cationic protein, peroxidases, hydrolases, lysophospholipase

FcεRI, Fcε receptor type I; IL, interleukin.

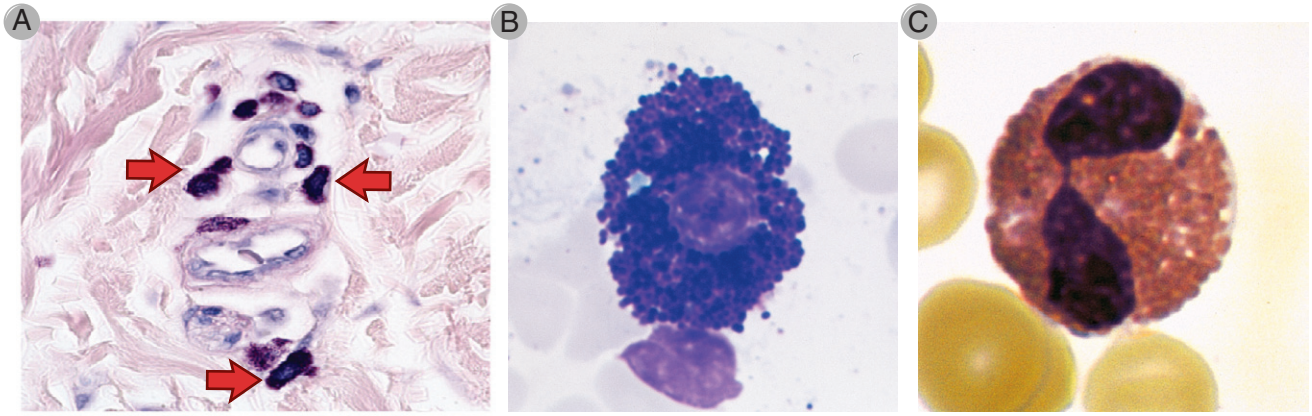


FIGURE 19–2 Morphology of mast cells, basophils, and eosinophils. Photomicrographs of Wright-Giemsa–stained perivascular dermal mast cells (**A,** *arrows*), peripheral blood basophil **(B)**, and peripheral blood eosinophil **(C)** are presented. Note the characteristic red staining of the cytoplasmic granules in the eosinophil and blue-staining cytoplasmic granules of the basophil. (***A*** *courtesy of Dr. George Murphy and* ***B*** *and* ***C*** *courtesy of Dr. Jonathan Hecht, Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts.)*

Activated mast cells secrete a variety of mediators that are responsible for the manifestations of allergic reactions (Table 19-2). These include substances that are stored in granules and rapidly released upon activation, and others that are synthesized upon activation. The production and actions of these mediators are described later.

Different mast cell populations exist that are distinguished by their anatomic locations, granule contents, and activities (Table 19-3). In rodents, two major subsets of mast cells have been described, one found in the mucosa of the gastrointestinal tract and the other in connective tissues. The mucosal mast cells have abundant chondroitin sulfate and little histamine in their granules. The development of mucosal mast cells in vivo depends on the cytokine IL-3 produced by T cells. Mast cells may be cultured from rodent bone marrow in the presence of IL-3, and such cultured mast cells resemble mucosal mast cells on the basis of the high granule content of chondroitin sulfate and low histamine concentration. The human counterpart of mucosal mast cells is most often identified by the presence of tryptase and the absence of other neutral proteases in the granules. In humans, the mucosal type of mast cells predominates in intestinal mucosa and alveolar spaces in the lung, and their presence is also T cell dependent. Connective tissue mast cells, also first identified in rodents, are found in the lung and in the serosa of body cavities. Their major granule proteoglycan is heparin, and they produce large quantities of histamine. Unlike mucosal mast cells, connective tissue mast cells show little T cell dependence. In humans, the corresponding subset is identified by the presence of several neutral proteases in the granules, including tryptase, chymase, cathepsin G–like protease, and carboxypeptidase. Human connective tissue mast cells are found in the skin and intestinal submucosa. Although the idea of these subsets has provided a valuable framework for studies of mast cells, it is clear that the populations are neither fixed nor clearly separable.

The locations, granule contents, and relative T cell dependence of different mast cell populations suggest that each may be important in a different set of disease processes. It is likely that mucosal-type mast cells are involved in T cell– and IgE-dependent immediate hypersensitivity diseases involving the airways, such as bronchial asthma, and other mucosal tissues. Conversely, connective tissue–type mast cells mediate immediate hypersensitivity reactions in the skin.

Basophils are blood granulocytes with structural and functional similarities to mast cells. Like other granulocytes, basophils are derived from bone marrow progenitors (a lineage different from that of mast cells), mature in the bone marrow, and circulate in the blood (Fig. 19-2B). Basophils constitute less than 1% of blood leukocytes. Although they are normally not present in tissues, basophils may be recruited to some inflammatory sites. Basophils contain granules that bind basic dyes, and they are capable of synthesizing many of the same mediators as mast cells (see Table 19-2). Like mast cells, basophils express FcεRI, bind IgE, and can be triggered by antigen binding to the IgE. Therefore, basophils that are recruited into tissue sites where antigen is present may contribute to immediate hypersensitivity reactions.

Binding of IgE to Mast Cells and Basophils: The Fcε Receptor

Mast cells and basophils express a high-affinity Fc receptor specific for ε heavy chains, called FcεRI, which binds IgE. IgE, like all other antibodies, is made exclusively by B cells, yet IgE functions as an antigen receptor on the surface of mast cells and basophils. This function is accomplished by IgE binding to FcεRI on these cells. The affinity of FcεRI for IgE is very high (dissociation constant [K_d] of about 1×10^{-10} M), much higher than that of any other Fc receptor for its antibody ligand. Therefore, the normal serum concentration of IgE, although low in comparison to other Ig isotypes (less than 5×10^{-10} M), is sufficient to allow occupancy of FcεRI receptors. In addition to mast cells and basophils, FcεRI has been detected on eosinophils, epidermal Langerhans cells, some dermal macrophages, and activated monocytes.

TABLE 19–2 Mediators Produced by Mast Cells, Basophils, and Eosinophils

Cell Type	Mediator Category	Mediator	Function/Pathologic Effects
Mast cells and basophils			
	Stored preformed in cytoplasmic granules	Histamine	Increases vascular permeability; stimulates smooth muscle cell contraction
		Enzymes: neutral proteases (tryptase and/or chymase), acid hydrolases, cathepsin G, carboxypeptidase	Degrade microbial structures; tissue damage/remodeling
	Major lipid mediators produced on activation	Prostaglandin D_2	Vasodilation, bronchoconstriction, neutrophil chemotaxis
		Leukotrienes C_4, D_4, E_4	Prolonged bronchoconstriction, mucus secretion, increased vascular permeability
		Platelet-activating factor	Chemotaxis and activation of leukocytes, bronchoconstriction, increased vascular permeability
	Cytokines produced on activation	IL-3	Mast cell proliferation
		TNF, MIP-1α	Inflammation/late-phase reaction
		IL-4, IL-13	IgE production, mucus secretion
		IL-5	Eosinophil production and activation
Eosinophils			
	Stored preformed in cytoplasmic granules	Major basic protein, eosinophil cationic protein	Toxic to helminths, bacteria, host cells
		Eosinophil peroxidase, lysosomal hydrolases, lysophospholipase	Degrades helminthic and protozoan cell walls; tissue damage/remodeling
	Major lipid mediators produced on activation	Leukotrienes C_4, D_4, E_4	Prolonged bronchoconstriction, mucus secretion, increased vascular permeability
	Cytokines produced on activation	IL-3, IL-5, GM-CSF	Eosinophil production and activation
		IL-8, IL-10, RANTES, MIP-1α, eotaxin	Chemotaxis of leukocytes

GM-CSF, granulocyte-monocyte colony-stimulating factor; IL, interleukin, MIP-1α, monocyte inflammatory protein 1α; RANTES, regulated by activation, normal T cell expressed and secreted; TNF, tumor necrosis factor.

The function of the receptor on many of these cells is not established.

Each FcεRI molecule is composed of an α chain that binds the Fc region of IgE and a β chain and two γ chains that are responsible for signaling (Fig. 19-3). The amino-terminal extracellular portion of the α chain includes two Ig-like domains that form the binding site for IgE. The β chain of FcεRI contains a single immunoreceptor tyrosine-based activation motif (ITAM) in the cytoplasmic carboxyl terminal domain. The two identical γ chain polypeptides are linked by a disulfide bond and are homologous to the ζ chain of the T cell antigen receptor complex (see Chapter 7). The cytoplasmic portion of each γ chain contains one ITAM. The same γ chain serves as

TABLE 19–3 Mast Cell Subsets

	Connective Tissue Mast Cells		Mucosal Mast Cells	
Characteristic	**Rodent**	**Human**	**Rodent**	**Human**
Location	Peritoneal cavity	Skin, intestinal submucosa	Intestinal mucosa	Alveoli, intestinal mucosa
T cell dependence for development of phenotype in tissues	No	No	Yes	Yes
Granule contents	High levels of histamine, heparin	Major neutral proteases: tryptase, chymase, carboxypeptidase, cathepsin G	Low levels of histamine; high levels of chondroitin sulfate	Major neutral protease: tryptase

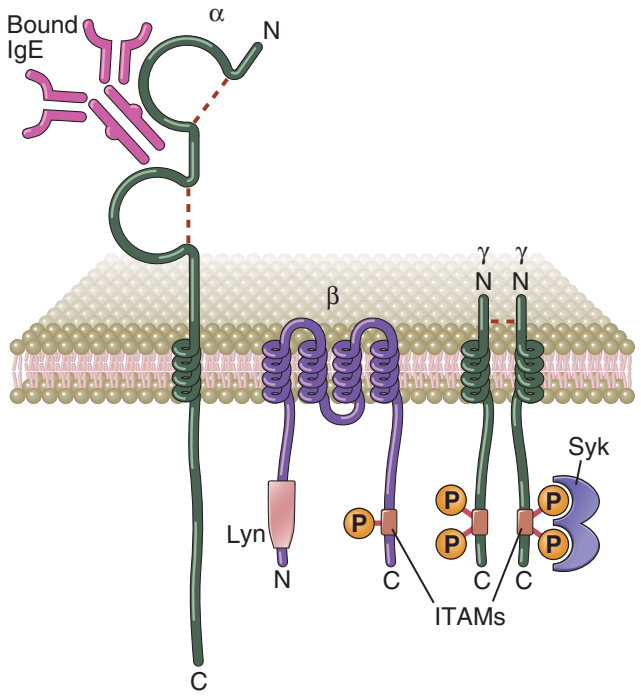


FIGURE 19–3 Polypeptide chain structure of the high-affinity IgE Fc receptor (FcεRI). IgE binds to the Ig-like domains of the α chain. The β chain and the γ chains mediate signal transduction. The boxes in the cytoplasmic region of the β and γ chains are ITAMs, similar to those found in the T cell receptor complex (see Fig. 7-5). Lyn and Syk are tyrosine kinases that bind to the β and γ chains, and participate in signaling events. A model structure of FcεRI is shown in Chapter 12.

the signaling subunit for FcγRI, FcγRIIIA, and FcαR and is called the FcR γ chain (see Chapter 12). Tyrosine phosphorylation of the ITAMs of the β and γ chains initiates the signaling cascade from the receptor that is required for mast cell activation, described next. The FcεRI on eosinophils and several other cell types lacks the β chain, so signaling is mediated only by the γ chains in these cells.

The importance of FcεRI in IgE-mediated immediate hypersensitivity reactions has been demonstrated in FcεRI α chain knockout mice. When these mice are given intravenous injections of IgE specific for a known antigen followed by that antigen, anaphylaxis does not develop or is mild, whereas it is a severe reaction in wild-type mice treated in the same way. FcεRI expression on the surface of mast cells and basophils is increased by IgE, thereby providing a mechanism for the amplification of IgE-mediated reactions.

Another IgE receptor called FcεRII, also known as CD23, is a protein related to C-type mammalian lectins whose affinity for IgE is much lower than that of FcεRI. The biologic role of FcεRII is not known.

Activation of Mast Cells

Mast cells are activated by cross-linking of FcεRI molecules, which occurs by binding of multivalent antigens to the IgE molecules that are attached to the Fc receptors (Fig. 19-4). In an individual allergic to a particular antigen, a large proportion of the IgE bound to FcεRI on the surface of mast cells is specific for that antigen. Exposure to the antigen will cross-link sufficient IgE molecules to trigger mast cell activation. In contrast, in nonatopic individuals, the IgE molecules bound to mast cells are specific for many different antigens, all of which may have induced low levels of IgE production. Therefore, no single antigen will cross-link enough of the IgE molecules to cause mast cell activation.

Immediate hypersensitivity reactions can be mimicked by injection of anti-IgE antibody instead of antigen.

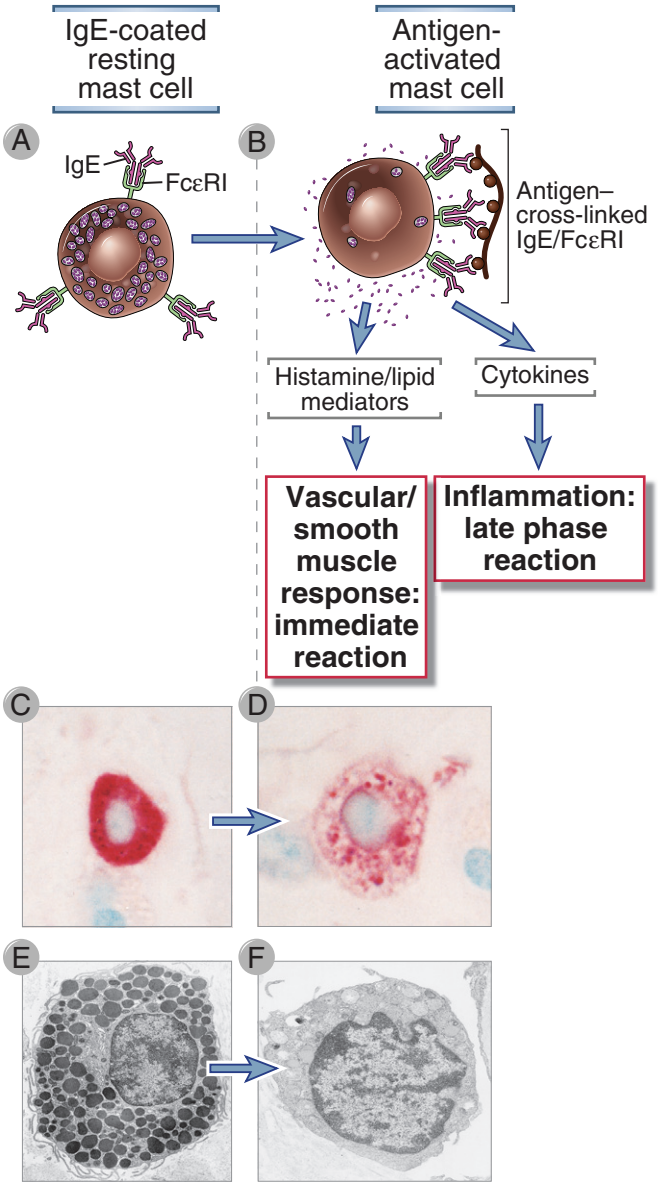


FIGURE 19–4 Mast cell activation. Antigen binding to IgE cross-links FcεRI molecules on mast cells, which induces the release of mediators that cause the hypersensitivity reaction **(A, B)**. Other stimuli, including the complement fragment C5a, can also activate mast cells. A light photomicrograph of a resting mast cell with abundant purple-staining cytoplasmic granules is shown in **C**. These granules are also seen in the electron micrograph of a resting mast cell shown in **E**. In contrast, the depleted granules of an activated mast cell are shown in the light photomicrograph **(D)** and electron micrograph **(F)**. *(Courtesy of Dr. Daniel Friend, Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts.)*

Anti-IgE antibodies act as an analogue of antigen and directly activate mast cells that have bound IgE on their surface. This use of anti-IgE to activate mast cells is similar to the use of anti-IgM antibodies as analogues of antigen to activate B cells, except that in the case of mast cells, secreted IgE, made by B cells, is bound to high-affinity Fc receptors on the cell surface rather than being synthesized as membrane IgE. Anti-IgE antibodies activate mast cells even in normal (nonatopic) individuals because, as mentioned earlier, mast cells are normally coated with IgE that can be cross-linked by the anti-IgE. In contrast, an antigen will activate mast cells only in individuals who are allergic to that antigen because only these individuals will produce enough specific IgE to be cross-linked by the antigen. Immediate hypersensitivity reactions can also be mimicked by the injection of other agents that directly activate mast cells, such as the complement fragments C5a, C4a, and C3a, called anaphylatoxins, or by local trauma, which also causes degranulation of mast cells. Conversely, these reactions can be inhibited by agents that prevent mast cell activation.

Activation of mast cells results in three types of biologic response: secretion of the preformed granule contents by exocytosis (degranulation), synthesis and secretion of lipid mediators, and synthesis and secretion of cytokines. The signaling cascades initiated by allergen-mediated FcεRI cross-linking are similar to the proximal signaling events initiated by antigen binding to lymphocytes (Fig. 19-5 and see Chapter 7). The Lyn tyrosine kinase is constitutively associated with the cytoplasmic tail of the FcεRI β chain. On cross-linking of FcεRI molecules by antigen, Lyn tyrosine kinase phosphorylates the ITAMs

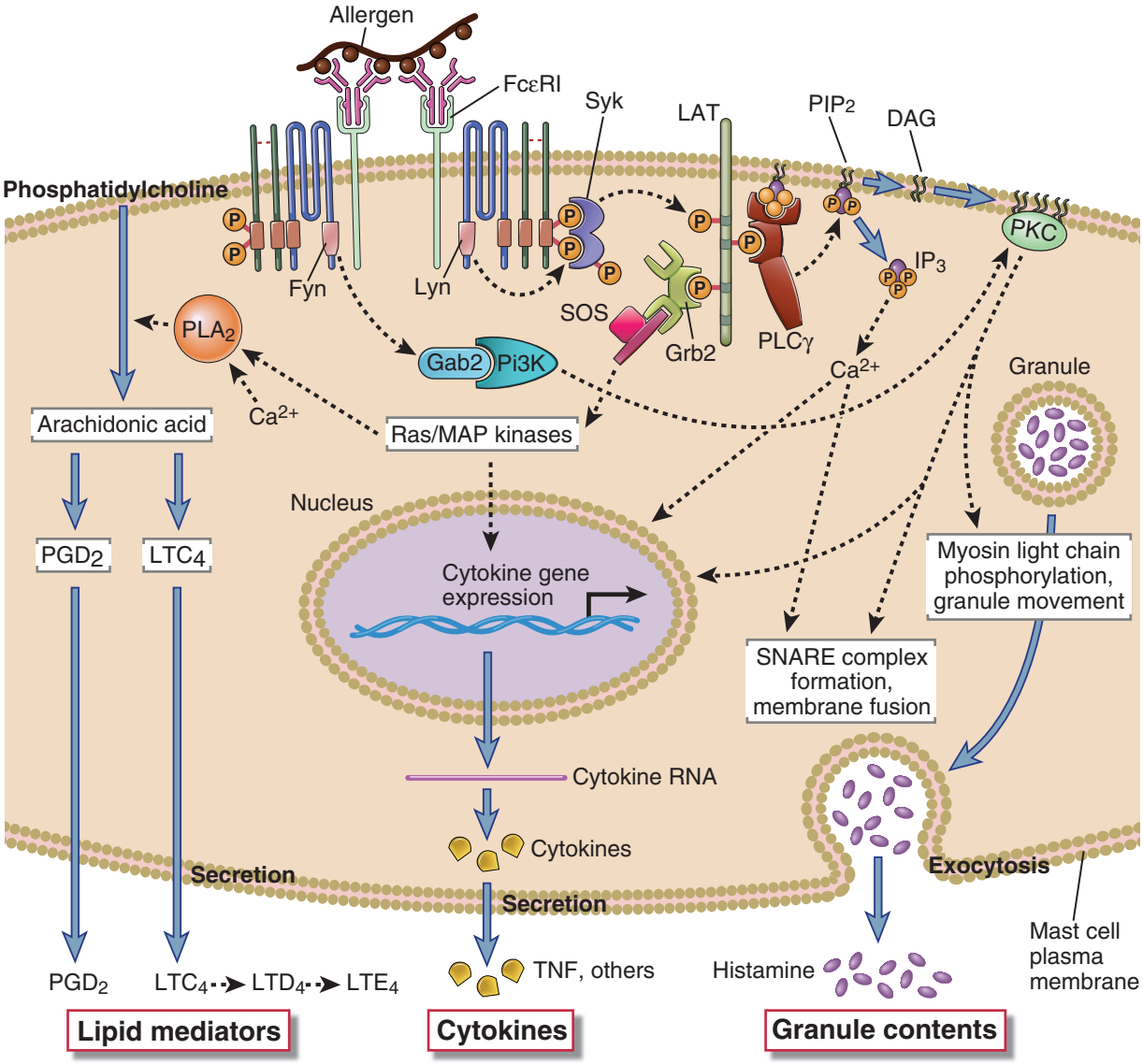


FIGURE 19–5 Biochemical events of mast cell activation. Cross-linking of bound IgE by antigen is thought to activate protein tyrosine kinases (Syk and Lyn), which in turn cause activation of a MAP kinase cascade and a phosphatidylinositol-specific phospholipase C (PI-PLCγ). PI-PLCγ catalyzes the release of IP3 and DAG from membrane PIP2. IP3 causes release of intracellular calcium from the endoplasmic reticulum. Calcium and DAG activate PKC, which phosphorylates substrates such as myosin light chain protein and thereby leads to the degradation and release of preformed mediators. Calcium and MAP kinases combine to activate the enzyme cytosolic phospholipase A_2 (PLA_2), which initiates the synthesis of lipid mediators, including prostaglandin D_2 (PGD_2) and leukotriene C_4 (LTC_4).

in the cytoplasmic domains of FcεRI β and γ chains. The Syk tyrosine kinase is then recruited to the ITAMs of the γ chain, becomes activated, and phosphorylates and activates other proteins in the signaling cascade, including several adaptor molecules and enzymes that participate in the formation of multicomponent signaling complexes, as described in T cells. Linker for activation of T cells (LAT) is one of the essential adaptor proteins involved in mast cell activation, and one of the enzymes recruited to LAT is the γ isoform of a phosphatidylinositol-specific phospholipase C (PLCγ). Once bound to LAT, PLCγ is phosphorylated and then catalyzes phosphatidylinositol bisphosphate breakdown to yield inositol trisphosphate (IP3) and diacylglycerol (DAG) (see Chapter 7). IP3 causes elevation of cytoplasmic calcium levels, and DAG activates protein kinase C (PKC). Another pathway of PKC activation in mast cells involves the tyrosine kinase Fyn, which phosphorylates the adaptor protein Grb-2–associated binder-like protein 2 (Gab2), which in turn binds phosphoinositide 3-kinase, leading to activation of PKC. Phosphorylation of the myosin light chains by activated PKC leads to disassembly of the actin-myosin complexes beneath the plasma membrane, thereby allowing granules to come in contact with the plasma membrane.

The mast cell granule membrane then fuses with the plasma membrane, a process that is mediated by members of the SNARE protein family, which are involved in many other membrane fusion events. Different SNARE proteins present on the granule and plasma membranes interact to form a multimeric complex that catalyzes fusion. The formation of SNARE complexes is regulated by several accessory molecules, including Rab3 guanosine triphosphatases and Rab-associated kinases and phosphatases. In resting mast cells, these regulatory molecules inhibit mast cell granule membrane fusion with the plasma membrane. On FcεRI cross-linking, the resulting increased cytoplasmic calcium concentrations and the activation of PKC block the regulatory functions of the accessory molecules. In addition, calcium sensor proteins, called synaptotagmins, respond to the elevated calcium concentrations by promoting SNARE complex formation and membrane fusion.

Following membrane fusion, the contents of the mast cell granules are released into the extracellular environment. This process can occur within seconds of FcεRI cross-linking, and can be visualized morphologically by loss of the dense granules of mast cells (see Fig. 19-4). The biologic actions of the mediators released upon mast cell degranulation are described later.

Synthesis of lipid mediators is controlled by activation of the cytosolic enzyme phospholipase A_2 (PLA_2) (see Fig. 19-5). This enzyme is activated by two signals: elevated cytoplasmic calcium and phosphorylation catalyzed by a mitogen-activated protein (MAP) kinase such as extracellular receptor-activated kinase (ERK). ERK is activated as a consequence of a kinase cascade initiated through the receptor ITAMs, probably using the same intermediates as in T cells (see Chapter 7). Once activated, PLA_2 hydrolyzes membrane phospholipids to release substrates that are converted by enzyme cascades into the ultimate mediators. The major substrate is arachidonic acid, which is converted by cyclooxygenase or lipoxygenase into different mediators (discussed later).

Cytokine production by activated mast cells is a consequence of newly induced cytokine gene transcription. The biochemical events that regulate cytokine gene transcription in mast cells appear to be similar to the events that occur in T cells. Recruitment and activation of various adaptor molecules and kinases in response to FcεRI cross-linking lead to nuclear translocation of nuclear factor of activated T cells (NFAT) and nuclear factor κB (NF-κB) as well as activation of activation protein 1 (AP-1) by protein kinases such as c-Jun N-terminal kinase. These transcription factors stimulate transcription of several cytokines (IL-4, IL-5, IL-6, IL-13, and tumor necrosis factor [TNF], among others) but, in contrast to T cells, not IL-2.

Mast cell activation through the FcεRI pathway is regulated by various inhibitory receptors, which contain an immunoreceptor tyrosine-based inhibition motif (ITIM) within their cytoplasmic tails (see Chapter 7). One such inhibitory receptor is FcγRIIB, which coaggregates with FcεRI during mast cell activation. The ITIM of FcγRIIB is phosphorylated by Lyn, and this leads to recruitment of the phosphatase called SH2 domain–containing inositol 5-phosphatase (SHIP) and inhibition of FcεRI signaling. Experiments in mice indicate that FcγRIIB regulates mast cell degranulation in vivo. Several other inhibitory receptors are also expressed on mast cells, but their importance in vivo is not yet known.

Mast cells can be directly activated by a variety of biologic substances independent of allergen-mediated cross-linking of FcεRI, including polybasic compounds, peptides, chemokines, and complement-derived anaphylatoxins. These additional modes of mast cell activation may be important in non–immune-mediated immediate hypersensitivity reactions, or they may amplify IgE-mediated reactions. Certain types of mast cells or basophils may respond to macrophage-derived chemokines, such as macrophage inflammatory protein 1α (MIP-1α), produced as part of innate immunity, and to T cell–derived chemokines, produced as part of adaptive cell-mediated immunity. The complement-derived anaphylatoxins, especially C5a, bind to specific receptors on mast cells and stimulate degranulation. These chemokines and complement fragments that activate mast cells are likely to be produced at sites of inflammation. Therefore, mast cell activation and release of mediators may amplify IgE-independent inflammatory reactions. Polybasic compounds, such as compound 48/40 and mastoparan, are used experimentally as pharmacologic triggers for mast cells. These agents contain a cationic region adjacent to a hydrophobic moiety, and they work by activating G proteins.

Many neuropeptides, including substance P, somatostatin, and vasoactive intestinal peptide, induce mast cell histamine release and may mediate neuroendocrine-linked mast cell activation. The nervous system is known to modulate immediate hypersensitivity reactions, and neuropeptides may be involved in this effect. The flare produced at the edge of the wheal in elicited immediate hypersensitivity reactions is in part mediated by the nervous system, as shown by the observation that it is

markedly diminished in skin sites lacking innervation. Cold temperatures and intense exercise may also trigger mast cell degranulation, but the mechanisms involved are not known.

Mast cells also express Fc receptors for IgG heavy chains, and the cells can be activated by cross-linking bound IgG. This IgG-mediated reaction is the likely explanation for the finding that Ig ε chain knockout mice are not completely resistant to antigen-induced mast cell–mediated anaphylaxis. However, IgE is the major antibody isotype involved in most immediate hypersensitivity reactions.

Mast cell activation is not an all-or-nothing phenomenon, and different types or levels of stimuli may elicit partial responses, with production of some mediators but not others. Such variations in activation and mediator release may account for variable clinical presentations.

Mediators Derived from Mast Cells

The effector functions of mast cells are mediated by soluble molecules released from the cells on activation (Fig. 19-6 and Table 19-2). These mediators may be divided into preformed mediators, which include biogenic amines and granule macromolecules, and newly synthesized mediators, which include lipid-derived mediators and cytokines.

Biogenic Amines

Many of the biologic effects of mast cell activation are mediated by biogenic amines that are stored in and released from cytoplasmic granules. Biogenic amines, sometimes called vasoactive amines, are low-molecular-weight compounds that contain an amine group. In human mast cells, the major mediator of this class is histamine, but in some rodents, serotonin may be of equal or greater import. Histamine acts by binding to target cell receptors, and different cell types express distinct classes of histamine receptors (e.g., H_1, H_2, H_3) that can be distinguished by their sensitivity to different pharmacologic inhibitors. The actions of histamine are short-lived because histamine is rapidly removed from the extracellular milieu by amine-specific transport systems. On binding to cellular receptors, histamine initiates intracellular events, such as phosphatidylinositol breakdown to IP3 and DAG, and these products cause different changes in different cell types. Binding of histamine to endothelium causes contraction of the endothelial cells, leading to increased interendothelial spaces, increased vascular permeability, and leakage of plasma into the tissues. Histamine also stimulates endothelial cells to synthesize vascular smooth muscle cell relaxants, such as prostacyclin (PGI_2) and nitric oxide, which cause vasodilation. These actions of histamine produce the wheal and flare response of immediate hypersensitivity (described later). H_1 histamine receptor antagonists (commonly called antihistamines) can inhibit the wheal and flare response to intradermal allergen or anti-IgE antibody. Histamine also causes constriction of intestinal and bronchial smooth muscle. Thus, histamine may contribute to the increased peristalsis and bronchospasm associated with ingested and inhaled allergens, respectively. However, in some allergic disorders, and especially in asthma, antihistamines are not effective at suppressing the reaction. Moreover, bronchoconstriction in asthma is more prolonged than are the effects of histamine, suggesting that other mast cell–derived mediators are important in some forms of immediate hypersensitivity.

Granule Enzymes and Proteoglycans

Neutral serine proteases, including tryptase and chymase, are the most abundant protein constituents of mast cell secretory granules and contribute to tissue damage in immediate hypersensitivity reactions. Tryptase is present in all human mast cells and is not known to be present in any other cell type. Consequently, the presence of tryptase in human biologic fluids is interpreted as a marker of mast cell activation. Chymase is found in some human mast cells, and its presence or absence is one criterion for characterizing human mast cell subsets, as discussed earlier. The functions of these enzymes in vivo are not known; however, several activities demonstrated in vitro suggest important biologic effects. For example, tryptase cleaves fibrinogen and activates collagenase, thereby causing tissue damage, whereas chymase can convert angiotensin I to angiotensin II, degrade epidermal basement membranes, and stimulate mucus secretion. Other enzymes found within mast cell granules include carboxypeptidase A and cathepsin G. Basophil granules also contain several enzymes, some of which are the same as those in mast cell granules, such as neutral proteases, and others are found in eosinophil granules, such as major basic protein and lysophospholipase.

Proteoglycans, including heparin and chondroitin sulfate, are also major constituents of both mast cell and basophil granules. These molecules are composed of a polypeptide core and multiple unbranched glycosaminoglycan side chains that impart a strong net negative charge to the molecules. Within the granules, proteoglycans serve as storage matrices for positively charged biogenic amines, proteases, and other mediators and prevent their accessibility to the rest of the cell. The mediators are released from the proteoglycans at different rates after granule exocytosis, biogenic amines dissociating much more rapidly than tryptase or chymase. In this way, the proteoglycans may control the kinetics of immediate hypersensitivity reactions.

Lipid Mediators

Mast cell activation results in the rapid de novo synthesis and release of lipid-derived mediators that have a variety of effects on blood vessels, bronchial smooth muscle, and leukocytes. The most important of these mediators are derived from arachidonic acid, which is generated by PLA_2-mediated hydrolysis of membrane phospholipids, as discussed earlier. Arachidonic acid is then metabolized by either the cyclooxygenase or lipoxygenase pathways to produce mediators of allergic reactions.

The major arachidonic acid–derived mediator produced by the cyclooxygenase pathway in mast cells is **prostaglandin D_2** (PGD_2). Released PGD_2 binds to receptors on smooth muscle cells and acts as a vasodilator

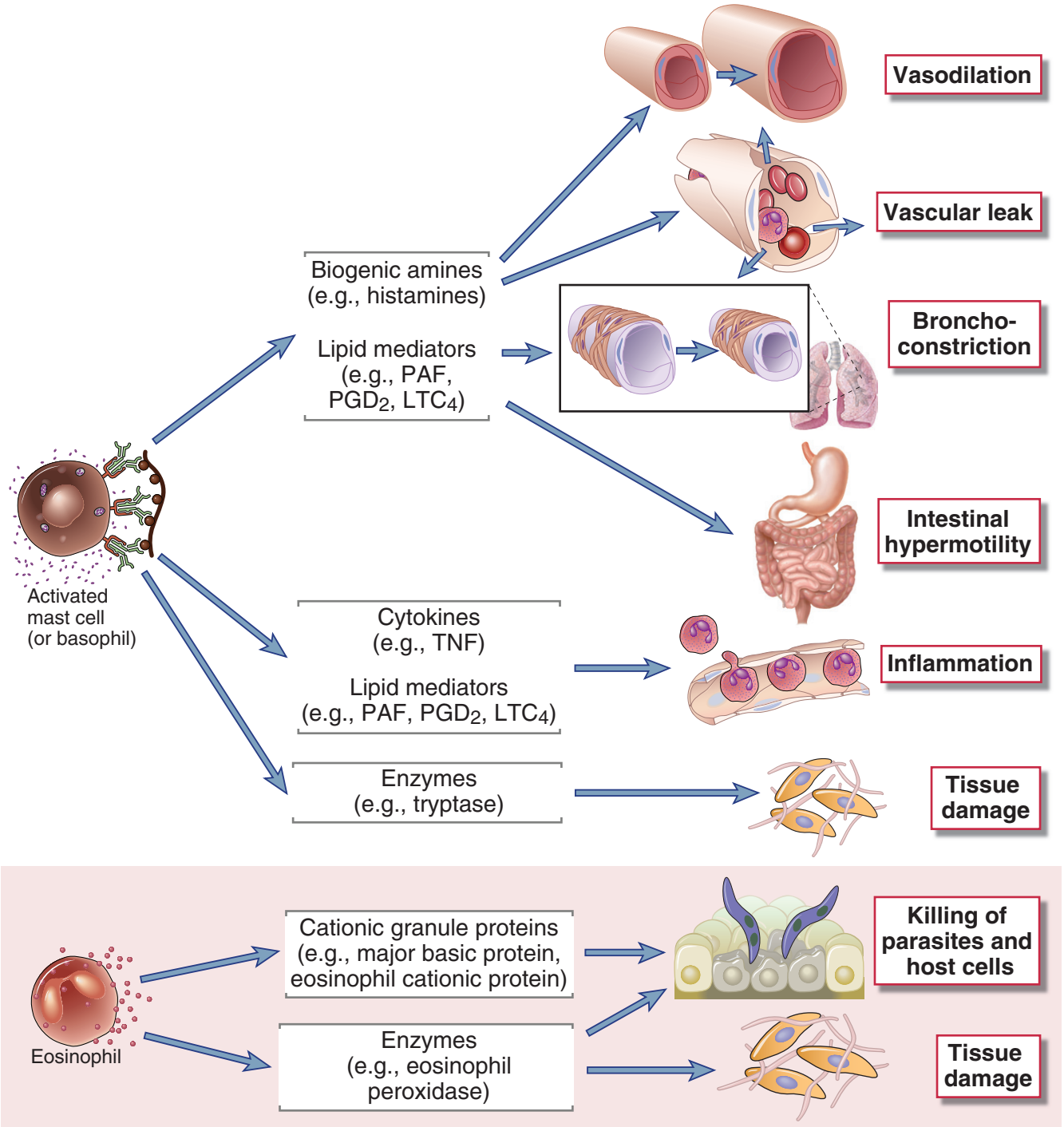


FIGURE 19–6 Biologic effects of mediators of immediate hypersensitivity. Mast cells and basophil mediators include biogenic amines and enzymes stored preformed in granules as well as cytokines and lipid mediators, which are largely newly synthesized on cell activation. The biogenic amines and lipid mediators induce vascular leakage, bronchoconstriction, and intestinal hypermotility, all components of the immediate response. Cytokines and lipid mediators contribute to inflammation, which is part of the late-phase reaction. Enzymes probably contribute to tissue damage. Activated eosinophils release preformed cationic proteins as well as enzymes that are toxic to parasites and host cells. Some eosinophil granule enzymes probably contribute to tissue damage in chronic allergic diseases.

and a bronchoconstrictor. PGD_2 also promotes neutrophil chemotaxis and accumulation at inflammatory sites. PGD_2 synthesis can be prevented by inhibitors of cyclooxygenase, such as aspirin and other nonsteroidal anti-inflammatory agents. These drugs may paradoxically exacerbate asthmatic bronchoconstriction because they shunt arachidonic acid toward production of leukotrienes, discussed next.

The major arachidonic acid–derived mediators produced by the lipoxygenase pathway are the **leukotrienes**, especially LTC_4 and its degradation products LTD_4 and LTE_4. LTC_4 is made by mucosal mast cells and

basophils but not by connective tissue mast cells. Mast cell–derived leukotrienes bind to specific receptors on smooth muscle cells, different from the receptors for PGD_2, and cause prolonged bronchoconstriction. Collectively, LTC_4, LTD_4, and LTE_4 constitute what was once called slow-reacting substance of anaphylaxis (SRS-A) and are thought to be important mediators of asthmatic bronchoconstriction. When injected into the skin, these leukotrienes produce a characteristic long-lived wheal and flare reaction. Pharmacologic inhibitors of 5-lipoxygenase also block anaphylactic reactions in experimental systems.

A third type of lipid mediator produced by mast cells is called **platelet-activating factor** (PAF) for its original bioassay as an inducer of rabbit platelet aggregation. In mast cells and basophils, PAF is synthesized by acylation of lysoglyceryl ether phosphorylcholine, a derivative of PLA_2-mediated hydrolysis of membrane phospholipids. PAF has direct bronchoconstricting actions. It also causes retraction of endothelial cells and can relax vascular smooth muscle. However, PAF is hydrophobic and is rapidly destroyed by a plasma enzyme called PAF hydrolase, which limits its biologic actions. Pharmacologic inhibitors of PAF receptors ameliorate some aspects of immediate hypersensitivity in the rabbit lung. Recent genetic evidence has pointed to PAF as a mediator of asthma. Asthma develops in early childhood in individuals with an inherited deficiency of PAF hydrolase. PAF may also be important in late-phase reactions, in which it can activate inflammatory leukocytes. In this situation, the source of PAF may be basophils or vascular endothelial cells (stimulated by histamine or leukotrienes), in addition to mast cells.

Cytokines

Mast cells (and basophils) produce many different cytokines that contribute to allergic inflammation (the late-phase reaction). These cytokines include TNF, IL-1, IL-4, IL-5, IL-6, IL-13, CCL3, CCL4, and various colony-stimulating factors such as IL-3 and granulocyte-monocyte colony-stimulating factor (GM-CSF). As mentioned before, mast cell activation induces transcription and synthesis of these cytokines, but preformed TNF may also be stored in granules and rapidly released on FcεRI cross-linking. T_H2 cells that are recruited into the sites of allergic reactions also produce some of these cytokines. The cytokines that are released from activated mast cells, basophils, and T_H2 cells are mainly responsible for the inflammation associated with late-phase reaction. TNF activates endothelial expression of adhesion molecules and together with chemokines accounts for neutrophil and monocyte infiltrates (see Chapter 3). In addition to allergic inflammation, mast cell cytokines also apparently contribute to the innate immune responses to infections. For example, as we will discuss later, mouse models indicate that mast cells are required for effective defense against some bacterial infections, and this effector function is mediated largely by TNF.

Properties of Eosinophils

Eosinophils are bone marrow–derived granulocytes that are abundant in the inflammatory infiltrates of late-phase reactions and are involved in many of the pathologic processes in allergic diseases. Eosinophils develop in the bone marrow, and after maturation they circulate in the blood. GM-CSF, IL-3, and IL-5 promote eosinophil maturation from myeloid precursors. Eosinophils are normally present in peripheral tissues, especially in mucosal linings of the respiratory, gastrointestinal, and genitourinary tracts, and their numbers can increase by recruitment in the setting of inflammation. The granules of eosinophils contain basic proteins that bind acidic dyes such as eosin (see Table 19-2 and Fig. 19-2C).

Cytokines produced by T_H2 cells promote the activation of eosinophils and their recruitment to late-phase reaction inflammatory sites. IL-5 is a potent eosinophil-activating cytokine, which enhances the ability of eosinophils to release granule contents. IL-5 also increases maturation of eosinophils from bone marrow precursors, and in the absence of this cytokine (e.g., in IL-5 knockout mice), there is a deficiency of eosinophil numbers and functions. Eosinophils are recruited into late-phase reaction sites as well as sites of helminthic infection, and their recruitment is mediated by a combination of adhesion molecule interactions and chemokines. Eosinophils bind to endothelial cells expressing E-selectin and VCAM-1, the ligand for the VLA-4 integrin. IL-4 produced by T_H2 cells may enhance expression of adhesion molecules for eosinophils. Eosinophil recruitment and infiltration into tissues also depend on the chemokine eotaxin (CCL11), which is produced by epithelial cells at sites of allergic reactions and binds to the chemokine receptor CCR3, which is expressed constitutively by eosinophils. In addition, the complement product C5a and the lipid mediators PAF and LTB_4, which are produced by mast cells, also function as chemoattractants for eosinophils.

Eosinophils release granule proteins that are toxic to parasitic organisms and may injure normal tissue. Eosinophils express Fc receptors for IgG, IgA, and IgE, and are presumably able to respond to cross-linking of these receptors by antigen binding the receptor-associated antibodies. FcεRI on human eosinophils lacks the β chain, a signaling component of the receptor, and it is unclear how efficiently these cells degranulate in response to IgE cross-linking. The granule contents of eosinophils include lysosomal hydrolases found in other granulocytes as well as eosinophil-specific proteins that are particularly toxic to helminthic organisms, including major basic protein and eosinophil cationic protein. These two cationic polypeptides have no known enzymatic activities, but they are toxic to helminths and bacteria, as well as normal tissue. In addition, eosinophilic granules contain eosinophil peroxidase, which is distinct from the myeloperoxidase found in neutrophils and catalyzes the production of hypochlorous or hypobromous acid. These products are also toxic to helminths, protozoa, and host cells.

Activated eosinophils, like mast cells and basophils, produce and release lipid mediators, including PAF, prostaglandins, and the leukotrienes LTC_4 and its derivatives LTD_4 and LTE_4. These eosinophil-derived lipid mediators may contribute to the pathologic processes of allergic diseases. Eosinophils also produce a variety of cytokines that may promote inflammatory responses and

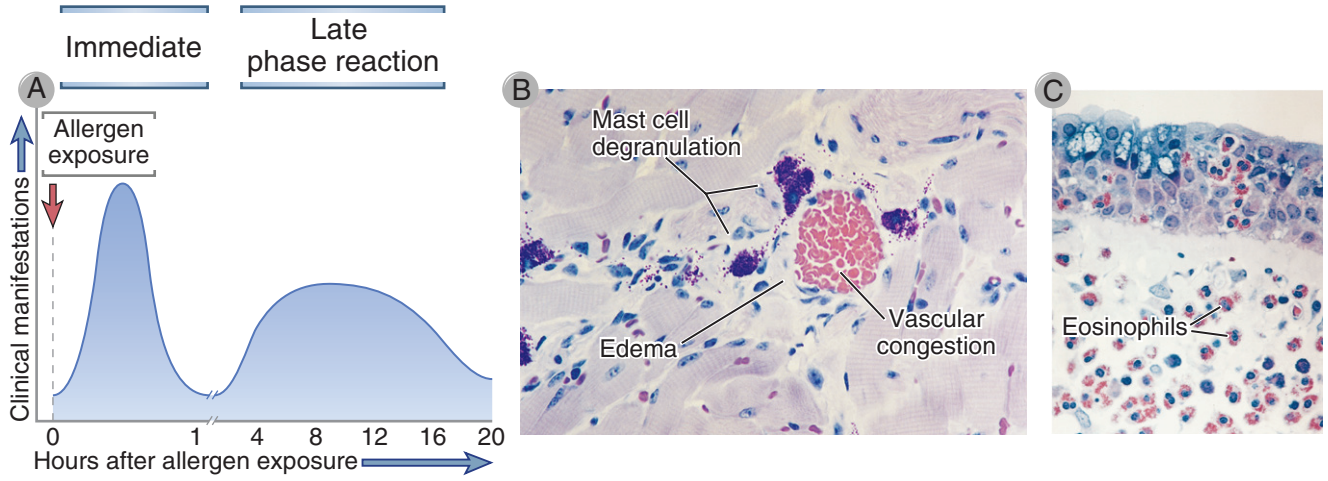


FIGURE 19–7 The immediate and late-phase reactions of allergy. A, Kinetics. The immediate vascular and smooth muscle reaction to allergen develops within minutes after challenge (allergen exposure in a previously sensitized individual), and the late-phase reaction develops 2 to 24 hours later. **B, C,** Morphology. The immediate reaction **(B)** is characterized by vasodilation, congestion, and edema, and the late-phase reaction **(C)** is characterized by an inflammatory infiltrate rich in eosinophils, neutrophils, and T cells. *(Courtesy of Dr. Daniel Friend, Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts.)*

tissue repair, but the biologic significance of eosinophil cytokine production is not known.

IgE- AND MAST CELL–DEPENDENT REACTIONS

The cells and mediators we have discussed are responsible for the immediate vascular changes and late inflammatory responses that typify allergic reactions. In the following sections we will describe in detail these immediate and late-phase reactions (Fig. 19-7).

The Immediate Reaction

The early vascular changes that occur during immediate hypersensitivity reactions are demonstrated by the wheal and flare reaction to the intradermal injection of an allergen (Fig. 19-8). When an individual who has previously encountered an allergen and produced IgE antibody is challenged by intradermal injection of the same antigen, the injection site becomes red from locally dilated blood vessels engorged with red blood cells. The site then rapidly swells as a result of leakage of plasma from the venules. This soft swelling is called a wheal and can involve an area of skin as large as several centimeters in diameter. Subsequently, blood vessels at the margins of the wheal dilate and become engorged with red blood cells and produce a characteristic red rim called a flare. The full wheal and flare reaction can appear within 5 to 10 minutes after administration of antigen and usually subsides in less than an hour.

The wheal and flare reaction is dependent on IgE and mast cells. Histologic examination shows that mast cells in the area of the wheal and flare have released preformed mediators; that is, their cytoplasmic granules have been discharged. A causal association of IgE and mast cells with immediate hypersensitivity was first deduced from experiments involving the passive transfer of IgE antibodies from an allergic individual into a normal recipient. For example, immediate hypersensitivity reactions against an allergen can be elicited in unresponsive individuals if the local skin site is first injected with IgE from an allergic individual. Such adoptive transfer experiments were first performed with serum from immunized individuals, and the serum factor responsible for the reaction was originally called reagin. For this reason, IgE molecules are still sometimes called reaginic antibodies. The antigen-initiated skin reaction that follows adoptive transfer of IgE is called passive cutaneous anaphylaxis.

The wheal and flare reaction results from sensitization of dermal mast cells by IgE bound to FcεRI, cross-linking of the IgE by the antigen, and activation of mast cells with release of mediators, notably histamine. Histamine binds to histamine receptors on venular endothelial cells; the endothelial cells synthesize and release PGI_2, nitric oxide, and PAF; and these mediators cause vasodilation and vascular leak, as described earlier. Skin mast cells appear to produce only small amounts of long-acting mediators such as leukotrienes, so the wheal and flare response subsides rapidly. Allergists often test patients for allergies to different antigens by examining the ability of these antigens applied in skin patches to elicit wheal and flare reactions.

The Late-Phase Reaction

The immediate wheal and flare reaction is followed 2 to 4 hours later by a late-phase reaction consisting of the accumulation of inflammatory leukocytes, including neutrophils, eosinophils, basophils, and T_H2 cells (see Fig. 19-7). The inflammation is maximal by about 24 hours and then gradually subsides. The capacity to mount a late-phase reaction can also be adoptively transferred with IgE, and the reaction can be mimicked by anti-IgE antibodies or mast cell–activating agents, like the immediate wheal and flare reaction. Cytokines produced by mast cells, including TNF, upregulate endothelial expression of leukocyte adhesion molecules, such as E-selectin

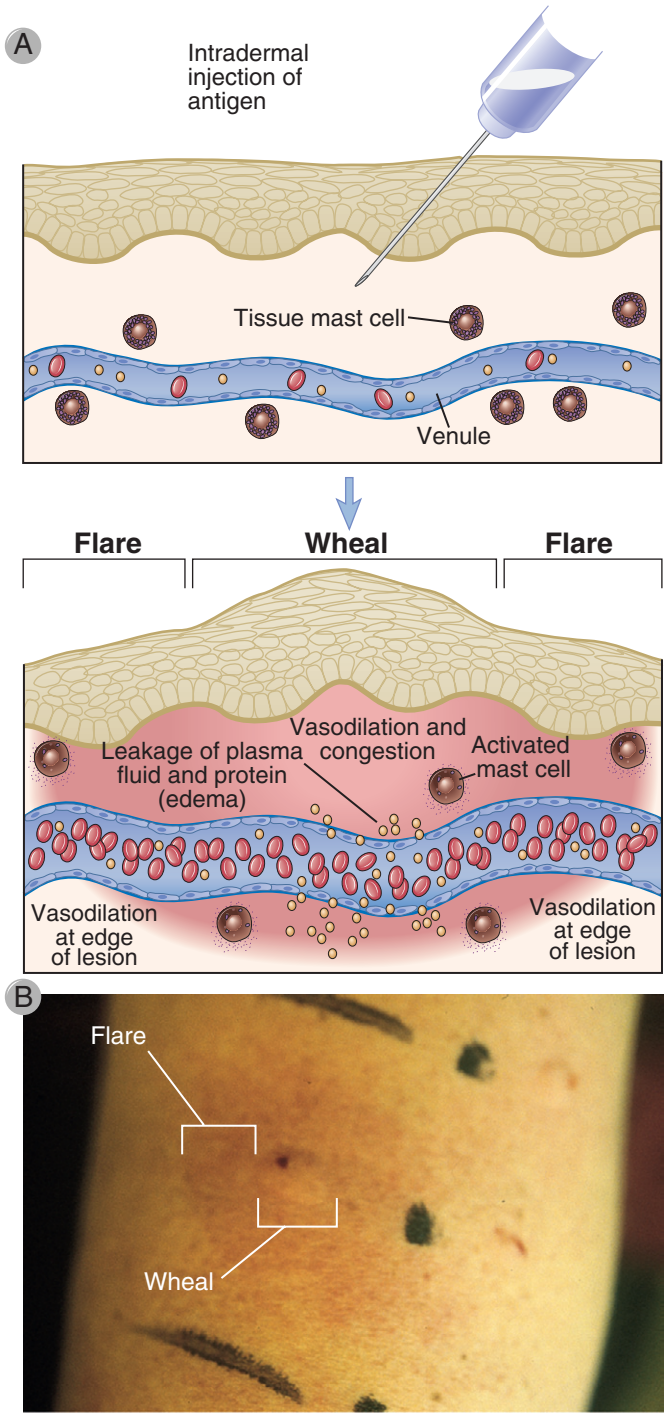


FIGURE 19–8 The wheal and flare reaction in the skin. A, In response to antigen-stimulated release of mast cell mediators, local blood vessels first dilate and then become leaky to fluid and macromolecules, which produces redness and local swelling (a wheal). Subsequent dilation of vessels on the edge of the swelling produces the appearance of a red rim (the flare). **B,** Photograph of a typical wheal and flare reaction in the skin in response to injection of an allergen. *(Courtesy of Dr. James D. Faix, Department of Pathology, Stanford University School of Medicine, Palo Alto, California.)*

and intercellular adhesion molecule 1 (ICAM-1), and chemokines that recruit blood leukocytes. Thus, mast cell activation promotes the recruitment of leukocytes into tissues. The types of leukocytes that are typical of late-phase reactions are eosinophils and T_H2 cells; in addition, neutrophils are often present in these reactions. Eosinophils and T_H2 cells both express CCR4 and CCR3, and the chemokines that bind to these receptors are produced by many cell types at sites of immediate hypersensitivity reactions, including epithelial cells. The late-phase reaction differs from delayed-type hypersensitivity reactions, in which macrophages and T_H1 cells are dominant.

The late-phase reaction may occur without a detectable preceding immediate hypersensitivity reaction. Bronchial asthma is a disease in which there may be repeated bouts of inflammation with accumulations of eosinophils and T_H2 cells without the vascular changes that are characteristic of the immediate response. In such disorders, there may be little mast cell activation, and the cytokines that sustain the late-phase reaction may be produced mainly by T cells.

GENETIC SUSCEPTIBILITY TO IMMEDIATE HYPERSENSITIVITY

The propensity to develop allergies is influenced by the inheritance of several genes. Abnormally high levels of IgE synthesis and associated atopy often run in families. Family studies have shown clear autosomal transmission of atopy, although the full inheritance pattern is multigenic. Within the same family, the target organ of atopic disease is variable. Thus, hay fever, asthma, and eczema can be present to various degrees in different members of the same kindred. All these individuals, however, will show higher than average plasma IgE levels.

Studies of the genetics of allergic diseases in populations have included genome-wide linkage analyses and, more recently, genome-wide association studies. Linkage analyses for atopy/asthma susceptibility loci identified several chromosomal regions of importance in allergic disease (Table 19-4). Each of these loci may contain several genes that may contribute to the disease. Some of the genes in these loci may regulate T_H2 responses and IgE production. Other genes may have tissue-specific influences, such as airway remodeling in the asthmatic lung. One of the susceptibility loci for atopy is on chromosome 5q, near the site of the gene cluster encoding the cytokines IL-3, IL-4, IL-5, IL-9, and IL-13 and the IL-4 receptor. This region is of great interest because of the connection between several of the genes located here and the mechanisms of IgE regulation and mast cell and eosinophil growth and differentiation. Furthermore, the homologous chromosomal region in mice has been linked to a propensity for $CD4^+$ T cells in some inbred strains of mice to differentiate into T_H2 cells in response to model protein antigens. Among the genes in this cluster, polymorphisms in the IL-13 gene appear to have the strongest association with asthma. The tendency to produce IgE antibodies against some but not all antigens, such as ragweed pollen, may be linked to particular class II major histocompatibility complex (MHC) alleles. This linkage

TABLE 19–4 Examples of Genes Associated with Atopy and Asthma

Candidate Genes or Encoded Protein	Chromosomal Location	Disease Association	Putative Role of Gene Products in Disease
Cytokine gene cluster (IL-4, IL-5, IL-13), CD14, β_2-adrenergic receptor	5q	Asthma	IL-4 and IL-13 promote IgE switching, IL-5 promotes eosinophil growth and activation; CD14 is a component of the LPS receptor that, through interaction with TLR4, may influence the balance between T_H1 and T_H2 responses to antigens; β_2-adrenergic receptor regulates bronchial smooth muscle contraction
Class II MHC	6p	Asthma	Some alleles may regulate T cell responses to allergens
FcεRI β chain	11q	Asthma	Mediates mast cell activation
Stem cell factor, interferon-γ, STAT6	12q	Asthma	Stem cell factor regulates mast cell growth and differentiation; interferon-γ opposes actions of IL-4; STAT6 mediates IL-4 signal transduction
IL-4 receptor α chain	16	Asthma	Subunit of both IL-4 and IL-13 receptors
ADAM33	20p	Asthma	Metalloproteinase involved in airway remodeling
DPP10		Asthma	Peptidase that may regulate chemokine and cytokine activity
PHF11	13q	Asthma	Transcriptional regulator involved in B cell clonal expansion and Ig expression
ORMDL3	17q	Asthma	Unknown
IL-1 receptor–like 1	2q	Asthma	Membrane receptor that mediates effects of IL-1 on T cells
Phosphodiesterase 4D	5q	Asthma	Degrades cAMP and regulates airway smooth muscle contractility
Filaggrin		Atopic dermatitis	Component of terminally differentiated keratinocytes

may be an example of an "immune response gene" (*Ir* gene) effect in which atopic individuals inherit class II MHC alleles that can bind and display dominant epitopes of certain allergens (see Chapter 6). Several other chromosomal regions have been shown to be associated with high serum IgE levels, atopy, and asthma. In addition to these linkage studies, individual asthma-associated genes have been identified by positional cloning. These include *ADAM33, DPP10,* and *PHF11* (see Table 19-4). Although there are highly significant associations of polymorphisms of these genes with asthma, the way in which the products of the genes contribute to disease is not yet understood.

Some genes whose products regulate the innate immune response to infections have been associated with allergy and asthma. These include CD14, a component of the lipopolysaccharide receptor, and TIM-1, a protein thought to influence helper T cell differentiation. Strong innate responses to infections generally favor development of T_H1 responses and inhibit T_H2 responses (see Chapter 9). Therefore, polymorphisms or mutations in genes that result in diminished innate responses to common infectious organisms may increase the risk for development of atopy.

Genome-wide association studies of asthma have found associations with variants of the genes encoding ORMDL3, a ubiquitous protein of unknown function; IL-1 receptor–like 1, which may mediate some effects of IL-1 on T cells; and phosphodiesterase 4D, which is a regulator of airway smooth muscle contractility (see Table 19-4).

ALLERGIC DISEASES IN HUMANS: PATHOGENESIS AND THERAPY

Mast cell degranulation is a central component of all allergic diseases, and the clinical and pathologic manifestations of the diseases depend on the tissues in which the mast cell mediators have effects as well as the chronicity of the resulting inflammatory process. Atopic individuals may have one or more manifestations of allergic disease. The most common forms of these diseases are allergic rhinitis (hay fever), bronchial asthma, atopic dermatitis (eczema), and food allergies. The clinical and pathologic features of allergic reactions vary with the anatomic site of the reaction, for several reasons. The point of contact with the allergen determines the organs or tissues that are involved. For example, inhaled antigens cause rhinitis or asthma, ingested antigens often cause vomiting and diarrhea, and injected antigens cause systemic effects on the circulation. The concentration of mast cells in various target organs influences the severity of responses. Mast cells are particularly abundant in the skin and the mucosa of the respiratory and gastrointestinal tracts, and these tissues frequently suffer the most injury in immediate hypersensitivity reactions. The local mast cell phenotype may influence the characteristics of the immediate hypersensitivity reaction. For example, connective tissue mast cells produce abundant histamine and are responsible for wheal and flare reactions in the skin. In the following section, we discuss the major features of allergic diseases manifested in different tissues.

Systemic Anaphylaxis

Anaphylaxis is a systemic immediate hypersensitivity reaction characterized by edema in many tissues and a fall in blood pressure, secondary to vasodilation. These effects usually result from the systemic presence of antigen introduced by injection, an insect sting, or absorption across an epithelial surface such as the skin or gut mucosa. The allergen activates mast cells in many tissues, resulting in the release of mediators that gain access to vascular beds throughout the body. The decrease in vascular tone and leakage of plasma caused by the released mediators can lead to a fall in blood pressure or shock, called anaphylactic shock, which is often fatal. The cardiovascular effects are accompanied by constriction of the upper and lower airways, laryngeal edema, hypermotility of the gut, outpouring of mucus in the gut and respiratory tract, and urticarial lesions (hives) in the skin. It is not known which mast cell mediators are the most important in anaphylactic shock. The mainstay of treatment is systemic epinephrine, which can be lifesaving by reversing the bronchoconstrictive and vasodilatory effects of mast cell mediators. Epinephrine also improves cardiac output, further aiding survival from threatened circulatory collapse. Antihistamines may also be beneficial in anaphylaxis, suggesting a role for histamine in this reaction. In some animal models, PAF receptor antagonists offer partial protection.

Bronchial Asthma

Asthma is an inflammatory disease caused by repeated immediate-phase hypersensitivity and late-phase allergic reactions in the lung leading to the clinicopathologic triad of intermittent and reversible airway obstruction, chronic bronchial inflammation with eosinophils, and bronchial smooth muscle cell hypertrophy and hyperreactivity to bronchoconstrictors (Fig. 19-9). Patients suffer paroxysms of bronchial constriction and increased production of thick mucus, which leads to bronchial obstruction and exacerbates respiratory difficulties. Asthma frequently coexists with bronchitis or emphysema, and the combination of these diseases can cause severe damage to lung tissue. Affected individuals may suffer considerable morbidity, and asthma can be fatal. Asthma affects about 10 million people in the United States, and the frequency of this disease has increased significantly in recent years. The prevalence rate is similar to that in other industrialized countries, but it may be lower in less developed areas of the world. One possible explanation for the increased prevalence of asthma and other atopic diseases in industrialized countries is that the frequency of infections in these countries is generally lower. As we discussed earlier, the innate immune responses associated with most infections promote T_H1 responses and suppress T_H2 responses required for development of atopy. A variety of epidemiologic data are consistent with the idea that reducing infections leads to increased prevalence of allergic disease. This idea has been given the catchy name *the hygiene hypothesis*.

About 70% of cases of asthma are associated with IgE-mediated immediate hypersensitivity. In the remaining 30% of patients, asthma may not be associated with atopy and may be triggered by nonimmune stimuli such as drugs, cold, and exercise. Even among nonatopic asthmatics, the pathophysiologic process of airway

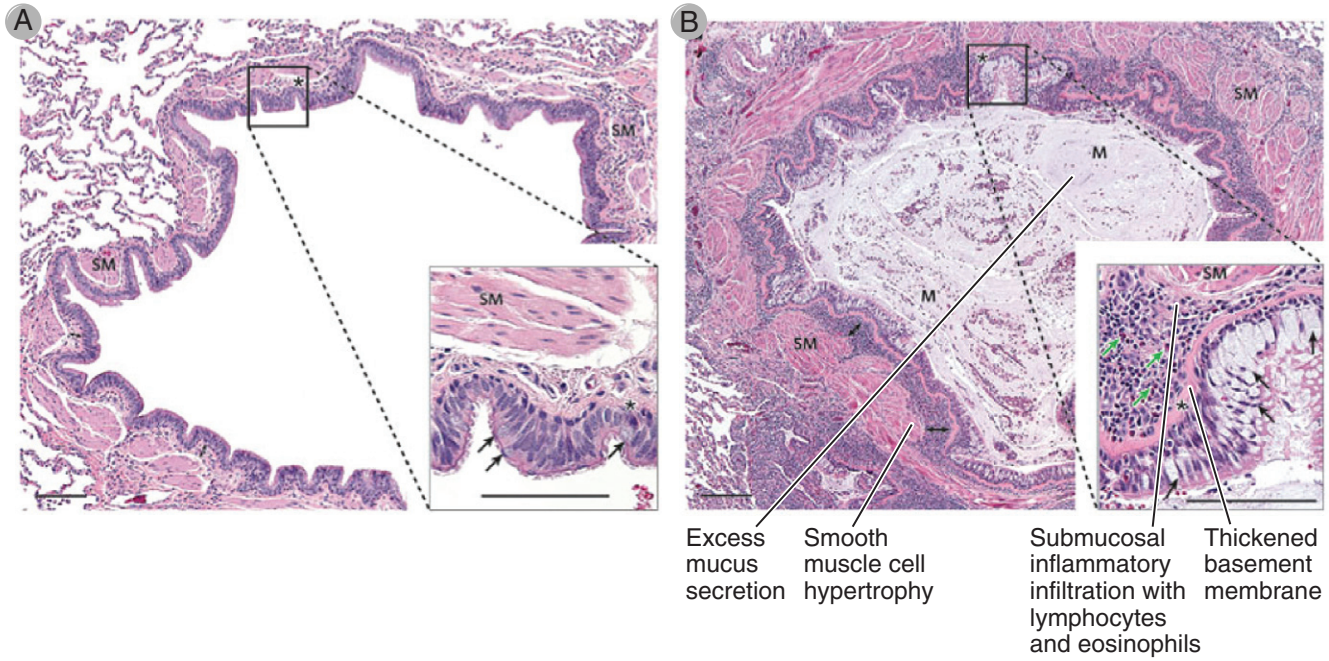


FIGURE 19–9 Histopathologic features of bronchial asthma. Atopic bronchial asthma results from repeated immediate hypersensitivity reactions in the lungs with chronic late-phase reactions. A cross-section of a normal bronchus is shown in **A;** a bronchus from a patient with asthma is shown in **B.** The diseased bronchus has excessive mucus (M) production, many submucosal inflammatory cells (including eosinophils), and smooth muscle (SM) hypertrophy, and many more goblet cells than in the normal bronchus (black arrows in insets). *(From Galli SJ, M Tsai, and AM Piliponsky. The development of allergic inflammation. Nature 454:445-454, 2008. Courtesy of G. J. Berry, Stanford University, California.)*

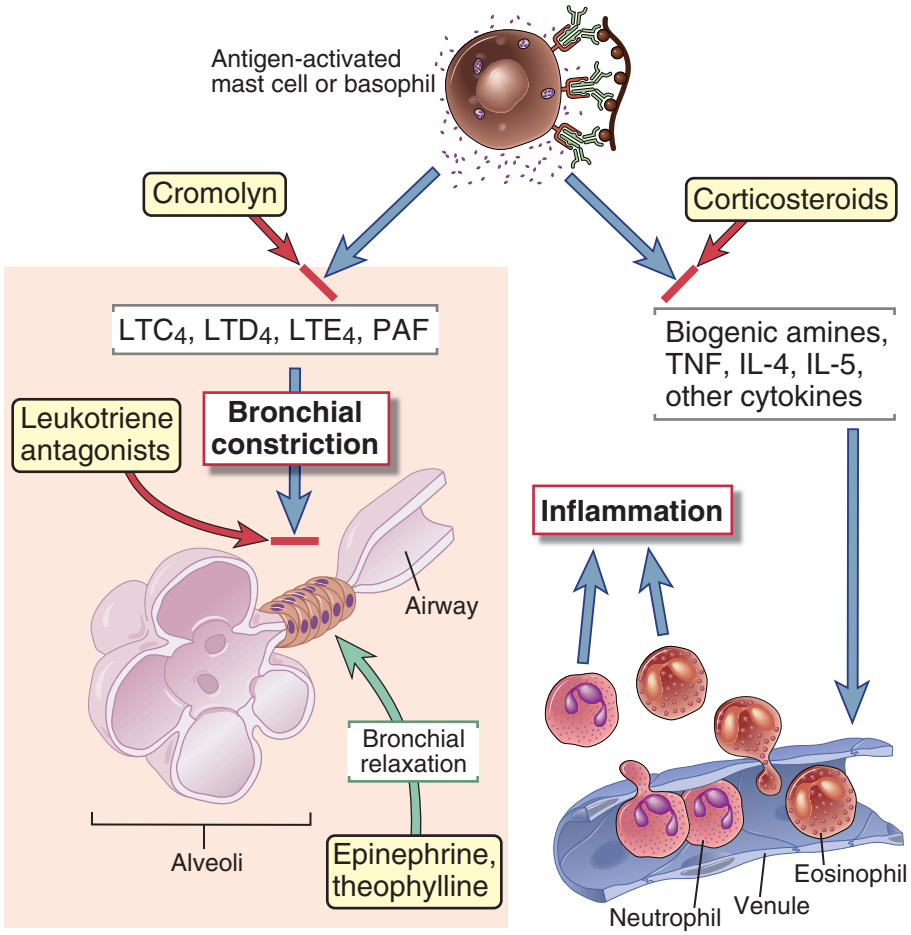


FIGURE 19–10 Mediators and treatment of asthma. Mast cell–derived leukotrienes and PAF are thought to be the major mediators of acute bronchoconstriction. Therapy is targeted both at reducing mast cell activation with inhibitors such as cromolyn and at countering mediator actions on bronchial smooth muscle by bronchodilators such as epinephrine and theophylline. These drugs also inhibit mast cell activation. Mast cell–derived cytokines are thought to be the major mediators of sustained airway inflammation, which is an example of a late-phase reaction, and corticosteroid therapy is used to inhibit cytokine synthesis. Cytokines are also produced by T_H2 cells (not shown).

constriction is similar, which suggests that alternative mechanisms of mast cell degranulation (e.g., by locally produced neurotransmitters) may underlie the disease.

The pathophysiologic sequence in atopic asthma is probably initiated by mast cell activation in response to allergen binding to IgE as well as by T_H2 cells reacting to allergens (Fig. 19-10). The lipid mediators and cytokines produced by the mast cells and T cells lead to the recruitment of eosinophils, basophils, and more T_H2 cells. The chronic inflammation in this disease may continue without mast cell activation. There is experimental evidence that other T cell subsets, including T_H1 and T_H17 cells, as well as IL-9–secreting T cells, contribute to the pathology of established disease. Smooth muscle cell hypertrophy and hyperreactivity are thought to result from leukocyte-derived mediators and cytokines. Mast cells, basophils, and eosinophils all produce mediators that constrict airway smooth muscle. The most important of the bronchoconstricting mediators are LTC_4, its breakdown products LTD_4 and LTE_4, and PAF. In clinical experiments, antagonists of LTC_4 synthesis or leukotriene receptor antagonists prevent allergen-induced airway constriction. Increased mucus secretion results from the action of cytokines, mainly IL-13, on bronchial epithelial cells.

Respiratory viral and bacterial infections are a predisposing factor in the development of asthma or exacerbations of preexisting asthma. For example, it is estimated that respiratory viral infections precede up to 80% of asthma attacks in children. It is not known if these infections increase the chances for development of asthma or just exacerbate preexisting asthma. The association of asthma with infections appears inconsistent with the hygiene hypothesis, mentioned earlier. It would be expected that infections stimulate innate immunity and thus suppress T_H2 responses. This apparent paradox reflects our incomplete understanding of the T cell populations that contribute to different stages of asthma.

Current therapy for asthma has two major targets: prevention and reversal of inflammation and relaxation of airway smooth muscle. In recent years, the balance of therapy has shifted toward anti-inflammatory agents as the primary mode of treatment. Several classes of drugs are in current use to treat asthma. Inhaled corticosteroids block the production of inflammatory cytokines. Corticosteroids may also be given systemically, especially once

an attack is under way, to reduce inflammation. Bronchial smooth muscle cell relaxation has been achieved principally by elevating intracellular cyclic adenosine monophosphate (cAMP) levels in smooth muscle cells, which inhibits contraction. The major drugs used are activators of adenylate cyclase, such as epinephrine and related β_2-adrenergic agents, and theophylline, which inhibits phosphodiesterase enzymes that degrade cAMP. Theophylline may also have anti-inflammatory effects unrelated to its effects on smooth muscle cell relaxation that contribute to its effectiveness. Leukotriene inhibitors block the binding of bronchoconstricting leukotrienes to airway smooth muscle cells. Humanized monoclonal anti-IgE antibody is an approved therapy that effectively reduces serum IgE levels in patients. Because histamine has little role in airway constriction, antihistamines (H_1 receptor antagonists) are not useful in the treatment of asthma. Indeed, because many antihistamines are also anticholinergics, these drugs may worsen airway obstruction by causing thickening of mucus secretions.

Immediate Hypersensitivity Reactions in the Upper Respiratory Tract, Gastrointestinal Tract, and Skin

Allergic rhinitis, also called hay fever, is perhaps the most common allergic disease and is a consequence of immediate hypersensitivity reactions to common allergens such as plant pollen or house dust mites localized to the upper respiratory tract by inhalation. The pathologic and clinical manifestations include mucosal edema, leukocyte infiltration with abundant eosinophils, mucus secretion, coughing, sneezing, and difficulty in breathing. Allergic conjunctivitis with itchy eyes is commonly associated with the rhinitis. Focal protrusions of the nasal mucosa, called nasal polyps, filled with edema fluid and eosinophils may develop in patients who suffer frequent repetitive bouts of allergic rhinitis. Antihistamines are the most common drugs used to treat allergic rhinitis.

Food allergies are immediate hypersensitivity reactions to ingested foods that lead to the release of mediators from intestinal mucosal and submucosal mast cells. Clinical manifestations include enhanced peristalsis, increased fluid secretion from intestinal lining cells, and associated vomiting and diarrhea. Urticaria is often associated with allergic reactions to food, and systemic anaphylaxis may occasionally ensue. Allergic reactions to many different types of food have been described, but some of the most common are peanuts and shellfish. Individuals may be so sensitive to these allergens that severe systemic reactions can occur in response to minute contaminants of the allergen introduced accidentally during food preparation.

Allergic reactions in the skin are manifested as **urticaria** and **eczema**. Urticaria, which is essentially an acute wheal and flare reaction induced by mast cell mediators, occurs in response to direct contact with the allergen or after an allergen enters the circulation through the intestinal tract or by injection. Because the reaction that ensues is mediated largely by histamine, antihistamines (H_1 receptor antagonists) can block this response almost completely. The urticaria may persist for several hours, probably because antigen persists in the plasma. Chronic eczema (also called atopic dermatitis) is a common skin disorder that may be caused by a late-phase reaction to an allergen in the skin. In the cutaneous late-phase reaction, TNF, IL-4, and other cytokines, probably derived from T_H2 cells and mast cells, act on the venular endothelial cells to promote inflammation. As may be expected for a cytokine-mediated response, the late-phase inflammatory reaction is not inhibited by antihistamines. It can be blocked by treatment with corticosteroids, which inhibit cytokine synthesis. Chronic contact sensitivity, which is a T_H1-mediated DTH reaction, is also often called eczema. Another term used for immune-mediated skin disorders is atopic dermatitis; not all the cases are examples of atopy.

Immunotherapy for Allergic Diseases

In addition to therapy aimed at the consequences of immediate hypersensitivity, mentioned before, clinical immunologists often try to limit the onset of allergic reactions by treatments aimed at reducing the quantity of IgE produced in the patient. Several empirical protocols have been developed to diminish synthesis of allergen-specific IgE. In one approach, called **desensitization**, small quantities of antigen are repeatedly administered subcutaneously. As a result of this treatment, specific IgE levels decrease and IgG titers often rise, perhaps further inhibiting IgE production by neutralizing the antigen and by antibody feedback (see Chapter 11). It is possible that desensitization may work by inducing specific T cell tolerance or by changing the predominant phenotype of antigen-specific T cells from T_H2 to T_H1; however, there is no clear evidence to support any of these hypotheses. The beneficial effects of desensitization may occur in a matter of hours, much earlier than changes in IgE levels. The precise mechanism is unknown, but this approach has been effective in preventing acute anaphylactic responses to protein antigens (e.g., insect venom) or vital drugs (e.g., penicillin). Although many people with more common chronic atopic conditions, such as hay fever and asthma, benefit from desensitization therapy, the overall effectiveness for these disorders is more variable.

Other approaches being used to reduce IgE levels include systemic administration of humanized monoclonal anti-IgE antibodies mentioned earlier.

THE PROTECTIVE ROLES OF IgE- AND MAST CELL–MEDIATED IMMUNE REACTIONS

Although most of our understanding of mast cell– and basophil-mediated responses comes from analysis of immediate hypersensitivity, it is logical to assume that these responses have evolved because they provide protective functions. In fact, some evidence shows that IgE- and mast cell–mediated responses are important for defense against certain types of infection. Much of this evidence comes from studies in mice deficient in IgE, T_H2 cytokines, or mast cells.

A major protective function of IgE-initiated immune reactions is the eradication of helminthic parasites. Eosinophil-mediated killing of helminths is an effective defense against these organisms (Fig. 19-11 and see

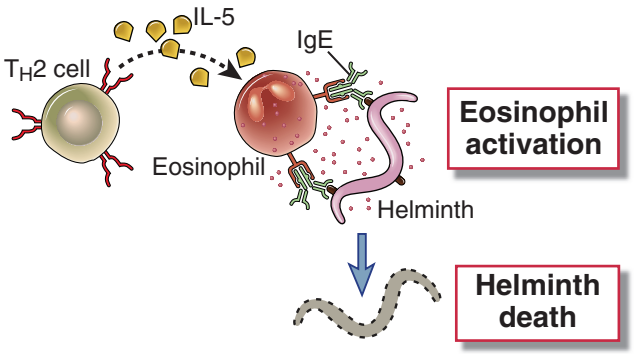


FIGURE 19–11 Activation of eosinophils to kill helminths. IL-5 secreted by T_H2 cells enhances the ability of helminths to kill the parasites. Crosslinking FcεR1 on eosinophils by IgE bound to helminth antigens may also induce eosinophil degranulation, releasing enzymes toxic to the parasites.

Chapter 10). The activities of IL-4 and IL-13 in IgE production and IL-5 in eosinophil activation contribute to a coordinated defense against helminths. In addition, IgE-dependent mast cell activation in the gastrointestinal tract promotes the expulsion of parasites by increasing peristalsis and by an outpouring of mucus. Studies in mice have highlighted the beneficial roles of IgE and mast cells. For example, mice treated with anti–IL-4 antibody and IL-4 knockout mice do not make IgE and appear to be more susceptible than normal animals to some helminthic infections. IL-5 knockout mice, which are unable to activate eosinophils, also show increased susceptibility to some helminths. Furthermore, genetically mast cell–deficient mice show increased susceptibility to infection by tick larvae, and immunity can be provided to these mice by adoptive transfer of specific IgE and mast cells (but not by either component alone). The larvae are eradicated by the late-phase reaction.

Mast cells play an important protective role as part of the innate immune response to bacterial infections. Studies in mice have indicated that mast cells can be activated by IgE-independent mechanisms in the course of an acute bacterial infection and that the mediators they release are critical for clearing the infection. Mast cell–deficient mice are less capable of clearing and are more likely to die of acute bacterial infection of the peritoneum than are normal mice. The protective role of mast cells in this setting is mediated by TNF and depends on TNF-stimulated influx of neutrophils into the peritoneum, specifically, the late-phase reaction. The mechanisms by which mast cells are activated during innate immune responses to bacterial infection are not known but may involve complement activation by the alternative pathway, leading to the release of C5a, which directly triggers mast cell degranulation. It is also possible that the classical pathway of complement could be activated by natural antibodies that are produced by B-1 B cells and that recognize common microbial pathogens. Bacterial products may also activate mast cells by binding to Toll-like receptors expressed by the mast cells.

Mast cell–derived proteases have been shown to destroy some snake and insect venoms in mice. This is an unusual form of "innate immunity" against a potentially lethal encounter with nonmicrobial organisms.

SUMMARY

- Immediate hypersensitivity is an immune reaction triggered by antigen binding to IgE pre-bound to mast cells, which leads to the release of inflammatory mediators.
- The steps in the development of immediate hypersensitivity are exposure to an antigen (allergen) that stimulates T_H2 responses and IgE production, binding of the IgE to Fcε receptors on mast cells, cross-linking of the IgE and the Fcε receptors by the allergen, activation of mast cells, and release of mediators.
- Individuals susceptible to immediate hypersensitivity reactions are called atopic and often have more IgE in the blood and more IgE-specific Fc receptors per mast cell than do nonatopic individuals. IgE synthesis is induced by exposure to antigen and T_H2 cytokines, particularly IL-4.
- Mast cells are derived from bone marrow precursors that mature in tissues. They express high-affinity receptors for IgE (FcεRI) and contain cytoplasmic granules in which are stored various inflammatory mediators. Subsets of mast cells, including mucosal and connective tissue mast cells, may produce different mediators. Basophils are a type of circulating granulocyte that expresses high-affinity Fcε receptors and contains granules with contents similar to mast cells.
- Eosinophils are a special class of granulocyte; they are recruited into inflammatory reactions by chemokines and IL-4 and are activated by IL-5. Eosinophils are effector cells that are involved in killing parasites. In allergic reactions, eosinophils contribute to tissue injury.
- On binding of antigen to IgE on the surface of mast cells or basophils, the high-affinity Fcε receptors become cross-linked and activate intracellular second messengers that lead to granule release and new synthesis of mediators. Activated mast cells and basophils produce three important classes of mediators: biogenic amines, such as histamine; lipid mediators, such as prostaglandins, leukotrienes, and PAF; and cytokines, such as TNF, IL-4, IL-13, and IL-5.
- Biogenic amines and lipid mediators cause the rapid vascular and smooth muscle reactions of immediate hypersensitivity, such as vasodilation, vascular leakage and edema, bronchoconstriction, and gut hypermotility. Cytokines released by mast cells and T_H2 cells mediate the late-phase reaction, which is an inflammatory reaction involving neutrophil and eosinophil infiltration.
- Various organs show distinct forms of immediate hypersensitivity involving different mediators and target cell types. The most severe form is a systemic reaction called anaphylactic shock. Asthma is a manifestation of immediate hypersensitivity and late-phase reactions in the lung. Allergic rhinitis (hay fever) is the most common allergic disease of the upper respiratory tract. Food

allergens can cause diarrhea and vomiting. In the skin, immediate hypersensitivity is manifested as wheal and flare and late-phase reactions and may lead to chronic eczema.

- Drug therapy is aimed at inhibiting mast cell mediator production and at blocking or counteracting the effects of released mediators on target organs. The goal of immunotherapy is to prevent or to reduce T_H2 cell responses to specific allergens and the production of IgE.
- Immediate hypersensitivity reactions provide protection against helminthic infections by promoting IgE- and eosinophil-mediated antibody-dependent cell-mediated cytotoxicity and gut peristalsis. Mast cells may also play a role in innate immune responses to bacterial infections.

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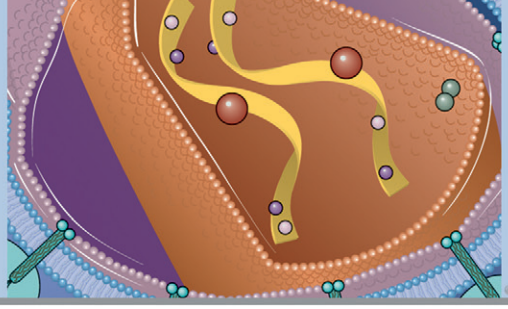
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CHAPTER

20

Congenital and Acquired Immunodeficiencies

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SUMMARY, 469

Integrity of the immune system is essential for defense against infectious organisms and their toxic products and therefore for the survival of all individuals. Defects in one or more components of the immune system can lead to serious and often fatal disorders, which are collectively called **immunodeficiency diseases**. These diseases are broadly classified into two groups. The **congenital**, or **primary, immunodeficiencies** are genetic defects that result in an increased susceptibility to infection that is frequently manifested early in infancy and childhood but is sometimes clinically detected later in life. It is estimated that in the United States, approximately 1 in 500 individuals is born with a defect in some component of the immune system, although only a small proportion are affected severely enough for development of life-threatening complications. **Acquired,** or **secondary, immunodeficiencies** are not inherited diseases but develop as a consequence of malnutrition, disseminated cancer, treatment with immunosuppressive drugs, or infection of cells of the immune system, most notably with the human immunodeficiency virus (HIV), the etiologic agent of acquired immunodeficiency syndrome (AIDS). This chapter describes the major types of congenital and acquired immunodeficiencies, with an emphasis on their pathogenesis and the components of the immune system that are involved in these disorders.

GENERAL FEATURES OF IMMUNODEFICIENCY DISEASES

Before beginning our discussion of individual diseases, it is important to summarize some general features of immunodeficiencies.

- ***The principal consequence of immunodeficiency is an increased susceptibility to infection.*** The nature of the infection in a particular patient depends largely on the component of the immune system that is defective (Table 20-1). Deficient humoral immunity usually results in increased susceptibility to infection by encapsulated, pus-forming bacteria and some viruses, whereas defects in cell-mediated immunity lead to infection by viruses and other intracellular microbes. Combined deficiencies in both humoral and cell-mediated immunity make patients susceptible to infection by all classes of microorganisms. Immunodeficient patients often present with infections by microbes that are commonly encountered but effectively eliminated by healthy persons; such infections are said to be opportunistic.

TABLE 20–1 Features of Immunodeficiencies Affecting T or B Lymphocytes

Feature	B Cell Deficiency	T Cell Deficiency
Susceptibility to infection	Pyogenic bacteria (otitis, pneumonia, meningitis, osteomyelitis), enteric bacteria and viruses, some parasites	*Pneumocystis jiroveci*, many viruses, atypical mycobacteria, fungi
Diagnosis		
Serum Ig levels	Reduced	Normal or reduced
DTH reactions to common antigens	Normal	Reduced
Morphology of lymphoid tissues	Absent or reduced follicles and germinal centers (B cell zones)	Usually normal follicles, may be reduced parafollicular cortical regions (T cell zones)

DTH, delayed-type hypersensitivity.

- ***Patients with immunodeficiencies are also susceptible to certain types of cancer.*** Many of these cancers appear to be caused by oncogenic viruses, such as the Epstein-Barr virus. An increased incidence of cancer is most often seen in T cell immunodeficiencies because, as discussed in Chapter 17, T cells play an important role in surveillance against oncogenic viruses and the tumors they cause.
- ***Paradoxically, certain immunodeficiencies are associated with an increased incidence of autoimmunity.*** The mechanism underlying this association is not known.
- ***Immunodeficiency may result from defects in lymphocyte development or activation or from defects in the effector mechanisms of innate and adaptive immunity.*** Immunodeficiency diseases are clinically and pathologically heterogeneous, in part because different diseases involve different components of the immune system.

In this chapter, we first describe congenital immunodeficiencies, including defects in components of the innate immune system, and defects in the humoral and cell-mediated arms of the adaptive immune system. We conclude with a discussion of acquired immunodeficiencies, with an emphasis on AIDS.

CONGENITAL (PRIMARY) IMMUNODEFICIENCIES

In different congenital immunodeficiencies, the causative abnormality may be in components of the innate causative system, at different stages of lymphocyte development, or in the responses of mature lymphocytes to antigenic stimulation. Inherited abnormalities affecting innate immunity most commonly affect the complement pathway or phagocytes. Abnormalities in lymphocyte development may be caused by mutations in genes encoding a variety of molecules, including enzymes, adaptors, transport proteins, and transcription factors. These inherited defects, and the corresponding targeted disruptions in mice, have been useful for elucidating the mechanisms of lymphocyte development (see Chapter 8). Abnormalities in B lymphocyte development and function result in deficient antibody production and are diagnosed by reduced levels of serum immunoglobulin (Ig), defective antibody responses to vaccination, and, in some cases, reduced numbers of B cells in the circulation or lymphoid tissues or absent plasma cells in tissues (see Table 20-1). Abnormalities in T lymphocyte maturation and function lead to deficient cell-mediated immunity and may also result in reduced antibody production. Primary T cell immunodeficiencies are diagnosed by reduced numbers of peripheral blood T cells, low proliferative responses of blood lymphocytes to polyclonal T cell activators such as phytohemagglutinin, and deficient cutaneous delayed-type hypersensitivity (DTH) reactions to ubiquitous microbial antigens, such as *Candida* antigens. Defects in both humoral and cell-mediated immunity are classified under severe combined immunodeficiencies. In the following sections, we describe immunodeficiencies caused by inherited mutations in genes encoding components of the innate immune system or in genes required for lymphocyte development and activation. We conclude with a brief discussion of therapeutic strategies for these diseases.

Defects in Innate Immunity

Innate immunity constitutes the first line of defense against infectious organisms. Two important mediators of innate immunity are phagocytes and complement, both of which also participate in the effector phases of adaptive immunity. Therefore, congenital disorders of phagocytes and the complement system result in recurrent infections. Complement deficiencies were described in Chapter 12. Deficiencies have been described in the classical and alternative complement pathways as well as in the lectin pathway. They typically present with recurrent bacterial infections, particularly by encapsulated bacteria and also *Neisseria* species, and often also contribute to susceptibility to autoimmune disorders, particularly systemic lupus erythematosus.

In this section of the chapter, we discuss some examples of congenital phagocyte disorders (Table 20-2) and inherited defects in Toll-like receptor (TLR) pathways and in the IL-12/IFN-γ pathway. Phagocyte defects generally result in infections of the skin and respiratory tract with bacteria or fungi, the latter predominantly involving

TABLE 20–2 Congenital Disorders of Innate Immunity

Disease	Functional Deficiencies	Mechanism of Defect
Chronic granulomatous disease	Defective production of reactive oxygen species by phagocytes; recurrent intracellular bacterial and fungal infections	Mutation in genes of phagocyte oxidase complex; phox-91 (cytochrome b_{588} α subunit) is mutated in X-linked form
Leukocyte adhesion deficiency type 1	Defective leukocyte adhesion and migration linked to decreased or absent expression of β_2 integrins; recurrent bacterial and fungal infections	Mutations in gene encoding the β chain (CD18) of β_2 integrins
Leukocyte adhesion deficiency type 2	Defective leukocyte rolling and migration linked to decreased or absent expression of leukocyte ligands for endothelial E- and P- selectins, causing failure of leukocyte migration into tissues; recurrent bacterial and fungal infections	Mutations in gene encoding a GDP-fucose transporter required for the synthesis of the sialyl Lewis X component of E- and P- selectin ligands
Leukocyte adhesion deficiency type 3	Defective leukocyte adhesion and migration linked to defective inside-out signaling and therefore defective integrin activation	Mutations in gene encoding KINDLIN-3
Chédiak-Higashi syndrome	Defective vesicle fusion and lysosomal function in neutrophils, macrophages, dendritic cells, natural killer cells, cytotoxic T cells, and many other cell types; recurrent infections by pyogenic bacteria	Mutation in LYST leading to defect in secretory granule exocytosis and lysosomal function
Toll-like receptor signaling defects	Recurrent infections because of defects in TLR and CD40 signaling and defective type I interferon production	Mutations in NEMO, UNC93B, MyD88, IκBα, and IRAK-4 compromise NF-κB activation downstream of Toll-like receptors

IRAK-4, IL-1 receptor–associated kinase 4; LYST, lysosomal trafficking protein; NEMO, NF-κB essential modulator.

Aspergillus and *Candida* species. Deep-seated abscesses and oral stomatitis are also common. Defects in TLR signaling and in type I interferon signaling may contribute to recurrent pyogenic infections as well as to severe viral infections; defects in IL-12 and the IFN-γ pathway are linked to susceptibility to intracellular pathogens, particularly mycobacterial infections.

Defective Microbicidal Activities of Phagocytes: Chronic Granulomatous Disease

Chronic granulomatous disease (CGD) is caused by mutations in components of the phagocyte oxidase (phox) enzyme complex. It is a rare disease, estimated to affect about 1 in 1 million individuals in the United States. About two thirds of cases show an X-linked recessive pattern of inheritance, and the remainder are autosomal recessive. The most common X-linked form of the disease is caused by a mutation in the gene encoding the 91-kD α subunit of cytochrome b_{558}, an integral membrane protein also known as phox-91. This mutation results in defective production of superoxide anion, one of several reactive oxygen species, which constitute a major microbicidal mechanism of phagocytes (see Chapter 4). Mutations in other components of the phox complex contribute to autosomal recessive variants of CGD. Defective production of reactive oxygen species results in a failure to kill phagocytosed microbes. The disease is characterized by recurrent infections with catalase-producing intracellular bacteria and fungi, usually from early childhood. Many of the organisms that are particularly troublesome in CGD patients produce catalase, which destroys the microbicidal hydrogen peroxide that may be produced by host cells from the residual reactive oxygen radical superoxide. Because the infections are not controlled by phagocytes, they stimulate chronic cell-mediated immune responses, resulting in T cell–mediated macrophage activation and the formation of granulomas composed of activated macrophages. Presumably, these activated macrophages try to limit or to eliminate the microbes despite defective production of reactive oxygen species. This histologic appearance is the basis for the name of the disorder. The disease is often fatal, even with aggressive antibiotic therapy.

The cytokine interferon-γ (IFN-γ) enhances transcription of the gene encoding phox-91 and also stimulates other components of the phagocyte oxidase enzyme complex. Therefore, IFN-γ stimulates the production of superoxide by normal neutrophils as well as by CGD neutrophils, especially in cases in which the coding portion of the phox-91 gene is intact but its transcription is reduced. Once neutrophil superoxide production is restored to about 10% of normal levels, resistance to infection is greatly improved. IFN-γ therapy is now commonly used for the treatment of X-linked CGD.

Leukocyte Adhesion Deficiencies

The leukocyte adhesion deficiencies are a group of autosomal recessive disorders caused by defects in leukocyte and endothelial adhesion molecules. These diseases are characterized by a failure of leukocyte, particularly neutrophil, recruitment to sites of infection, resulting in severe periodontitis and other recurrent infections starting early in life, and the inability to make pus. Different types of leukocyte adhesion deficiencies are caused by mutations in different genes.

- Leukocyte adhesion deficiency type 1 (LAD-1) is a rare autosomal recessive disorder characterized by recurrent bacterial and fungal infections and impaired wound healing. In these patients, most adhesion-dependent functions of leukocytes are abnormal. These functions include adherence to endothelium, neutrophil aggregation and chemotaxis, phagocytosis, and cytotoxicity mediated by neutrophils, natural killer (NK) cells, and T lymphocytes. The molecular basis of the defect is absent or deficient expression of the β_2 integrins (heterodimers of CD18 and the CD11 family of glycoproteins) due to various mutations in the CD18 gene. The β_2 integrins include leukocyte function-associated antigen 1 (LFA-1 or CD11aCD18), Mac-1 (CD11bCD18), and p150,95 (CD11cCD18). These proteins participate in the adhesion of leukocytes to other cells, notably endothelial cells, and the binding of T lymphocytes to antigen-presenting cells (APCs) (see Chapter 3).
- Leukocyte adhesion deficiency type 2 (LAD-2) is another rare disorder that is clinically similar to LAD-1 but is not due to integrin defects. In contrast, LAD-2 results from an absence of sialyl Lewis X, the tetrasaccharide carbohydrate ligand on neutrophils and other leukocytes that is required for binding to E-selectin and P-selectin on cytokine-activated endothelium (see Chapter 3). This defect is caused by a mutation in a GDP-fucose transporter responsible for the transport of fucose into the Golgi, resulting in an inability to synthesize sialyl Lewis X. The absence of sialyl Lewis X results in defective binding of leukocytes to endothelium, the absence of leukocyte "rolling," and therefore the defective recruitment of leukocytes to sites of infection. This abnormality in fucosylation seen in LAD-2 also contributes to a Bombay blood group phenotype caused by the absence of all ABO blood group antigens as well as to mental retardation and other developmental defects. Fucose is an essential component of the H glycolipid that forms the core antigen in the ABO system.
- Leukocyte adhesion deficiency type 3 (LAD-3) involves a defect in inside-out signaling and thus a defect in chemokine-induced integrin activation that is required for leukocytes to bind firmly to endothelium (see Chapter 3). In a subset of patients it is caused by mutations in the gene encoding KINDLIN-3. KINDLIN-3 is a protein that binds to the cytoplasmic tail of some integrins and is involved in signaling. Increased bleeding is also observed in subjects with KINDLIN-3 mutations because of integrin dysfunction in platelets.

Defects in NK Cells and Other Leukocytes: The Chédiak-Higashi Syndrome

The Chédiak-Higashi syndrome is a rare autosomal recessive disorder characterized by recurrent infections by pyogenic bacteria, partial oculocutaneous albinism, and infiltration of various organs by non-neoplastic lymphocytes. The neutrophils, monocytes, and lymphocytes of these patients contain giant lysosomes. This disease is caused by mutations in the gene encoding the lysosomal trafficking regulator protein LYST, resulting in defective phagosome-lysosome fusion in neutrophils and macrophages (causing reduced resistance to infection), defective melanosome formation in melanocytes (causing albinism), and lysosomal abnormalities in cells of the nervous system (causing nerve defects) and platelets (leading to bleeding disorders). Giant lysosomes form in neutrophils during the maturation of these cells from myeloid precursors. Some of these neutrophil precursors die prematurely, resulting in moderate leukopenia. Surviving neutrophils may contain reduced levels of the lysosomal enzymes that normally function in microbial killing. These cells are also defective in chemotaxis and phagocytosis, further contributing to their deficient microbicidal activity. NK cell function in these patients is impaired, probably because of an abnormality in the cytoplasmic granules that store proteins mediating cytotoxicity. The severity of the defect in cytotoxic T lymphocyte (CTL) function is variable among patients. A mutant mouse strain called the beige mouse is an animal model for the Chédiak-Higashi syndrome. This strain is characterized by deficient NK cell function and giant lysosomes in leukocytes. The beige mutation has been mapped to the mouse *Lyst* locus.

Other mutations that affect both CTL and NK cell function will be considered later when we discuss defects in T lymphocyte activation and function. A mutation in CD16/FcγRIII, the Fc receptor on NK cells that is required for antibody-dependent cellular cytotoxicity (see Chapter 12), has been described in a patient with recurrent viral infections.

Inherited Defects in TLR Pathways, Nuclear Factor κB Signaling, and Type I Interferons

Inherited defects in TLR-dependent responses are rare and have been recognized only recently. The major signaling pathway downstream of most TLRs as well as of the interleukin-1 receptor (IL-1R) involves the MyD88 adaptor and the IRAK-4 and IRAK-1 kinases (see Chapter 4), and this pathway results in the nuclear factor κB (NF-κB)–dependent induction of proinflammatory cytokines. TLR 3, 7, 8, and 9 recognize nucleic acids, are located in endosomes, and require a protein called UNC93B for their function. UNC93B is an endoplasmic reticulum membrane protein that interacts with endosomal TLRs when they are synthesized in the endoplasmic reticulum and helps deliver these TLRs to the endosomes. The UNC93B protein is also critical for signaling by nucleic acid–specific TLRS. Signaling downstream of the endosomal TLRs results in the synthesis and secretion of type I interferons. Defects in TLR signaling tend to have a fairly circumscribed clinical phenotype. Severe invasive bacterial infections early in life, especially pneumococcal disease, are observed in individuals with mutations in *MYD88* and *IRAK4*. Later in life, infections tend to be less severe. Heterozygous mutations in *TLR3* as well as homozygous mutations in *UNC93B* result in reduced type I interferon generation and susceptibility to herpes simplex encephalitis. Type I interferon receptors activate the STAT1 transcription factor. Loss-of-function *STAT1* mutations (which interfere with interferon signaling) have also been linked to severe viral infections, notably herpes simplex encephalitis.

Some immune deficiencies are caused by defects in signaling pathways downstream of TLRs. Point mutations in the inhibitor of κB kinase γ (IKKγ), also known as nuclear factor κB essential modulator (NEMO), a component of the IκB kinase complex that is required for NF-κB activation, contribute to the X-linked recessive condition known as anhidrotic ectodermal dysplasia with immunodeficiency (EDA-ID). In this disorder, differentiation of ectoderm-derived structures is abnormal, and immune function is impaired in a number of ways. Responses to TLR signals as well as CD40 signals are compromised. These patients suffer from infections with encapsulated pyogenic bacteria as well as with intracellular bacterial pathogens including mycobacteria, viruses, and fungi such as *Pneumocystis jiroveci* (see also discussion later in the section on hyper-IgM syndromes). An autosomal recessive form of EDA-ID has been described in which a hypermorphic point mutation in IκBα prevents the phosphorylation, ubiquitination, and degradation of IκBα, thus leading to impaired NF-κB activation.

Defects in the IL-12/IFN-γ Pathway

IL-12 is secreted by dendritic cells and macrophages, and IL-12R signaling induces the synthesis of IFN-γ by helper T cells, cytotoxic T cells, and NK cells (see Chapter 4). Mutations in the genes encoding IL-12p40, the IL-12Rβ1 chain, and both chains of the IFN-γ receptor, as well as some hypomorphic mutations in STAT1, all result in susceptibility to environmental *Mycobacterium* species (often called atypical mycobacteria), such as *Mycobacterium avium, Mycobacterium kansasii,* and *Mycobacterium fortuitum*. IKKγ/NEMO mutations also lead to susceptibility to intracellular pathogens including mycobacteria, as discussed in the previous section.

Severe Combined Immunodeficiencies

Congenital immunodeficiencies that affect both humoral and cell-mediated immunity are called combined immunodeficiencies, and a subset of these in which most peripheral T cells are missing or defective are known as severe combined immunodeficiencies (SCIDs) (Table 20-3). These diseases are characterized by deficiencies of both B and T cells or only of T cells; in the latter cases, the defect in humoral immunity is due to the absence of T cell help. Children with SCID usually have infections during the first year of life, *Pneumocystis jiroveci* pneumonia being particularly common, and they succumb to these infections unless they are treated.

SCID results from impaired T lymphocyte development with or without defects in B cell maturation (Fig. 20-1). The thymic epithelium contributes in a major way to early T cell development. The process of T (and B) lymphocyte maturation from hematopoietic stem cells to functionally competent mature lymphocytes involves proliferation of early lymphocyte progenitors, rearrangement of the locus encoding one chain of the antigen receptor followed by selection of cells that have made in-frame productive rearrangements at a pre-antigen receptor checkpoint, expression of both chains of the antigen receptor, and selection of cells with useful specificities (see Chapter 8). Defects in many of these steps have been described in different forms of SCID. About 50% of SCIDs are autosomal recessive; the rest are X-linked. The most common cause of autosomal recessive SCID is deficiency of the enzyme adenosine deaminase, required for purine metabolism. X-linked SCID is caused by mutations in the gene encoding a cytokine receptor component called the common γ chain. The individual disorders are described here.

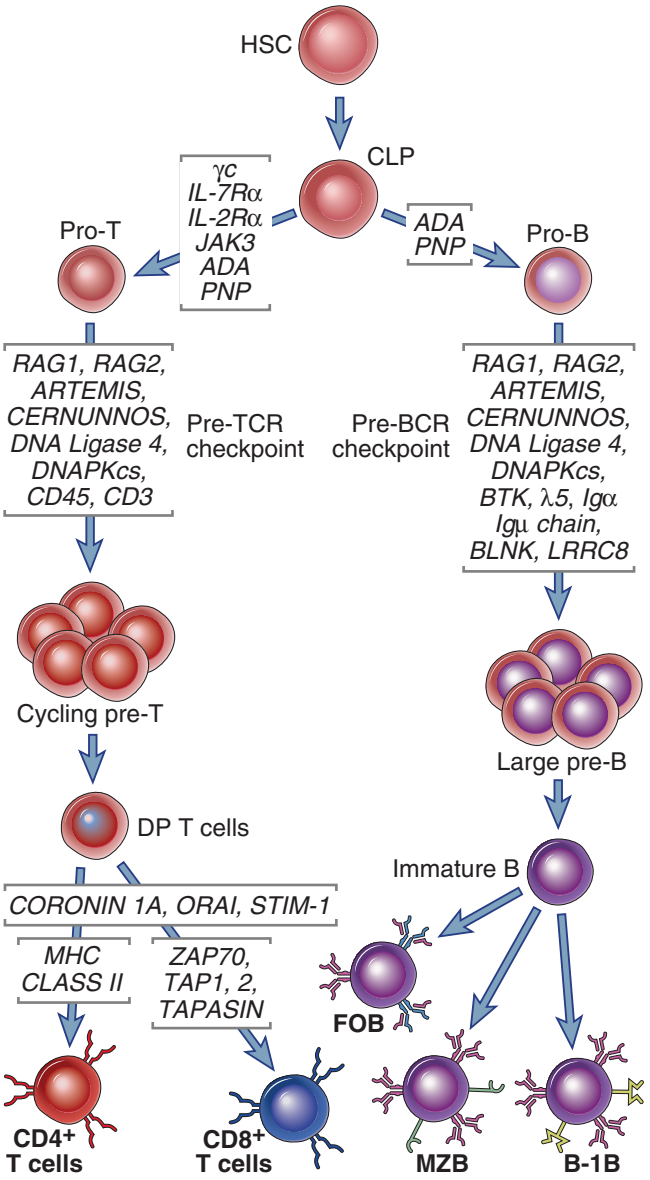


FIGURE 20–1 Immunodeficiency caused by defects in B and T cell maturation. Primary immunodeficiencies caused by genetic defects in lymphocyte maturation are shown. These defects may affect T cell maturation alone, B cell maturation alone, or both. CLP, common lymphoid progenitor; DP, double-positive; FoB, follicular B cells; HSC, hematopoietic stem cell; MZB, marginal zone B cells.

The DiGeorge Syndrome and Other Forms of SCID due to Defective Thymic Epithelial Development

Failure or incomplete development of the thymic anlage can lead to defective T cell development. The most

TABLE 20–3 Severe Combined Immunodeficiencies

Disease	Functional Deficiencies	Mechanism of Defect
Defects in cytokine signaling		
X-linked SCID	Marked decrease in T cells; normal or increased B cells; reduced serum Ig	Cytokine receptor common γ chain mutations; defective T cell development in the absence of IL-7–derived signals
Autosomal recessive forms	Marked decrease in T cells; normal or increased B cells; reduced serum Ig	Mutations in *IL2RA, IL7RA, JAK3*
Defects in nucleotide salvage pathways		
ADA deficiency	Progressive decrease in T, B, and NK cells; reduced serum Ig	ADA deficiency caused by mutations in the gene, leading to accumulation of toxic metabolites in lymphocytes
PNP deficiency	Progressive decrease in T, B, and NK cells; reduced serum Ig	PNP deficiency caused by mutations in the gene, leading to accumulation of toxic metabolites in lymphocytes
Defects in V(D)J recombination		
RAG1 or RAG2 deficiency recombination*	Decreased T and B cells; reduced serum Ig; absence or deficiency of T and B cells	Cleavage defect during V(D)J recombination; mutations in *RAG1* or *RAG2*
Double-stranded break repair and checkpoint	Decreased T and B cells; reduced serum Ig; absence or deficiency of T and B cells	Failure to resolve hairpins during V(D)J recombination; mutations in *ARTEMIS,* DNA-PKcs, *CERNUNNOS, LIG4, NBS1, MRE11, ATM*
Defective thymus development		
Defective pre-TCR checkpoint	Decreased T cells; normal or reduced B cells; reduced serum Ig	Mutations in *CD45, CD3D, CD3E, ORAI1* (CRAC channel component), *STIM1*
DiGeorge syndrome	Decreased T cells; normal B cells; normal or reduced serum Ig	22ql 1 deletion; T-box 1 (*TBX1*) transcription factor mutations
FoxN1 deficiency	Thymic aplasia with defective thymic cell development	Recessive mutation in *FOXN1*
Other defects		
Reticular dysgenesis	Decreased T, B, and myeloid cells	Mutation in *AK2*

ADA, adenosine deaminase; AK2, adenylate kinase 2; ATM, ataxia-telangiectasia mutated; CRAC, calcium release activated channel; DNA-PKcs, DNA-dependent protein kinase catalytic subunit; LIG4, DNA ligase 4; MRE11, meiotic recombination homologue 11; NBS1, Nijmegen breakpoint syndrome 1; PNP, purine nucleoside phosphorylase.
*Hypomorphic mutations in *RAG* genes and in *ARTEMIS* can contribute to Omenn's syndrome.

common defect in thymic development linked to SCID is seen in children with the **DiGeorge syndrome**. This selective T cell deficiency is due to a congenital malformation that results in defective development of the thymus and the parathyroid glands as well as other structures that develop from the third and fourth pharyngeal pouches during fetal life. The congenital defect is manifested by hypoplasia or agenesis of the thymus leading to deficient T cell maturation, absent parathyroid glands causing abnormal calcium homeostasis and muscle twitching (tetany), abnormal development of the great vessels, and facial deformities. Different patients may show varying degrees of these abnormalities. The disease is caused most frequently by a deletion in chromosome 22q11. Mutations in the murine homologue of a gene encoding a transcription factor called T-box 1 (TBX1), which lies within the region deleted in DiGeorge syndrome, also result in a similar defect in thymic development. It is likely that the immunodeficiency associated with DiGeorge syndrome can be explained, at least in part, by the deletion of the *TBX1* gene. In this syndrome, peripheral blood T lymphocytes are absent or greatly reduced in number, and the cells do not respond to polyclonal T cell activators or in mixed leukocyte reactions. Antibody levels are usually normal but may be reduced in severely affected patients. As in other severe T cell deficiencies, patients are susceptible to mycobacterial, viral, and fungal infections.

The immunodeficiency associated with DiGeorge syndrome can be corrected by fetal thymic transplantation or by bone marrow transplantation. Such treatment is usually not necessary, however, because T cell function tends to improve with age in a large fraction of patients with this syndrome and is often normal by 5 years. Improvement with age probably occurs because of the presence of some thymic tissue or because some as yet undefined extrathymic sites assume the function of T cell maturation. It is also possible that as these patients grow older, thymus tissue develops at ectopic sites (i.e., other than the normal location).

An animal model of T cell immunodeficiency resulting from abnormal development of the thymus is the nude

(athymic) mouse. These mice have an inherited defect of certain types of epithelial cells in the skin, leading to hairlessness, and in the lining of the third and fourth pharyngeal pouches, causing thymic hypoplasia. The disorder is due to a mutation in the *FoxN1* gene encoding a Forkhead family transcription factor that is required for the normal development of certain ectoderm-derived cell types. Affected mice have rudimentary thymuses in which T cell maturation cannot occur normally. As a result, few or no mature T cells are present in peripheral lymphoid tissues, and cell-mediated immune reactions cannot occur. Autosomal recessive *FOXN1* mutations have been described in a small number of patients who present with SCID, alopecia (hair loss), and nail dystrophy. An even rarer defect in the thymus has been described involving a mutation in *CORONIN-1A,* which encodes a protein that regulates the actin cytoskeleton. The absence of functional CORONIN-1A results in defective egress of mature T cells from the thymus.

ADA Deficiency and Other Forms of SCID Caused by Defects in Nucleotide Metabolism

The most common cause of autosomal recessive SCID is deficiency of an enzyme called adenosine deaminase (ADA) due to mutations in the ADA gene. ADA functions in the salvage pathway of purine synthesis and catalyzes the irreversible deamination of adenosine and 2′-deoxyadenosine to inosine and 2′-deoxyinosine, respectively. Deficiency of the enzyme leads to the accumulation of deoxyadenosine and its precursors *S*-adenosylhomocysteine and deoxyadenosine triphosphate (dATP). These byproducts have many toxic effects, including inhibition of DNA synthesis. Although ADA is present in most cells, developing lymphocytes are less efficient than most other cell types at degrading dATP into 2′-deoxyadenosine, and therefore lymphocyte maturation is particularly sensitive to ADA deficiency. Other features of the disease can include deafness, costochondral abnormalities, liver damage, and behavioral problems. ADA deficiency leads to reduced numbers of B and T cells; lymphocyte cell numbers are usually normal at birth but fall off precipitously during the first year of life. A few patients may have a nearly normal number of T cells, but these cells do not proliferate in response to antigenic stimulation.

A rarer autosomal recessive form of SCID is due to the deficiency of purine nucleoside phosphorylase (PNP), an enzyme that is also involved in purine catabolism. PNP catalyzes the conversion of inosine to hypoxanthine and guanosine to guanine, and deficiency of PNP leads to the accumulation of deoxyguanosine and deoxyguanosine triphosphate, with toxic effects on immature lymphocytes, mainly T cells. Autoimmune hemolytic anemia and progressive neurologic deterioration are also features of this disorder.

A particularly severe form of SCID is seen in a disease called reticular dysgenesis. This rare disorder is characterized by the absence of T and B lymphocytes and most myeloid cells, including granulocytes, and is due to a defect in the development of lymphoid and myeloid progenitors. This autosomal recessive disease is due to a mutation in the *adenylate kinase 2 (AK2)* gene. The AK2 protein regulates the level of adenosine diphosphate, and in the absence of AK2 there is increased apoptosis of lymphoid and myeloid precursors.

X-Linked SCID

X-linked SCID is caused by mutations in the gene encoding the common γ (γ_c) chain shared by the receptors for the interleukins IL-2, IL-4, IL-7, IL-9, and IL-15 (see Chapters 4 and 9). X-linked SCID is characterized by impaired maturation of T cells and NK cells and greatly reduced numbers of mature T cells and NK cells, but the number of B cells is usually normal or increased. The humoral immunodeficiency in this disease is due to a lack of T cell help for antibody production. This disease is a result of the inability of the lymphopoietic cytokine IL-7, whose receptor uses the γ_c chain for signaling, to stimulate the growth of immature thymocytes. In addition, the receptor for IL-15, which is a potent stimulus for the proliferation of NK cells, also uses the γ_c signaling chain, and the failure of IL-15 function accounts for the deficiency of NK cells.

Heterozygous females are usually phenotypically normal carriers, whereas males who inherit the abnormal X chromosome manifest the disease. Because developing cells in females randomly inactivate one of the two X chromosomes, the normal allele encoding a functional γ_c protein will not be expressed in half the lymphocyte precursors in a female carrier. These cells will fail to mature, and consequently, all the mature lymphocytes in a female carrier will have inactivated the same X chromosome (carrying the mutant allele). In contrast, half of all nonlymphoid cells will have inactivated one X chromosome, and half the other. A comparison of X chromosome inactivation in lymphoid cells versus nonlymphoid cells may be used to identify carriers of the mutant allele. The nonrandom use of X chromosomes in mature lymphocytes is also characteristic of female carriers of other X-linked mutations of genes that affect lymphocyte development, as discussed later.

Autosomal Recessive Mutations in Cytokine Signaling Components

Some patients with a disease identical to X-linked SCID show an autosomal recessive inheritance. These patients have mutations in the *IL-7 receptor α chain* or the *JAK3 kinase*, which associates with the γ_c chain and is required for signaling by this receptor (see Chapter 7). Patients with mutations in the gene encoding the IL-7Rα chain have a defect in T cell development but exhibit normal NK cell development, because IL-15 signaling is unaffected, and have normal numbers of B cells.

Severe Combined Immunodeficiency Caused by Defects in V(D)J Recombination and Pre-TCR Checkpoint Signaling

Absence of V(D)J recombination leads to a failure to express the pre-TCR and the pre-BCR and a block in T and B cell development. Mutations in the *RAG1* or *RAG2* genes (whose protein products mediate the cleavage step during V(D)J recombination) or the *ARTEMIS* gene, which encodes an endonuclease that resolves coding-end hairpins during V(D)J recombination, all result in a failure of V(D)J recombination. These diseases are rare,

but they account for a large number of the autosomal recessive forms of SCID. The normal functions of these genes are discussed in Chapter 8. In children with these mutations, B and T lymphocytes are absent and immunity is severely compromised. Mutations in genes encoding proteins involved in double-stranded break repair/nonhomologous end joining of DNA also lead to SCID because of defects in V(D)J recombination. Homozygous mutations in the gene encoding the catalytic subunit of the DNA-dependent protein kinase (DNA-PK), *CERNUNNOS/XLF,* and *DNA LIGASE 4* all lead to SCID. Among the many functions of DNA-PK is the phosphorylation and activation of ARTEMIS, and CERNUNNOS interacts with the XRCC4/DNA ligase 4 complex and presumably facilitates the ligation event that completes the nonhomologous end-joining process. Genetic defects in this end-joining process also result in increased cellular sensitivity to radiation and can result in other manifestations, such as microcephaly, facial dysmorphisms, and defective tooth development.

Hypomorphic mutations (that only partially reduce function) in the *RAG* genes, in *ARTEMIS,* or in the *IL7RA* gene are the cause of a disorder characterized by restricted generation of T and B cells, immunodeficiency, and immune dysregulation. This disorder is known as **Omenn's syndrome**. It is phenotypically different from the diseases described above because in this disease immunodeficiency coexists with exaggerated immune activation and autoimmunity. This may be caused by relative absence of regulatory T cells, or in cases with decreased V(D)J recombination, defective receptor editing in immature B cells.

Although most autosomal recessive forms of SCID are linked to mutations in *ADA, RAG1, RAG2,* and *ARTEMIS,* rare forms of this syndrome are caused by mutations in the genes encoding the CD45 phosphatase (that is a positive regulator of Src family kinases, such as Fyn, Lck, and Lyn) and mutations in the CD3 δ or ε chains or in the CD3-associated ζ chain. These mutations contribute to defective pre-TCR signaling and result in a block in αβ T cell development.

The Bare Lymphocyte Syndrome and Other Defects in T Cell Positive Selection

The generation of single-positive $CD4^+$ and $CD8^+$ T cells from double-positive thymocytes depends on positive selection and lineage commitment events. Specific inherited mutations in genes that regulate the process of positive selection abrogate the development of $CD4^+$ T cells or of $CD8^+$ T cells.

Class II major histocompatibility complex (MHC) deficiency, also called bare lymphocyte syndrome, is a rare heterogeneous group of autosomal recessive diseases in which patients express little or no HLA-DP, HLA-DQ, or HLA-DR on B lymphocytes, macrophages, and dendritic cells and fail to express class II MHC molecules in response to IFN-γ. They express normal or only slightly reduced levels of class I MHC molecules and β_2-microglobulin. Most cases of the bare lymphocyte syndrome are due to mutations in genes encoding proteins that regulate class II MHC gene transcription. For example, mutations affecting the constitutively expressed transcription factor RFX5 or the IFN-γ–inducible transcriptional activator CIITA lead to reduced class II MHC expression and a failure of APCs to activate $CD4^+$ T lymphocytes. Failure of antigen presentation may result in defective positive selection of T cells in the thymus, with a reduction in the number of mature $CD4^+$ T cells or defective activation of cells in the periphery. Affected individuals are deficient in DTH responses and in antibody responses to T cell–dependent protein antigens. The disease appears within the first year of life and is usually fatal unless it is treated by bone marrow transplantation.

Autosomal recessive class I MHC deficiencies have also been described and are characterized by decreased $CD8^+$ T cell numbers and function. In some cases, the failure to express class I MHC molecules is due to mutations in the *TAP-1* or *TAP-2* genes, which encode the subunits of the TAP (transporter associated with antigen processing) complex, which normally transports peptides from the cytosol into the endoplasmic reticulum, where they are required for class I MHC assembly (see Chapter 6). These TAP-deficient patients express few cell surface class I MHC molecules, a phenotype similar to *TAP* gene knockout mice. Such patients suffer mainly from necrotizing granulomatous skin lesions and respiratory tract bacterial infections, but not viral infections, which is surprising considering that a principal function of $CD8^+$ T cells is defense against viruses. A similar deficiency of class I MHC expression has been observed in patients with mutations in the gene encoding the tapasin protein (see Chapter 6).

Patients with ZAP-70 deficiency have a lineage commitment defect resulting in reduced $CD8^+$ T cells but not $CD4^+$ T cells; the reason for the selective loss is not clear. This specific tyrosine kinase defect does not compromise $CD4^+$ T cell development or emigration to the periphery. However, these $CD4^+$ T cells fail to proliferate normally when challenged with antigens.

SCID Caused by Defective T Cell Activation

Another rare form of SCID is caused by mutation in a gene encoding Orai1, a component of the CRAC channel (see Chapter 7). Antigen receptor signaling leads to the activation of the γ isoform of phospholipase C (PLCγ) and the inositol trisphosphate (IP3)–dependent release of calcium ions from the endoplasmic reticulum and mitochondria (see Chapter 7). The released calcium is replenished by store-operated CRAC channels that facilitate an influx of extracellular calcium. This process is crucial for lymphocyte activation, and it is defective in cells with mutant *ORAI1*. A similar phenotype is observed in patients with mutations in *STIM1,* which encodes an endoplasmic reticulum protein that senses the depletion of calcium stores and contributes to the opening of the CRAC channel. Patients with *ORAI1* and *STIM1* mutations do not exhibit a defect in T cell development, but their T cells cannot be properly activated.

Antibody Deficiencies: Defects in B Cell Development and Activation

Whereas defects in T cell development or in both T and B cell development contribute to the SCID phenotype,

TABLE 20–4 Antibody Deficiencies

Disease	Functional Deficiencies	Mechanism of Defect
Agammaglobulinemias		
X-linked	Decrease in all serum Ig isotypes; reduced B cell numbers	Pre-B receptor checkpoint defect; Btk mutation
Autosomal recessive forms	Decrease in all serum Ig isotypes; reduced B cell numbers	Pre-B receptor checkpoint defect; mutations in IgM heavy chain (μ), surrogate light chains (λ5), Igα, BLNK
Hypogammaglobulinemias/isotype defects		
Selective IgA deficiency	Decreased IgA; may be associated with increased susceptibility to bacterial infections and protozoa such as *Giardia lamblia*	Mutations in *TACI* in some patients
Selective IgG2 deficiency	Increased susceptibility to bacterial infections	Small subset have deletion in IgH γ2 locus
Common variable immunodeficiency	Hypogammaglobulinemia; normal or decreased B cell numbers	Mutations in *ICOS* and *TACI* in some patients
ICF syndrome	Hypogammaglobulinemia, occasional mild T cell defects	Mutations in *DNMT3B*
Hyper-IgM syndromes		
X-linked	Defects in T helper cell–mediated B cell, macrophage, and dendritic cell activation; defects in somatic mutation, class switching, and germinal center formation; defective cell-mediated immunity	Mutation in *CD40L*
Autosomal recessive with cell-mediated immune defects	Defects in T helper cell–mediated B cell, macrophage, and dendritic cell activation; defects in somatic mutation, class switching, and germinal center formation; defective cell-mediated immunity	Mutations in *CD40, NEMO*
Autosomal recessive with antibody defect only	Defects in somatic mutation and isotype switching	Mutations in *AID, UNG*

AID, activation-induced cytidine deaminase; DNMT3B, DNA methyltransferase 3B; ICF, immunodeficiencies-centromeric instability-facial anomalies; ICOS, inducible costimulator; NEMO, NF-κB essential modulator; TACI, transmembrane activator and calcium modulator and cyclophilin ligand interactor; UNG, uracil N-glycosylase.

more circumscribed defects in B cells result in disorders in which the primary abnormality is in antibody synthesis (Table 20-4). Some of these disorders are caused by defects in B cell development (see Fig. 20-1) and others by abnormal B cell activation and antibody production (Fig. 20-2). However, in one subset of hyper-IgM syndromes discussed later, antibody deficiencies are also accompanied by defects in macrophage and APC activation, which in turn result in attenuated cell-mediated immunity.

X-Linked Agammaglobulinemia: An X-linked Pre-BCR Signaling Defect

X-linked agammaglobulinemia, also called Bruton's agammaglobulinemia, is caused by mutations or deletions in the gene encoding an enzyme called Bruton tyrosine kinase (Btk) that results in a failure of B cells to mature beyond the pre-B cell stage in the bone marrow (see Fig. 20-1). The disease is characterized by the absence of gamma globulin in the blood, as the name implies. It is one of the most common congenital immunodeficiencies and the prototype of a failure of B cell maturation. Btk is involved in transducing signals from the pre-B cell receptor (pre-BCR) that are required for the survival and differentiation of pre-B cells (see Chapter 8). In female carriers of this disease, only B cells that have inactivated the X chromosome carrying the mutant allele mature. Patients with X-linked agammaglobulinemia usually have low or undetectable serum Ig, reduced or absent B cells in peripheral blood and lymphoid tissues, no germinal centers in lymph nodes, and no plasma cells in tissues. The maturation, numbers, and functions of T cells are generally normal. Some studies have revealed reduced numbers of activated T cells in patients, which may be a consequence of reduced antigen presentation caused by the lack of B cells. Autoimmune disorders develop in almost 20% of patients, for unknown reasons. The infectious complications of X-linked agammaglobulinemia are greatly reduced by periodic (e.g., weekly or monthly) injections of pooled gamma globulin preparations. Such preparations contain preformed antibodies against common pathogens and provide effective passive immunity.

Knockout mice lacking Btk, as well as naturally Btk mutant *Xid* mice, show a less severe defect in B cell maturation than humans do because a Btk-like tyrosine kinase called Tec is active in mouse pre-B cells that lack Btk and partially compensates for the mutant Btk. The

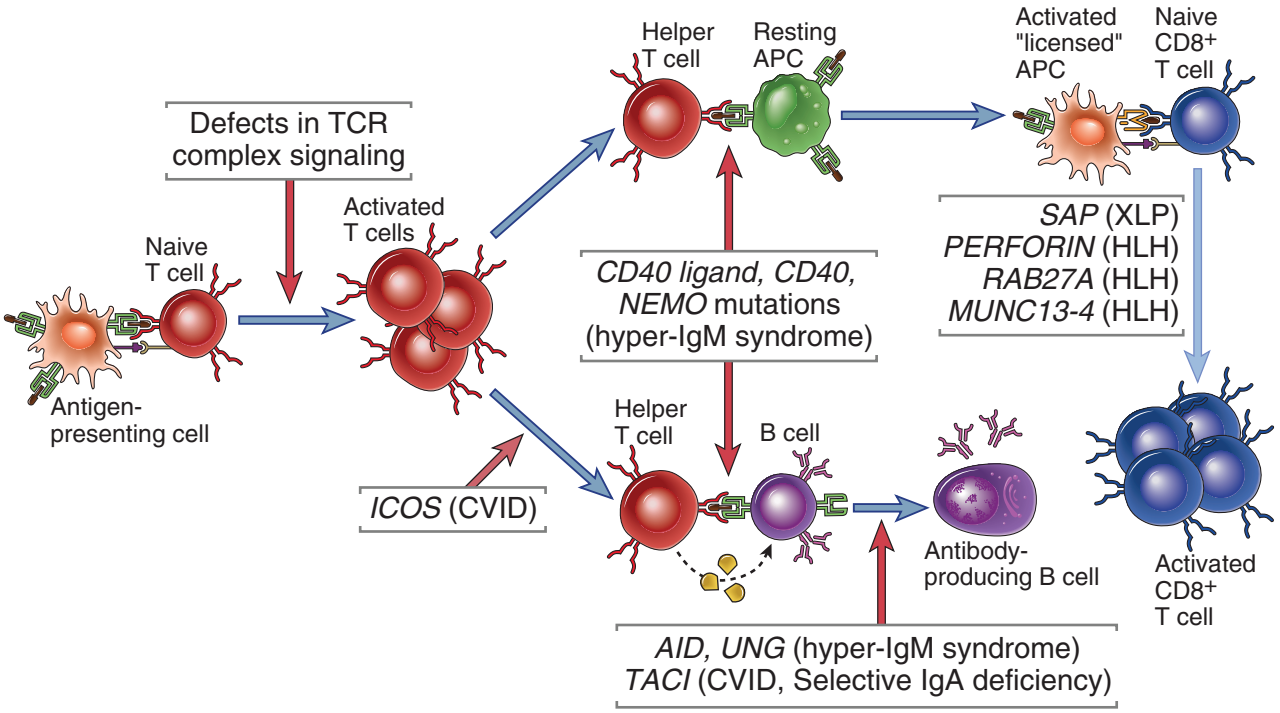


FIGURE 20–2 Immunodeficiency caused by defects in B and T cell activation. Primary immunodeficiencies may be caused by genetic defects in molecules required for T or B lymphocyte antigen receptor signaling, for helper T cell–mediated activation of B cells and APCs, or for activation of cytotoxic T lymphocytes and NK cells. CVID, common variable immunodeficiency; HLH, hemophagocytic lymphohistiocytosis.

main abnormalities in *Xid* mice are defective antibody responses to some polysaccharide antigens and a deficiency in mature follicular and B-1 B cells.

Autosomal Recessive Pre-BCR Checkpoint Defects

Autosomal recessive forms of agammaglobulinemia have been described, most of which can be linked to defects in pre-BCR signaling. Mutant genes that have been identified in this context include genes encoding the μ (IgM) heavy chain, the λ5 surrogate light chain, Igα (a signaling component of the pre-BCR and BCR), and BLNK (an adaptor protein downstream of the pre-BCR and BCR).

Selective Immunoglobulin Isotype Deficiencies

Many immunodeficiencies that selectively involve one or a few Ig isotypes have been described. The most common is selective IgA deficiency, which affects about 1 in 700 Caucasians and is thus the most common primary immunodeficiency known. IgA deficiency usually occurs sporadically, but many familial cases with either autosomal dominant or recessive patterns of inheritance are also known. The clinical features are variable. Many patients are entirely normal; others have occasional respiratory infections and diarrhea; and rarely, patients have severe, recurrent infections leading to permanent intestinal and airway damage, with associated autoimmune disorders. IgA deficiency is characterized by low serum IgA, usually less than 50 μg/mL (normal, 2 to 4 mg/mL), with normal or elevated levels of IgM and IgG. The defect in these patients is a block in the differentiation of B cells to IgA antibody–secreting plasma cells. The α heavy chain genes and the expression of membrane-associated IgA are normal. No gross abnormalities in the numbers, phenotypes, or functional responses of T cells have been noted in these patients. In a small proportion of patients with selective IgA deficiency, mutations have been described in *TACI* (transmembrane activator and calcium modulator and cyclophilin ligand interactor), one of the three types of receptors for the cytokines BAFF (B cell–activating factor) and APRIL (a proliferation-inducing ligand). *TACI* mutations are also an important cause of common variable immunodeficiency, discussed later. IgA deficiency may represent a *forme fruste* of common variable immunodeficiency.

Selective IgG subclass deficiencies have been described in which total serum IgG levels are normal but concentrations of one or more subclasses are below normal. Deficiency of IgG3 is the most common subclass deficiency in adults, and IgG2 deficiency associated with IgA deficiency is the most common in children. Some individuals with these deficiencies have recurrent bacterial infections, but many do not have any clinical problems. Selective IgG subclass deficiencies are usually due to abnormal B cell differentiation and rarely to homozygous deletions of various constant region (C_γ) genes.

Defects in B Cell Differentiation: Common Variable Immunodeficiency

Common variable immunodeficiency is a group of heterogeneous disorders defined by reduced levels of serum Ig, impaired antibody responses to infection or vaccines, and increased incidence of infections. The diagnosis is usually one of exclusion when other primary

immunodeficiency diseases are ruled out. The presentation and pathogenesis are, as the name implies, highly variable. Although Ig deficiency and associated pyogenic infections, typically with *Haemophilus influenzae* and *Streptococcus pneumoniae*, are major components of these disorders, autoimmune diseases, including pernicious anemia, hemolytic anemia, inflammatory bowel disease, and rheumatoid arthritis, may be just as clinically significant. A high incidence of malignant tumors, particularly lymphomas, is also associated with common variable immunodeficiency. These disorders may be diagnosed early in childhood or late in life. Both sporadic and familial cases occur, the latter with both autosomal dominant and recessive inheritance patterns. Mature B lymphocytes are present in these patients, but plasma cells are absent in lymphoid tissues, which suggests a block in B cell differentiation to antibody-secreting cells. The defective antibody production has been attributed to multiple abnormalities, including intrinsic B cell defects, deficient T cell help, and excessive "suppressor cell" activity. A small proportion of patients with common variable immunodeficiency have a mutation in the *ICOS* (inducible T cell costimulator) gene. ICOS is required for T follicular helper cell generation (see Chapter 11). A more common cause of this syndrome is the existence of mutations in *TACI*, described before in the context of selective IgA deficiency. A few cases of common variable immunodeficiency are linked to mutations in the *CD19* gene. CD19 is a signaling component of the CR2 (CD21) coreceptor complex (see Chapter 7).

Defects in T Cell–Dependent B Cell Activation: Hyper-IgM Syndromes

The X-linked hyper-IgM syndrome is caused by mutations in the gene encoding the T cell effector molecule CD40 ligand (CD154). It is a rare disorder associated with defective switching of B cells to the IgG and IgA isotypes; these antibodies are therefore reduced, and the major isotype detected in the blood is IgM. The mutant forms of CD40 ligand produced in these patients do not bind to or transduce signals through CD40 and therefore do not stimulate B cells to undergo heavy chain isotype switching, which requires T cell help (see Chapter 11). Patients suffer from infections similar to those seen in other hypogammaglobulinemias. Patients with X-linked hyper-IgM syndrome also show defects in cell-mediated immunity, with an increased susceptibility to infection by the intracellular fungal microbe *Pneumocystis jiroveci*. This defective cell-mediated immunity occurs because CD40 ligand is also involved in T cell–dependent activation of macrophages and dendritic cells (see Chapter 10). Knockout mice lacking CD40 or CD40 ligand have a phenotype similar to that of the human disease.

Rare cases of hyper-IgM syndrome show an autosomal recessive inheritance pattern. In these patients, the genetic defects may be in CD40 or in the enzyme activation-induced deaminase (AID), which is involved in heavy chain isotype switching and somatic mutation (see Chapter 11). Mutations in *AID* are generally homozygous recessive. A small fraction of mutations in the region of the *AID* gene that corresponds to the C-terminal part of this enzyme exhibit an autosomal dominant inheritance pattern. One form of the hyper-IgM syndrome is caused by autosomal recessive mutations in uracil N-glycosylase (UNG; see Chapter 11), an enzyme that removes U residues from Ig genes during class switching and somatic mutation. An inherited disorder, EDA-ID, in which hypomorphic NEMO mutations contribute to a hyper-IgM state as well as defects in ectodermal structures, is described earlier in the section on TLR signaling defects.

AID and *UNG* mutations affect class switch recombination and somatic hypermutation in distinct ways. In the absence of AID, both switching and hypermutation are defective because AID is absolutely required for both processes. In the absence of UNG, isotype switching is defective but somatic hypermutation is largely preserved, although it exhibits less A:T mutations without the activity of UNG. The role of DNA repair gene mutations in class switching defects will be considered in the section on ataxia-telangiectasia later in this chapter.

Defects in T Lymphocyte Activation and Function

Congenital abnormalities in the activation of T lymphocytes are being increasingly recognized as our understanding of the molecular basis of lymphocyte activation improves (Table 20-5). Included in this broad category are some disorders of CTL and NK cell granule composition or exocytosis. Although we classify disorders linked to defective MHC expression with disorders of T cell development, these abnormalities also result in defective activation of T cells that do mature and emerge from the thymus.

Defects in TCR Signal Transduction

Many examples of rare immunodeficiency diseases caused by defects in the expression of molecules required for T cell activation and function have been identified, and some have already been discussed in the context of SCID. Biochemical and molecular analyses of affected individuals have revealed mutations in the genes encoding various T cell proteins (see Table 20-5). Examples include impaired TCR complex expression or function caused by mutations in the CD3 ε or γ genes, defective TCR-mediated signaling caused by mutations in the *ZAP70* gene, reduced synthesis of cytokines such as IL-2 and IFN-γ (in some cases caused by defects in transcription factors), and lack of expression of IL-2 receptor chains. These defects are often found in only a few isolated cases or in a few families, and the clinical features and severity vary widely. Patients with these abnormalities may have deficiencies predominantly in T cell function or have mixed T cell and B cell immunodeficiencies despite normal or even elevated numbers of blood lymphocytes. We have previously considered the importance of the CD3 complex at the pre-TCR checkpoint, the role of *ZAP70* mutations in $CD8^+$ T cell development, and the relevance of *ORAI1* and *STIM1* in T cell activation, all in the clinical context of SCID. Other syndromes involving the defective activation of mature T cells are considered here.

TABLE 20–5 Defects in T Cell Activation

Disease	Functional Deficiencies	Mechanism of Defect
Defects in MHC expression		
Bare lymphocyte syndrome	Defective MHC class II expression and deficiency in $CD4^+$ T cells; defective cell-mediated immunity and T-dependent humoral immune responses	Defects in transcription factors regulating MHC class II gene expression, including *CIITA, RFXANK, RFX5,* and *RFXAP*
MHC class I deficiency	Decreased MHC class I levels; reduced $CD8^+$ T cells	Mutations in *TAP1, TAP2,* and *TAPASIN*
Defective T cell signaling		
Proximal TCR signaling defects	Defects in cell-mediated immunity and T-dependent humoral immunity	Mutations in *CD3* genes, *CD45, STIM1, ORAI1*
Wiskott-Aldrich syndrome	Defective T cell activation, leukocyte mobility	TCR-dependent actin-cytoskeletal rearrangements are defective because of mutations in *WASP*
Familial hemophagocytic lymphohistiocytoses		
X-linked lymphoproliferative syndrome	Uncontrolled EBV-induced B cell proliferation, uncontrolled macrophage and CTL activation, defective NK cell and CTL function	Mutations in *SAP*
Perforin deficiencies	Uncontrolled macrophage and CTL activation, defective NK cell and CTL function	Mutations in *PERFORIN*
Granule fusion	Uncontrolled macrophage and CTL activation, defective NK cell and CTL function	Defective cytotoxic granule exocytosis; mutations in *RAB27A, MUNC13-4, SYNTAXIN, AP3* (and in *LYST* in Chédiak-Higashi syndrome—see Table 20-2)

AP3, adaptor-related protein complex 3; LYST, lysosomal trafficking regulator protein; SAP, SLAM-associated protein; TAP, transporter associated with antigen processing; WASP, Wiskott-Aldrich syndrome protein.

Wiskott-Aldrich Syndrome

Variable degrees of T and B cell immunodeficiency occur in certain congenital diseases with a wide spectrum of abnormalities involving multiple organ systems. One such disorder is Wiskott-Aldrich syndrome, an X-linked disease characterized by eczema, thrombocytopenia (reduced blood platelets), and susceptibility to bacterial infection. Some of the abnormalities in this disorder can be traced to defective T cell activation, although intrinsic loss of B cell function also contributes to the pathogenesis. In the initial stages of the disease, lymphocyte numbers are normal, and the principal defect is an inability to produce antibodies in response to T cell–independent polysaccharide antigens, because of which these patients are especially susceptible to infections with encapsulated bacteria. The lymphocytes (and platelets) are smaller than normal. With increasing age, the patients show reduced numbers of lymphocytes and more severe immunodeficiency. The defective gene responsible for Wiskott-Aldrich syndrome encodes a cytoplasmic protein called WASP (Wiskott-Aldrich syndrome protein), expressed exclusively in bone marrow–derived cells, which interacts with several proteins, including adaptor molecules downstream of the antigen receptor, such as Grb-2 (see Chapter 7), the Arp2/3 complex involved in actin polymerization, and small G proteins of the Rho family that regulate actin cytoskeletal rearrangement. Defective activation and synapse formation in lymphocytes and defective mobility of all leukocytes may account for the immunodeficiency observed in this syndrome.

The X-Linked Lymphoproliferative Syndrome

X-linked lymphoproliferative (XLP) disease is a disorder characterized by an inability to eliminate Epstein-Barr virus (EBV), eventually leading to fulminant infectious mononucleosis and the development of B cell tumors and associated hypogammaglobulinemia. In about 80% of cases, the disease is due to mutations in the gene encoding an adaptor molecule called SAP (SLAM-associated protein) that binds to a family of cell surface molecules involved in the activation of NK cells and T and B lymphocytes, including the signaling lymphocyte activation molecule (SLAM). SAP links the membrane proteins SLAM and 2B4 (see Chapter 7) to the Src family kinase Fyn. Defects in SAP contribute to attenuated NK and T cell activation and result in increased susceptibility to viral infections. As discussed in Chapter 11, SAP is required for T_{FH} cell development, and the inability of XLP patients to generate germinal centers and high-affinity antibodies also likely contributes to susceptibility to viral infection. In about 20% of cases of XLP, the genetic defect resides not in SAP but in the gene encoding XIAP (X-linked inhibitor of apoptosis). The resulting enhanced apoptosis of T cells and NKT cells leads to a marked depletion of these cell types. This

immunodeficiency is most commonly manifested by severe EBV infections, which probably arise opportunistically because of the ubiquitous nature of EBV.

Defective CTL and NK Cell Activation: The Familial Hemophagocytic Lymphohistiocytosis Syndromes

The hemophagocytic lymphohistiocytosis (HLH) syndromes are a group of life-threatening immunodeficiency disorders in which NK cell and CTL granule secretion is defective. As a result, viral infections are not held in check, and uncontrolled macrophage activation is a feature of these syndromes. A late but striking feature of these disorders is the ingestion of red blood cells by activated macrophages (hemophagocytosis). Mutations in the perforin gene, as well as mutations in genes encoding the cellular machinery involved in granule exocytosis, can contribute to the phenotypes observed in this syndrome. Specifically, mutations in *RAB27A,* a small guanosine triphosphatase involved in vesicular fusion, and in *MUNC13-4,* which encodes an adaptor that participates in granule exocytosis, compromise the fusion of lytic granules with the plasma membrane and thus contribute to various subtypes of HLH. Similarly, mutations in the gene for one component of the AP-3 cytosolic adaptor protein complex can also disrupt intracellular transport and contribute to a form of HLH. It is believed that T cells and macrophages respond strongly to microbes to compensate for the CTL and NK cell defects, and these compensatory responses are manifested by hemophagocytosis and lymphadenopathy in the context of immunodeficiency.

Multisystem Disorders with Immunodeficiency: Ataxia-Telangiectasia

Immunodeficiency is often one of a constellation of symptoms in a number of inherited disorders. Examples of such syndromes discussed before include Chédiak-Higashi syndrome, Wiskott-Aldrich syndrome, and DiGeorge syndrome. Ataxia-telangiectasia is an autosomal recessive disorder characterized by abnormal gait (ataxia), vascular malformations (telangiectases), neurologic deficits, increased incidence of tumors, and immunodeficiency. The immunologic defects are of variable severity and may affect both B and T cells. The most common humoral immune defects are IgA and IgG2 deficiency, probably because of the crucial role the ATM protein plays in class switch recombination (discussed later). The T cell defects, which are usually less pronounced, are associated with thymic hypoplasia. Patients experience upper and lower respiratory tract bacterial infections, multiple autoimmune phenomena, and increasingly frequent cancers with advancing age. The gene responsible for this disorder is located on chromosome 11 and encodes a protein called ATM (ataxia-telangiectasia mutated) that is related structurally to phosphatidylinositol 3-kinase but is a protein kinase. The ATM protein can activate cell cycle checkpoints and apoptosis in response to double-stranded DNA breaks and has also been shown to contribute to the stability of DNA double-stranded break complexes during V(D)J recombination. Because of these abnormalities in DNA repair, the generation of antigen receptors may also be abnormal.

DNA repair during class switch recombination not only involves the nonhomologous end-joining pathway but also requires the ATM protein, the MRE11 (meiotic recombination 11) protein, and the NBS1 (Nijmegen breakpoint syndrome 1) protein. Patients with mutations in the genes encoding these proteins or ATM often exhibit decreased levels of IgG, IgA, and IgE.

Therapeutic Approaches for Congenital Immunodeficiencies

The current treatment of immunodeficiencies has two aims: to minimize and control infections and to replace the defective or absent components of the immune system by adoptive transfer or transplantation. Passive immunization with pooled gamma globulin is very beneficial for agammaglobulinemic patients and has been lifesaving for many boys with X-linked agammaglobulinemia. Hematopoietic stem cell transplantation is currently the treatment of choice for many immunodeficiency diseases and has been successful in the treatment of SCID with ADA deficiency, Wiskott-Aldrich syndrome, bare lymphocyte syndrome, and leukocyte adhesion deficiencies. It is most successful with careful T cell depletion from the marrow and HLA matching to prevent graft-versus-host disease (see Chapter 16). Enzyme replacement therapy for ADA and PNP deficiencies has been attempted, with red blood cell transfusions used as a source of the enzymes. This approach has produced temporary clinical improvement in several patients with autosomal SCID. Injection of bovine ADA conjugated to polyethylene glycol to prolong its serum half-life has proved successful in some cases, but the benefits are usually short-lived.

In theory, the therapy of choice for congenital disorders of lymphocytes is to replace the defective gene in self-renewing stem cells. Gene replacement remains a distant goal for most human immunodeficiencies at present, despite considerable effort. The main obstacles to this type of gene therapy are difficulties in purifying self-renewing stem cells, which are the ideal target for introduction of the replacement gene, and in introducing genes into cells to achieve stable, long-lived, and high-level expression. Some progress has been made in gene therapy for ADA deficiency by use of a milder conditioning approach to deplete host bone marrow cells, which facilitates the grafting and proliferation of modified stem cells introduced into the host. A small number of patients with X-linked SCID have been successfully treated by transplantation of autologous bone marrow cells engineered to express a normal γ_c gene. However, a few treated patients have developed leukemia, apparently because the introduced γ_c gene inserted adjacent to an oncogene and activated this gene. As a result, the future of gene therapy for this disease is uncertain.

ACQUIRED (SECONDARY) IMMUNODEFICIENCIES

Deficiencies of the immune system often develop because of abnormalities that are not genetic but acquired during life (Table 20-6). The most prominent of these

TABLE 20–6 Acquired Immunodeficiencies

Cause	Mechanism
HIV infection	Depletion of $CD4^+$ T cells
Protein-calorie malnutrition	Metabolic derangements inhibit lymphocyte maturation and function
Irradiation and chemotherapy for cancer	Decreased bone marrow lymphocyte precursors
Cancer metastases and leukemia involving bone marrow	Reduced site of leukocyte development
Immunosuppression for transplants, autoimmune diseases	Reduced lymphocyte activation
Removal of spleen	Decreased phagocytosis of microbes

abnormalities is HIV infection, and this is described in the next section. Acquired immunodeficiency diseases are caused by two main types of pathogenic mechanisms. First, immunosuppression may occur as a biologic complication of another disease process. Second, so-called iatrogenic immunodeficiencies may develop as complications of therapy for other diseases.

Diseases in which immunodeficiency is a common complicating element include malnutrition, neoplasms, and infections. Protein-calorie malnutrition is common in developing countries and is associated with impaired cellular and humoral immunity to microorganisms. Much of the morbidity and mortality that afflict malnourished people is due to infections. The basis for the immunodeficiency is not well defined, but it is reasonable to assume that the global metabolic disturbances in these individuals, caused by deficient intake of protein, fat, vitamins, and minerals, will adversely affect maturation and function of the cells of the immune system.

Patients with advanced widespread cancer are often susceptible to infection because of impaired cell-mediated and humoral immune responses to a variety of organisms. Bone marrow tumors, including cancers metastatic to marrow and leukemias that arise in the marrow, may interfere with the growth and development of normal lymphocytes and other leukocytes. In addition, tumors may produce substances that interfere with lymphocyte development or function. An example of malignancy-associated immunodeficiency is the impairment in T cell function commonly observed in patients with a type of lymphoma called Hodgkin's disease. This defect was first characterized as an inability to mount a DTH reaction on intradermal injection of various common antigens to which the patients were previously exposed, such as *Candida* or tetanus toxoid. Other in vitro measures of T cell function, such as proliferative responses to polyclonal activators, are also impaired in patients with Hodgkin's disease. Such a generalized deficiency in DTH responses is called anergy. The cause of these T cell abnormalities is unknown.

Various types of infections lead to immunosuppression. Viruses other than HIV are known to impair immune responses; examples include the measles virus and human T cell lymphotropic virus 1 (HTLV-1). Both viruses can infect lymphocytes, which may be a basis for their immunosuppressive effects. Like HIV, HTLV-1 is a retrovirus with tropism for $CD4^+$ T cells; however, instead of killing helper T cells, it transforms them and produces an aggressive T cell malignant neoplasm called adult T cell leukemia/lymphoma (ATL). Patients with ATL typically have severe immunosuppression with multiple opportunistic infections. Chronic infections with *Mycobacterium tuberculosis* and various fungi frequently result in anergy to many antigens. Chronic parasitic infections may also lead to immunosuppression. For example, African children with chronic malarial infections have depressed T cell function, and this may be one reason why these children have an increased propensity to develop EBV-associated malignant tumors.

Iatrogenic immunosuppression is most often due to drug therapies that kill or functionally inactivate lymphocytes. Some drugs are given intentionally to immunosuppress patients, either for the treatment of inflammatory diseases or to prevent rejection of organ allografts. The most commonly used anti-inflammatory and immunosuppressive drugs are corticosteroids and cyclosporine, respectively. Various chemotherapeutic drugs are administered to patients with cancer, and these drugs are usually cytotoxic to mature and developing lymphocytes as well as to granulocyte and monocyte precursors. Thus, cancer chemotherapy is almost always accompanied by a period of immunosuppression and risk for infection. Iatrogenic immunosuppression and tumors involving the bone marrow are the most common causes of immunodeficiency in developed countries.

One other form of acquired immunosuppression results from the absence of a spleen caused by surgical removal of the organ after trauma and as treatment of certain hematologic diseases or by infarction in sickle cell disease. Patients without spleens are more susceptible to infection by some organisms, particularly encapsulated bacteria such as *Streptococcus pneumoniae*. This enhanced susceptibility is partly due to defective phagocytic clearance of opsonized blood-borne microbes, an important physiologic function of the spleen, and partly because of defective antibody responses resulting from the absence of marginal zone B cells.

HUMAN IMMUNODEFICIENCY VIRUS AND THE ACQUIRED IMMUNODEFICIENCY SYNDROME

AIDS is the disease caused by infection with HIV and is characterized by profound immunosuppression with associated opportunistic infections and malignant tumors, wasting, and central nervous system (CNS) degeneration. HIV infects a variety of cells of the immune system, including $CD4^+$ helper T cells, macrophages, and dendritic cells. HIV evolved as a human pathogen very recently relative to most other known human pathogens, and the HIV epidemic was first identified only in the 1980s. However, the degree of morbidity and mortality caused by HIV and the global impact of HIV infection on health care resources and economics are already

enormous and continue to grow. HIV has infected 50 to 60 million people and has caused the death of more than 22 million adults and children. Approximately 35 million people are living with HIV infection and AIDS, of which approximately 70% are in Africa and 20% in Asia, and almost 2 million die of the disease every year. The disease is especially devastating because about half of the approximately 3 million new cases every year occur in young adults (15 to 24 years old). AIDS has left approximately 14 million orphans and resulted in the death of approximately 30 million people. Currently, there is no effective vaccine or cure for AIDS, but quite effective antiretroviral therapies have been developed. In this section of the chapter, we describe the molecular and biologic properties of HIV, the pathogenesis of HIV-induced immunodeficiency, and the clinical and epidemiologic features of HIV-related diseases.

Molecular and Biologic Features of HIV

HIV is a member of the lentivirus family of animal retroviruses. Lentiviruses, including visna virus of sheep and the bovine, feline, and simian immunodeficiency viruses, are capable of long-term latent infection of cells and short-term cytopathic effects, and they all produce slowly progressive, fatal diseases that include wasting syndromes and CNS degeneration. Two closely related types of HIV, designated HIV-1 and HIV-2, have been identified. HIV-1 is by far the most common cause of AIDS; HIV-2, which differs in genomic structure and antigenicity, causes a form of AIDS with slower progression than HIV-1–linked disease.

HIV Structure and Genes

An infectious HIV particle consists of two identical strands of RNA packaged within a core of viral proteins and surrounded by a phospholipid bilayer envelope derived from the host cell membrane but including virally encoded membrane proteins (Fig. 20-3). The RNA genome of HIV is approximately 9.2 kb long and has the basic arrangement of nucleic acid sequences characteristic of all known retroviruses (Fig. 20-4). Long terminal repeats (LTRs) at each end of the genome regulate viral gene expression, viral integration into the host genome, and viral replication. The *gag* sequence encodes core structural proteins. The *env* sequence encodes the envelope glycoproteins gp120 and gp41, which noncovalently associate with each other and are required for infection of cells. The *pol* sequence encodes reverse transcriptase, integrase, and viral protease enzymes required for viral replication. In addition to these typical retrovirus genes, HIV-1 also includes six other regulatory genes, namely, the *tat, rev, vif, nef, vpr,* and *vpu* genes, whose products regulate viral reproduction and host immune evasion in various ways. The functions of these genes are summarized in Figure 20-4.

Viral Life Cycle

HIV infection of cells begins when the envelope glycoprotein (Env) of the virus binds to both CD4 and a coreceptor that is a member of the chemokine receptor family (Fig. 20-5). The viral particles that initiate infection are usually in the blood, semen, or other body fluids of one individual and are introduced into another individual by sexual contact, needle stick, or transplacental passage.

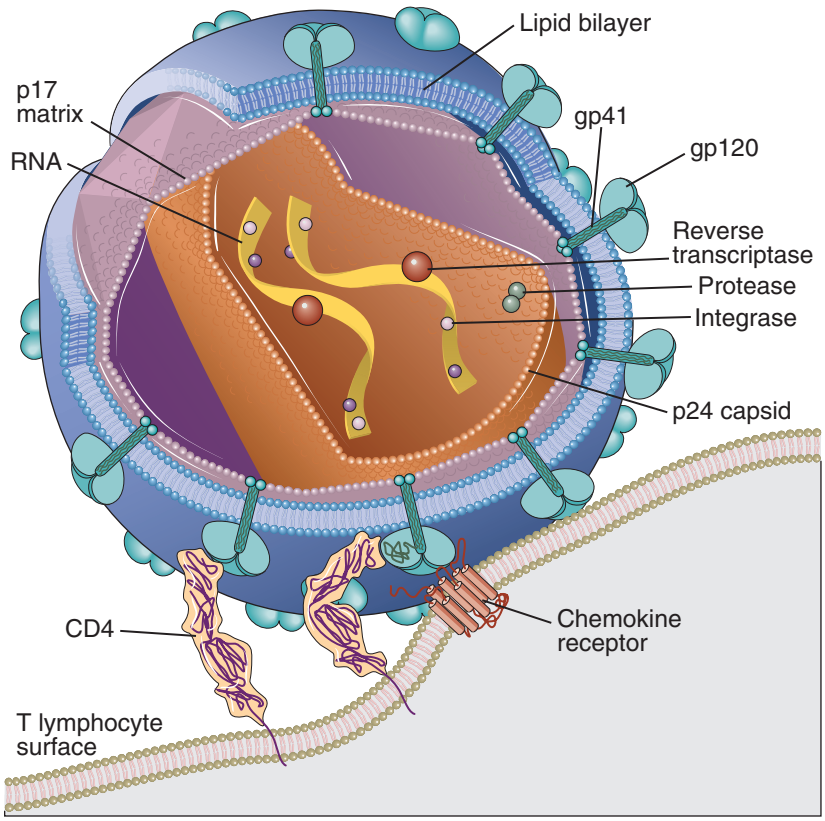
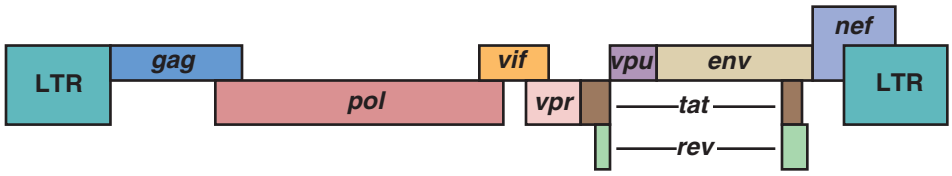


FIGURE 20–3 Structure of HIV-1. An HIV-1 virion is shown next to a T cell surface. HIV-1 consists of two identical strands of RNA (the viral genome) and associated enzymes, including reverse transcriptase, integrase, and protease, packaged in a cone-shaped core composed of p24 capsid protein with a surrounding p17 protein matrix, all surrounded by a phospholipid membrane envelope derived from the host cell. Virally encoded membrane proteins (gp41 and gp120) are bound to the envelope. CD4 and chemokine receptors on the host cell surface function as HIV-1 receptors. *(© 2000 Terese Winslow.)*



LTR	Transcription of viral genome; integration of viral DNA into host cell genome; binding site for host transcription factors
gag	Nucleocapsid core and matrix proteins
pol	Reverse transcriptase, protease, integrase, and ribonuclease
env	Viral coat proteins (gp120 and gp41)
vif	Overcomes inhibitory effect of host cell enzyme (APOBEC3G), promotes viral replication
vpr	Increases viral replication; promotes HIV infection of macrophages; blocks cell cycle progression
tat	Required for elongation of viral transcripts
rev	Promotes nuclear export of incompletely spliced viral RNAs
vpu	Downregulates host cell CD4 expression; enhances release of virus from cells; counteracts host restriction
nef	Downregulates host cell CD4 and class I MHC expression; enhances release of infectious virus factor tetherin

Abbreviations: LTR, long terminal repeat; gag, group-specific antigen; pol, polymerase; env, envelope; vif, viral infectivity factor; vpr, viral protein r; tat, transcriptional activator; rev, regulator of viral gene expression; vpu, viral protein u; nef, negative effector

FIGURE 20–4 HIV-1 genome. The genes along the linear genome are indicated as differently colored blocks. Some genes use some of the same sequences as other genes, as shown by overlapping blocks, but are read differently by host cell RNA polymerase. Similarly shaded blocks separated by lines indicate genes whose coding sequences are separated in the genome and require RNA splicing to produce functional mRNA. *(Modified from Greene W. AIDS and the immune system. Copyright 1993 by Scientific American, Inc. All rights reserved.)*

Env is a complex composed of a transmembrane gp41 subunit and an external, noncovalently associated gp120 subunit. These subunits are produced by proteolytic cleavage of a gp160 precursor. The Env complex is expressed as a trimeric structure of three gp120/gp41 pairs. This complex mediates a multistep process of fusion of the virion envelope with the membrane of the target cell (Fig. 20-6). The first step of this process is the binding of gp120 subunits to CD4 molecules, which induces a conformational change that promotes secondary gp120 binding to a chemokine coreceptor. Coreceptor binding induces a conformational change in gp41 that exposes a hydrophobic region, called the fusion peptide, which inserts into the cell membrane and enables the viral membrane to fuse with the target cell membrane. After the virus completes its life cycle in the infected cell (described later), free viral particles are released from one infected cell and bind to an uninfected cell, thus propagating the infection. In addition, gp120 and gp41, which are expressed on the plasma membrane of infected cells before virus is released, can mediate cell-cell fusion with an uninfected cell that expresses CD4 and coreceptors, and HIV genomes can then be passed between the fused cells directly.

The most important chemokine receptors that act as coreceptors for HIV are CXCR4 and CCR5. More than seven different chemokine receptors have been shown to serve as coreceptors for HIV entry into cells, and several other proteins belonging to the seven-transmembrane–spanning G protein–coupled receptor family, such as the leukotriene B_4 receptor, can also mediate HIV infection of cells. Different isolates of HIV have distinct tropisms for different cell populations that are related to the specificity of gp120 variants for different chemokine receptors. All HIV strains can infect and replicate in freshly isolated human $CD4^+$ T cells that are activated in vitro. In contrast, some strains will infect primary cultures of human macrophages but not continuous T cell lines (macrophage-tropic, or M-tropic, virus), whereas other strains will infect T cell lines but not macrophages (T-tropic virus). Some virus strains also infect both T cell lines and macrophages (dual-tropic virus). Macrophage-tropic virus isolates express a gp120 that binds to CCR5, which is expressed on macrophages (and some memory T cells), whereas T cell–tropic viruses bind to CXCR4, which is expressed on T cell lines. HIV variants are described as X4 for CXCR4 binding, R5 for CCR5 binding, or R5X4 for the ability to bind to both chemokine receptors. In many HIV-infected individuals, there is a change from the production of virus that uses CCR5 and is predominantly macrophage tropic early in the disease to virus that binds to CXCR4 and is T cell line tropic late in the disease. The T-tropic strains tend to be more virulent, presumably because they infect and deplete T cells more than do M-tropic strains. The importance of CCR5 in HIV infection in vivo is supported by the finding that individuals

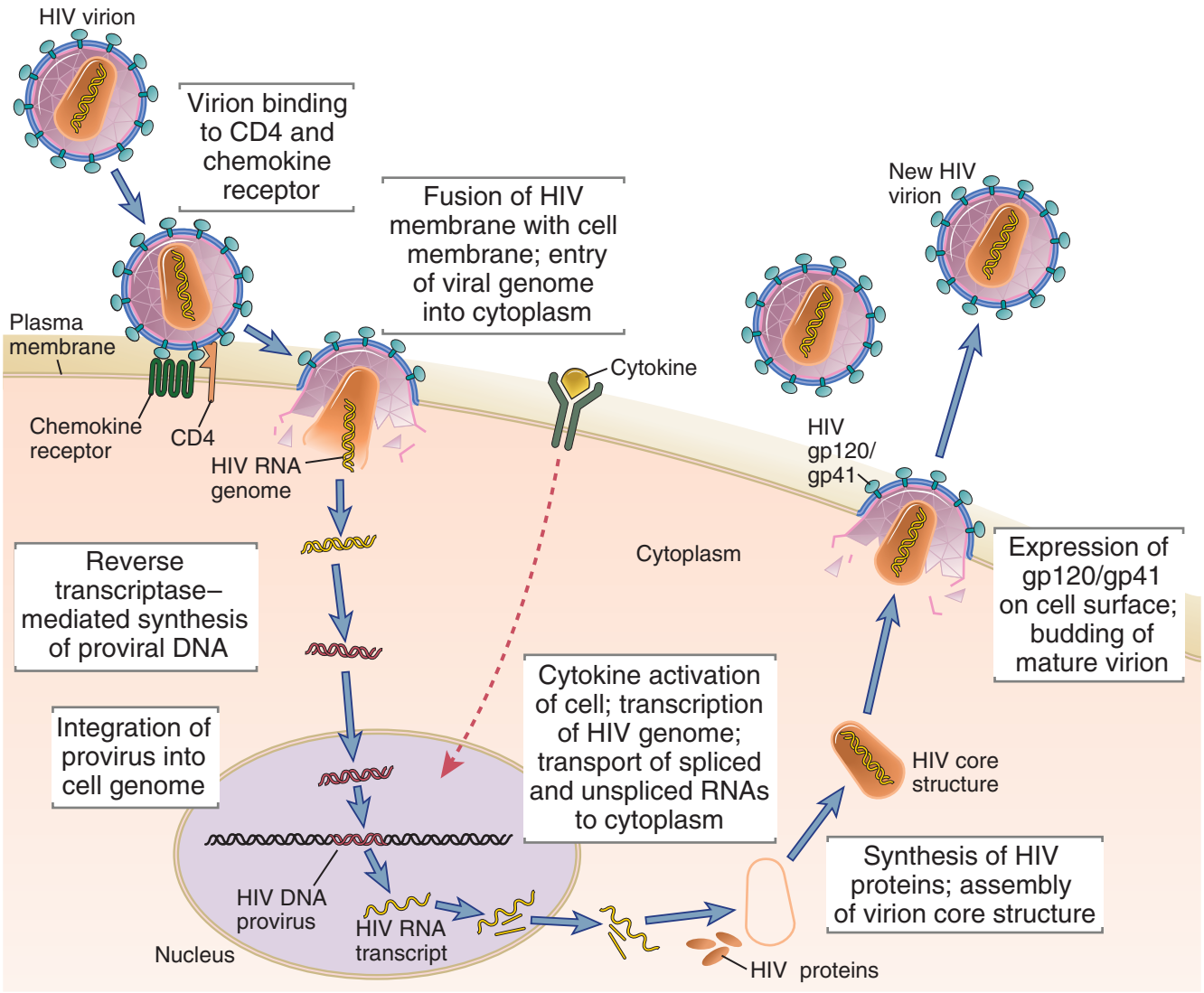


FIGURE 20–5 HIV life cycle. The sequential steps in the life cycle of HIV are shown, from initial infection of a host cell to viral replication and release of a new virion. For the sake of clarity, the production and release of only one new virion are shown. An infected cell actually produces many virions, each capable of infecting cells, thereby amplifying the infectious cycle.

who do not express this receptor on the cell surface because of an inherited homozygous 32-bp deletion in the *CCR5* gene are resistant to HIV infection.

Once an HIV virion enters a cell, the enzymes within the nucleoprotein complex become active and begin the viral reproductive cycle (see Fig. 20-5). The nucleoprotein core of the virus becomes disrupted, the RNA genome of HIV is reverse-transcribed into a double-stranded DNA form by viral reverse transcriptase, and the viral DNA enters the nucleus. The viral integrase also enters the nucleus and catalyzes the integration of viral DNA into the host cell genome. The integrated HIV DNA is called the provirus. The provirus may remain transcriptionally inactive for months or years, with little or no production of new viral proteins or virions, and in this way HIV infection of an individual cell can be latent.

Transcription of the genes of the integrated DNA provirus is regulated by the LTR upstream of the viral structural genes, and cytokines or other physiologic stimuli that trigger T cells and macrophages enhance viral gene transcription. The LTRs contain polyadenylation signal sequences, the TATA box promoter sequence, and binding sites for two host cell transcription factors, NF-κB and SP1. Initiation of HIV gene transcription in T cells is linked to activation of the T cells by antigen or cytokines. For example, polyclonal activators of T cells, such as phytohemagglutinin, and cytokines such as IL-2, tumor necrosis factor (TNF), and lymphotoxin stimulate HIV gene expression in infected T cells, and IL-1, IL-3, IL-6, TNF, lymphotoxin, IFN-γ, and granulocyte-macrophage colony-stimulating factor (GM-CSF) stimulate HIV gene expression and viral replication in infected monocytes and macrophages. TCR and cytokine stimulation of HIV gene transcription probably involves the activation of NF-κB and its binding to sequences in the LTR. This phenomenon is significant to the pathogenesis of AIDS because the normal response of a latently infected T cell to a microbe may be the way in which latency is ended

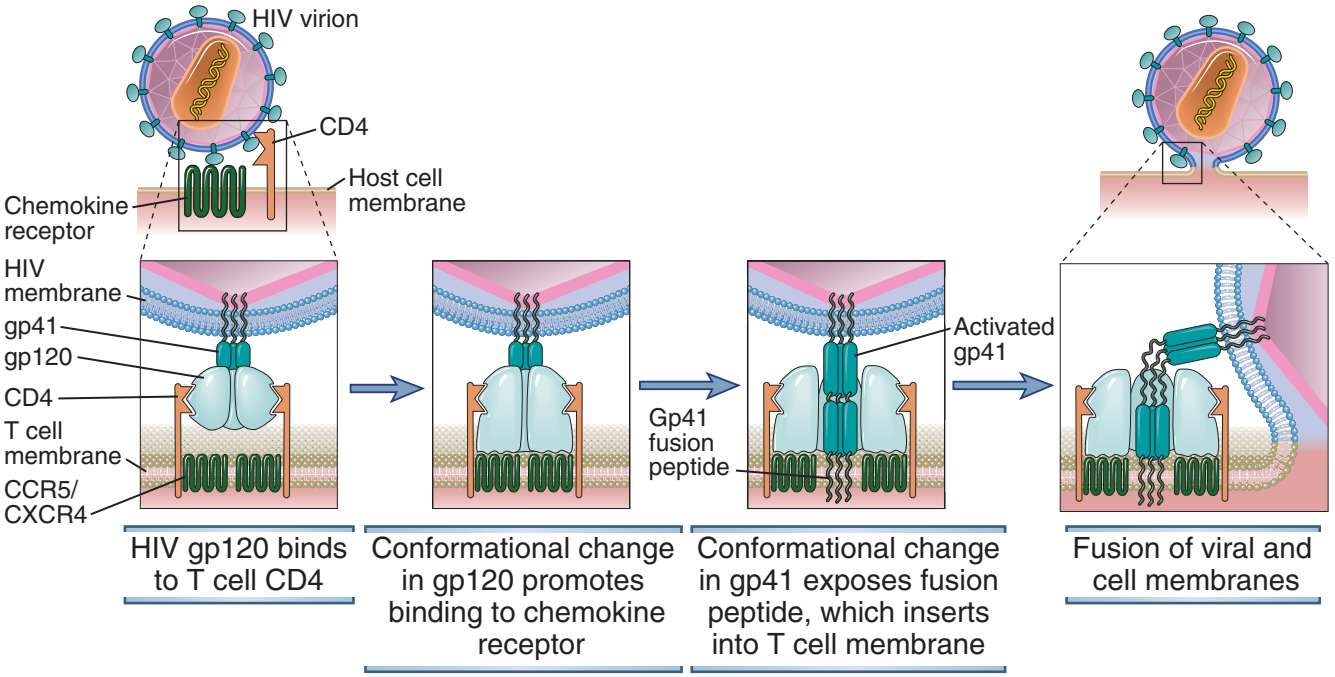


FIGURE 20–6 Mechanism of HIV entry into a cell. In the model depicted, sequential conformational changes in gp120 and gp41 are induced by binding to CD4. These changes promote binding of the virus to the coreceptor (a chemokine receptor) and fusion of the HIV-1 and host cell membranes. The fusion peptide of activated gp41 contains hydrophobic amino acid residues that mediate insertion into the host cell plasma membrane.

and virus production begins. The multiple infections that AIDS patients acquire thus stimulate HIV production and infection of additional cells.

The Tat protein is required for HIV gene expression and acts by enhancing the production of complete viral mRNA transcripts. Even in the presence of optimal signals to initiate transcription, few if any HIV mRNA molecules are actually synthesized without the action of Tat because transcription of HIV genes by mammalian RNA polymerase is inefficient and the polymerase complex usually stops before the mRNA is completed. Tat protein binds to the nascent mRNA and increases the "processivity" of RNA polymerase by several hundred-fold, which allows transcription to be completed to produce a functional viral mRNA.

Synthesis of mature, infectious viral particles begins after full-length viral RNA transcripts are produced and the viral genes are expressed as proteins. The mRNAs encoding the various HIV proteins are derived from a single full-genome-length transcript by differential splicing events. HIV gene expression may be divided into an early stage, during which regulatory genes are expressed, and a late stage, during which structural genes are expressed and full-length viral genomes are packaged. The Rev, Tat, and Nef proteins are early gene products encoded by fully spliced mRNAs that are exported from the nucleus and translated into proteins in the cytoplasm soon after infection of a cell. Late genes include *env, gag,* and *pol,* which encode the structural components of the virus and are translated from singly spliced or unspliced RNA. The Rev protein initiates the switch from early to late gene expression by promoting the export of these incompletely spliced late gene RNAs out of the nucleus. The *pol* gene product is a precursor protein that is sequentially cleaved to form reverse transcriptase, protease, ribonuclease, and integrase enzymes. As mentioned before, reverse transcriptase and integrase proteins are required to produce a DNA copy of the viral RNA genome and to integrate it as a provirus into the host genome. The *gag* gene encodes a 55-kD protein that is proteolytically cleaved into p24, p17, and p15 polypeptides by the action of the viral protease encoded by the *pol* gene. These polypeptides are the core proteins that are required for assembly of infectious viral particles. The primary product of the *env* gene is a 160-kD glycoprotein (gp160) that is cleaved by cellular proteases within the endoplasmic reticulum into the gp120 and gp41 proteins required for HIV binding to cells, as discussed earlier. Current antiviral drug therapy for HIV disease includes inhibitors of the enzymes reverse transcriptase, protease, and integrase.

After transcription of various viral genes, viral proteins are synthesized in the cytoplasm. Assembly of infectious viral particles then begins by packaging full-length RNA transcripts of the proviral genome within a nucleoprotein complex that includes the *gag* core proteins and the *pol*-encoded enzymes required for the next cycle of integration. This nucleoprotein complex then buds from the plasma membrane, capturing Env and host glycoproteins as part of its envelope. The rate of virus production can reach sufficiently high levels to cause cell death, as discussed later.

A host factor that prevents virion release in certain cell types is a protein called tetherin. Tetherin prevents the

pinching off of certain viruses including HIV, and its inhibition of the budding process can be antagonized by an HIV protein called Vpu.

Pathogenesis of HIV Infection and AIDS

HIV disease begins with acute infection, which is only partly controlled by the adaptive immune response, and advances to chronic progressive infection of peripheral lymphoid tissues (Fig. 20-7). The virus typically enters through mucosal epithelia. The subsequent events in the infection can be divided into several phases.

Acute (early) infection is characterized by infection of memory CD4$^+$ T cells in mucosal lymphoid tissues and death of many infected cells. Because the mucosal tissues are the largest reservoir of T cells in the body and the major site of residence of memory T cells, this local loss is reflected in considerable depletion of lymphocytes. In fact, within 2 weeks of infection, a large fraction of CD4$^+$ T cells may be destroyed.

The transition from the acute phase to a chronic phase of infection is characterized by dissemination of the virus, viremia, and the development of host immune responses. Dendritic cells in epithelia at sites of virus entry capture the virus and then migrate into the lymph nodes. Dendritic cells express a protein with a mannose-binding lectin domain, called DC-SIGN, which may be particularly important in binding the HIV envelope and transporting the virus. Once in lymphoid tissues, dendritic cells may pass HIV on to CD4$^+$ T cells through direct cell-cell contact. Within days after the first exposure to HIV, viral replication can be detected in the lymph nodes. This replication leads to viremia, during which large numbers of HIV particles are present in the patient's blood, accompanied by an acute HIV syndrome that includes a variety of nonspecific signs and symptoms typical of many viral infections (described later). The viremia allows the virus to disseminate throughout the body and to infect helper T cells, macrophages, and dendritic cells in peripheral lymphoid tissues. As the HIV infection spreads, the adaptive immune system mounts both humoral and cell-mediated immune responses directed at viral antigens, which we will describe later. These immune responses partially control the infection and viral production, and such control is reflected by a drop in viremia to low but detectable levels by approximately 12 weeks after the primary exposure.

In the next, chronic phase of the disease, lymph nodes and the spleen are sites of continuous HIV replication and cell destruction (see Fig. 20-7). During this period of the disease, the immune system remains competent at handling most infections with opportunistic microbes, and few or no clinical manifestations of the HIV infection are

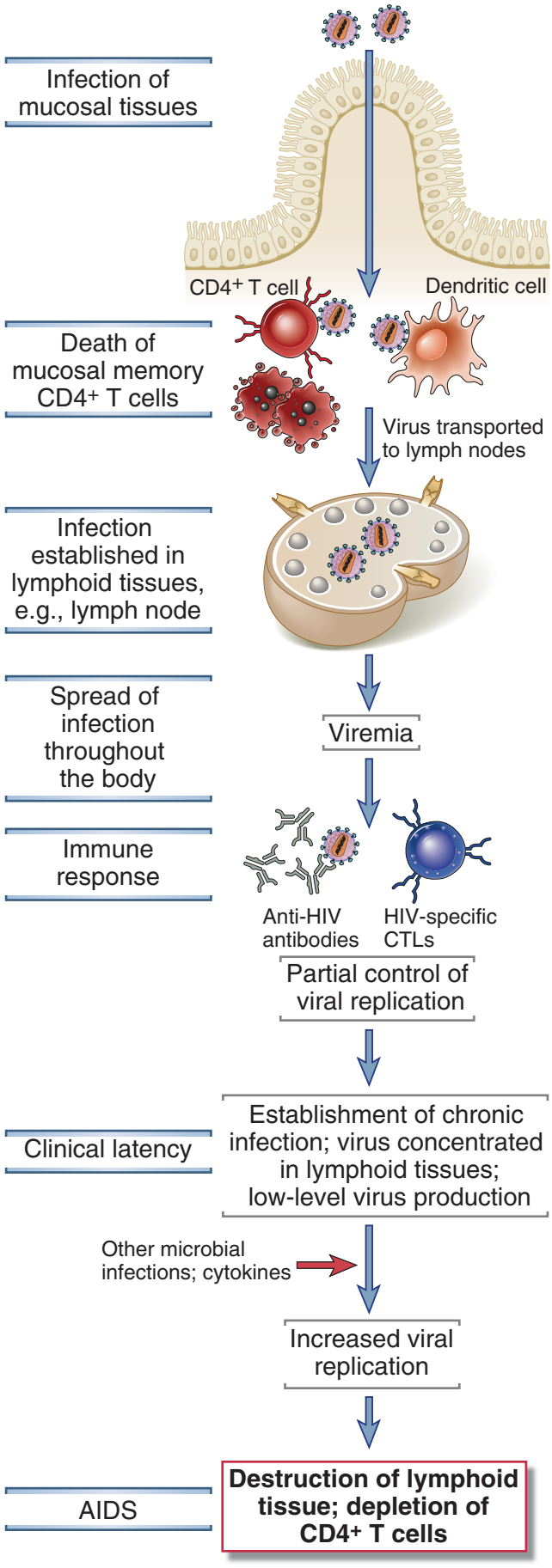


FIGURE 20–7 Progression of HIV infection. The progression of HIV infection correlates with spread of the virus from the initial site of infection to lymphoid tissues throughout the body. The immune response of the host temporarily controls acute infection but does not prevent the establishment of chronic infection of cells in lymphoid tissues. Cytokine stimuli induced by other microbes serve to enhance HIV production and progression to AIDS.

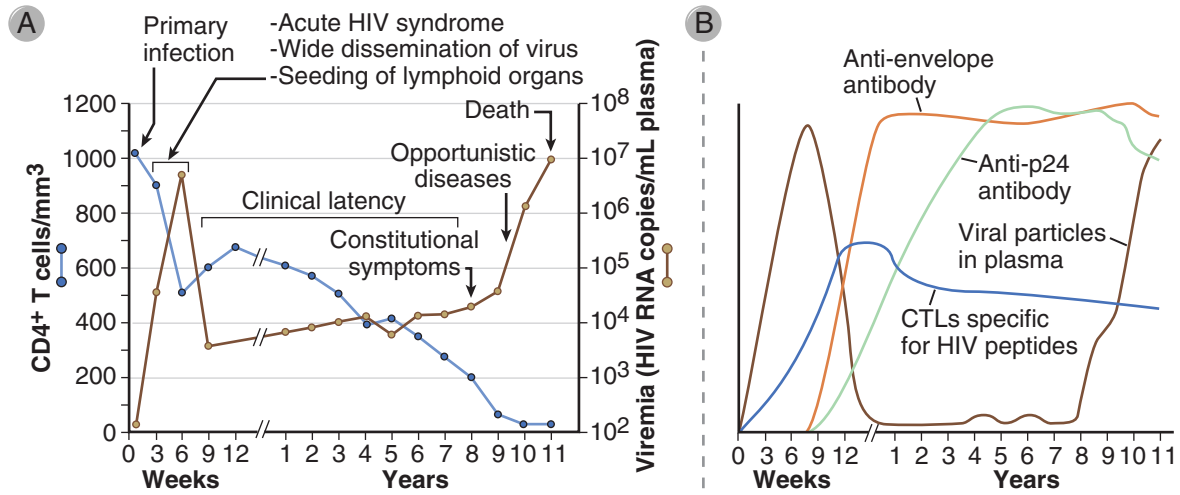


FIGURE 20–8 Clinical course of HIV disease. A, Plasma viremia, blood $CD4^+$ T cell counts, and clinical stages of disease. About 12 weeks after infection, blood-borne virus (plasma viremia) is reduced to very low levels (detectable only by sensitive reverse transcriptase–polymerase chain reaction assays) and stays this way for many years. Nonetheless, $CD4^+$ T cell counts steadily decline during this clinical latency period because of active viral replication and T cell infection in lymph nodes. When $CD4^+$ T cell counts drop below a critical level (about 200/mm^3), the risk for infection and other clinical features of AIDS is high. *(From Pantaleo G, C Graziosi, and AS Fauci. New concepts in the immunopathogenesis of human immunodeficiency virus infection. New England Journal of Medicine 328:327-335, 1993. Copyright © 1993 Massachusetts Medical Society. All rights reserved.)* **B,** Immune response to HIV infection. A CTL response to HIV is detectable by 2 to 3 weeks after the initial infection and peaks by 9 to 12 weeks. Marked expansion of virus-specific $CD8^+$ T cells occurs during this time, and up to 10% of a patient's CTLs may be HIV specific at 12 weeks. The humoral immune response to HIV peaks at about 12 weeks.

present. Therefore, this phase of HIV disease is called the clinical latency period. Although the majority of peripheral blood T cells do not harbor the virus, destruction of $CD4^+$ T cells within lymphoid tissues steadily progresses during the latent period, and the number of circulating blood $CD4^+$ T cells steadily declines (Fig. 20-8). More than 90% of the body's approximately 10^{12} T cells are normally found in peripheral and mucosal lymphoid tissues, and it is estimated that HIV destroys up to 1 to 2×10^9 $CD4^+$ T cells every day. Early in the course of the disease, the body may continue to make new $CD4^+$ T cells, and therefore these cells can be replaced almost as quickly as they are destroyed. At this stage, up to 10% of $CD4^+$ T cells in lymphoid organs may be infected, but the number of circulating $CD4^+$ T cells that are infected at any one time may be less than 0.1% of the total $CD4^+$ T cells in an individual. Eventually, over a period of years, the continuous cycle of virus infection, T cell death, and new infection leads to a steady decline in the number of $CD4^+$ T cells in the lymphoid tissues and the circulation.

Mechanisms of Immunodeficiency Caused by HIV

HIV infection ultimately results in impaired function of both the adaptive and innate immune systems. The most prominent defects are in cell-mediated immunity, and they can be attributed to several mechanisms, including direct cytopathic effects of the virus and indirect effects.

An important cause of the loss of $CD4^+$ T cells in HIV-infected people is the direct cytopathic effect of infection of these cells by HIV. Death of $CD4^+$ T cells is associated with production of virus in infected cells and is a major cause of the decline in the numbers of these cells, especially in the early (acute) phase of the infection. Several direct toxic effects of HIV on infected $CD4^+$ cells have been described.

- The process of virus production, with expression of gp41 in the plasma membrane and budding of viral particles, may lead to increased plasma membrane permeability and the influx of lethal amounts of calcium, which induces apoptosis, or osmotic lysis of the cell caused by the influx of water.
- Viral production can interfere with cellular protein synthesis and thereby lead to cell death.
- The plasma membranes of HIV-infected T cells fuse with uninfected $CD4^+$ T cells by virtue of gp120-CD4 interactions, and multinucleated giant cells or syncytia are formed. The process of HIV-induced syncytia formation can be lethal to HIV-infected T cells as well as to uninfected $CD4^+$ T cells that fuse to the infected cells. However, this phenomenon has largely been observed in vitro, and syncytia are rarely seen in the tissues of patients with AIDS.

Mechanisms in addition to direct lysis of infected $CD4^+$ T cells by virus have been proposed for the depletion and loss of function of these cells in HIV-infected individuals. One mechanism that has been suggested is related to chronic activation of uninfected cells by the infections that are common in patients infected with HIV and also by cytokines produced in response to these infections. Chronic activation of the T cells may predispose the cells to apoptosis; the molecular pathway involved in this type of activation-induced cell death is not yet defined. Apoptotic death of activated lymphocytes may account for the observation that the loss of T cells greatly exceeds the numbers of HIV-infected cells. HIV-specific CTLs are

present in many patients with AIDS, and these cells can kill infected $CD4^+$ T cells. In addition, antibodies against HIV envelope proteins may bind to HIV-infected $CD4^+$ T cells and target the cells for antibody-dependent cell-mediated cytotoxicity. Binding of gp120 to newly synthesized intracellular CD4 may interfere with normal protein processing in the endoplasmic reticulum and block cell surface expression of CD4, making the cells incapable of responding to antigenic stimulation. It has also been suggested that the maturation of $CD4^+$ T cells in the thymus becomes defective in infected individuals. The relative importance of these indirect mechanisms of $CD4^+$ T cell depletion in HIV-infected patients is uncertain and controversial.

Functional defects in the immune system of HIV-infected individuals exacerbate the immune deficiency caused by depletion of $CD4^+$ T cells. These functional defects include a decrease in T cell responses to antigens and weak humoral immune responses even though total serum Ig levels may be elevated. The defects may be a result of the direct effects of HIV infection on $CD4^+$ T cells, including the effects of soluble gp120 released from infected cells binding to uninfected cells. For example, CD4 that has bound gp120 may not be available to interact with class II MHC molecules on APCs, and thus T cell responses to antigens would be inhibited. Alternatively, gp120 binding to CD4 may deliver signals that down-regulate helper T cell function. HIV-infected T cells are unable to form tight synapses with APCs, and this may also interfere with T cell activation. Some studies have demonstrated that patients with HIV infection have increased numbers of $CD4^+CD25^+$ regulatory T cells, but it is not yet clear if this is a consistent finding or if these cells actually contribute to defective immunity.

The Tat protein may play some role in the pathogenesis of immunodeficiency caused by HIV. Within T cells, Tat can interact with a variety of regulatory proteins, and these interactions can interfere with normal T cell functions such as cytokine synthesis. Remarkably, Tat not only enters the nucleus of infected T cells but can also escape across the plasma membrane and enter neighboring cells, thus interfering with activation of uninfected T cells in a paracrine fashion.

Macrophages, dendritic cells, and follicular dendritic cells are infected or injured by HIV, and their abnormalities also contribute to the progression of immunodeficiency.

- Macrophages express much lower levels of CD4 than helper T lymphocytes do, but they do express CCR5 coreceptors and are susceptible to HIV infection. However, macrophages are relatively resistant to the cytopathic effects of HIV. Macrophages may also be infected by a gp120/gp41-independent route, such as phagocytosis of other infected cells or Fc receptor–mediated endocytosis of antibody-coated HIV virions. Because macrophages can be infected but are not generally killed by the virus, they may become a reservoir for the virus. In fact, the quantity of macrophage-associated HIV exceeds T cell–associated virus in most tissues from patients with AIDS, including the brain and lung. HIV-infected macrophages may be impaired in antigen presentation functions and cytokine secretion.
- Dendritic cells can also be infected by HIV. Like macrophages, dendritic cells are not directly injured by HIV infection. However, these cells form intimate contact with naive T cells during the course of antigen presentation. It is proposed that dendritic cells infect naive T cells during these encounters and may thus be an important pathway for T cell injury.
- Follicular dendritic cells (FDCs) in the germinal centers of lymph nodes and the spleen trap large amounts of HIV on their surfaces, in part by Fc receptor–mediated binding of antibody-coated virus. Although FDCs are not efficiently infected, they contribute to the pathogenesis of HIV-associated immunodeficiency in at least two ways. First, the FDC surface is a reservoir for HIV that can infect macrophages and $CD4^+$ T cells in the lymph nodes. Second, the normal functions of FDCs in immune responses are impaired, and they may eventually be destroyed by the virus. Although the mechanisms of HIV-induced death of FDCs are not understood, the net result of loss of the FDC network in the lymph nodes and spleen is a profound dissolution of the architecture of the peripheral lymphoid system.

HIV Reservoirs and Viral Turnover

The virus detected in patients' blood is produced mostly by short-lived infected $CD4^+$ T cells and in smaller amounts by other infected cells. Three phases of decay of plasma viremia have been observed in patients treated with antiretroviral drugs or predicted by mathematical modeling, and these decay curves have been used to surmise the distribution of HIV in different cellular reservoirs. More than 90% of plasma virus is believed to be produced by short-lived cells (half-lives of ~1 day), which are most likely activated $CD4^+$ T cells that are major reservoirs and sources of the virus in infected patients. About 5% of plasma virus is produced by macrophages, which have a slower turnover (half-life of about 2 weeks). It is hypothesized that a small fraction of the virus, perhaps as little as 1%, is present in latently infected memory T cells. Because of the long life span of memory cells, it could take decades for this reservoir of virus to be eliminated even if all new rounds of infection were blocked.

Clinical Features of HIV Disease

A vast amount of information has accumulated about the epidemiology and clinical course of HIV infection. As antiretroviral drug therapy is improving, many of the clinical manifestations are changing. In the following section, we describe the "classical" features of HIV infection and refer to the changing pictures when relevant.

Transmission of HIV and Epidemiology of AIDS

HIV is transmitted from one individual to another by three major routes:

- ***Sexual contact is the most frequent mode of transmission,*** either between heterosexual couples (the most

frequent mode of transmission in Africa and Asia) or between homosexual male partners. In sub-Saharan Africa, where the infection rate is the highest in the world (estimated to be about 10,000 new cases every day), more than half the infected individuals are women.

- ***Mother-to-child transmission*** of HIV accounts for the majority of pediatric cases of AIDS. This type of transmission occurs most frequently in utero or during childbirth, although transmission through breast milk is also possible.
- ***Inoculation of a recipient with infected blood or blood products*** is also a frequent mode of HIV transmission. Needles shared by intravenous drug abusers account for most cases of this form of transmission. With the advent of routine laboratory screening, transfusion of blood or blood products in a clinical setting accounts for a small portion of HIV infections.

Clinical Course of HIV Infection

The course of HIV disease can be followed by measuring the amount of virus in the patient's plasma and by the blood $CD4^+$ T cell count (see Fig. 20-8).

- ***The acute phase*** of the illness, also called the acute HIV syndrome, is the period of viremia characterized by nonspecific symptoms of infection. It develops in 50% to 70% of infected adults typically 3 to 6 weeks after infection. There is a spike of plasma virus and a modest reduction in $CD4^+$ T cell counts, but the number of blood $CD4^+$ T cells often returns to normal. In many patients, however, the infection is occult and there are no symptoms.
- ***The chronic phase of clinical latency*** may last for many years. During this time, the virus is contained within lymphoid tissues, and the loss of $CD4^+$ T cells is corrected by replenishment from progenitors. Patients are asymptomatic or suffer from minor infections. Within 2 to 6 months after infection, the concentration of plasma virus stabilizes at a particular set-point, which differs among patients. The level of the viral set-point and the number of blood $CD4^+$ T cells are clinically useful predictors of the progression of disease. As the disease progresses, patients become susceptible to other infections, and immune responses to these infections may stimulate HIV production and accelerate the destruction of lymphoid tissues. As discussed earlier, HIV gene transcription can be enhanced by stimuli that activate T cells, such as antigens and a variety of cytokines. Cytokines, such as TNF, which are produced by the innate immune system in response to microbial infections, are particularly effective in boosting HIV production. Thus, as the immune system attempts to eradicate other microbes, it brings about its own destruction by HIV.
- ***HIV disease progresses to the final and once almost invariably lethal phase, called AIDS, when the blood $CD4^+$ T cell count drops below 200 cells/mm^3.*** HIV viremia may climb dramatically as viral replication in reservoirs other than T cells accelerates unchecked. Patients with AIDS suffer from combinations of opportunistic infections, neoplasms, cachexia (HIV wasting syndrome), kidney failure (HIV nephropathy), and CNS degeneration (AIDS encephalopathy) (Table 20-7). Because $CD4^+$ helper T cells are essential for both cell-mediated and humoral immune responses to various microbes, the loss of these lymphocytes is the main reason that patients with AIDS become susceptible to many different types of infections. Furthermore, many of the tumors that arise in patients with AIDS have a viral etiology, and their prevalence in the setting of AIDS reflects an inability of the HIV-infected patient to mount an effective immune response against oncogenic viruses. Cachexia is often seen in patients with chronic inflammatory diseases and may result from effects of inflammatory cytokines (such as TNF) on appetite and metabolism. The CNS disease in AIDS may be due to neuronal damage by the virus or by shed viral proteins such as gp120 and Tat, as well as the effects of cytokines elaborated by infected microglial cells. Many of these devastating consequences of HIV infection, including opportunistic infections and tumors, have been significantly reduced by highly active antiretroviral therapy.

TABLE 20–7 Clinical Features of HIV Infection

Phase of Disease	Clinical Feature
Acute HIV disease	Fever, headaches, sore throat with pharyngitis, generalized lymphadenopathy, rashes
Clinical latency period	Declining blood $CD4^+$ T cell count
AIDS	Opportunistic infections Protozoa *(Toxoplasma, Cryptosporidium)* Bacteria *(Mycobacterium avium, Nocardia, Salmonella)* Fungi *(Candida, Cryptococcus neoformans, Coccidioides immitis, Histoplasma capsulatum, Pneumocystis)* Viruses (cytomegalovirus, herpes simplex, varicella-zoster) Tumors Lymphomas (including EBV-associated B cell lymphomas) Kaposi's sarcoma Cervical carcinoma Encephalopathy Wasting syndrome

Although this summary of the clinical course is true for the most severe cases, the rate of progression of the disease is highly variable, and some individuals are long-term nonprogressors. The immunologic correlates of variable progression remain unknown. Also, recent antiretroviral therapy has changed the course of the disease and greatly reduced the incidence of severe opportunistic infections (such as *Pneumocystis*) and tumors (such as Kaposi's sarcoma).

Immune Responses to HIV

HIV-specific humoral and cell-mediated immune responses develop after infection but generally provide limited protection. The early response to HIV infection is, in fact, similar in many ways to the immune response to other viruses and serves to clear most of the virus present in the blood and in circulating T cells. Nonetheless, it is clear that these immune responses fail to eradicate all virus, and the infection eventually overwhelms the immune system in most individuals. Despite the poor effectiveness of immune responses to the virus, it is important to characterize them for three reasons. First, the immune responses may be detrimental to the host, for example, by stimulating the uptake of opsonized virus into uninfected cells by Fc receptor–mediated endocytosis or by eradication of $CD4^+$ T cells expressing viral antigens by $CD8^+$ CTLs. Second, antibodies against HIV are diagnostic markers of HIV infection that are widely used for screening purposes. Third, the design of effective vaccines for immunization against HIV requires knowledge of the types of immune responses that are most likely to be protective (the "correlates of protection").

Many innate immune responses against HIV have been described. These include antimicrobial peptides (defensins), NK cells, dendritic cells (particularly plasmacytoid dendritic cells producing type I interferons), and the complement system. The role of these responses in combating the infection is not established.

The initial adaptive immune response to HIV infection is characterized by expansion of $CD8^+$ T cells specific for HIV peptides. As many as 10% or more of circulating $CD8^+$ T cells may be specific for HIV during the early stages of infection. These CTLs control infection in the acute phase (see Fig. 20-8) but ultimately prove ineffective because of the emergence of viral escape mutants (variants with mutated antigens). $CD4^+$ T cells also respond to the virus, and these $CD4^+$ T cells may contribute to viral control in a number of ways. An effective $CD4^+$ T cell response is required as a source of help for the generation of $CD8^+$ memory T cells, but $CD4^+$ T cells have also been shown to mediate cytolytic responses against HIV-infected cells, perhaps using Fas ligand to target Fas on infected $CD4^+$ T cells.

The importance of CTL responses in HIV control is underscored by the evolution of the virus under immune pressure, resulting in viral isolates that have lost their original CTL epitopes. The evolution of the virus also results in the loss of epitopes recognized by $CD4^+$ T cells, indicating that both $CD8^+$ and $CD4^+$ cells contribute to host defense against the virus.

Antibody responses to a variety of HIV antigens are detectable within 6 to 9 weeks after infection. The most immunogenic HIV molecules that elicit antibody responses appear to be the envelope glycoproteins, and high titers of anti-gp120 and anti-gp41 antibodies are present in most HIV-infected individuals. Other anti-HIV antibodies found frequently in patients' sera include antibodies to p24, reverse transcriptase, and *gag* and *pol* products (see Fig. 20-8). The effect of these antibodies on the clinical course of HIV infection is uncertain. The early antibodies are not neutralizing and are generally poor inhibitors of viral infectivity or cytopathic effects. Neutralizing antibodies against gp120 develop 2 to 3 months after primary infection, but even these antibodies cannot cope with a virus that is able to rapidly change the most immunodominant epitopes of its envelope glycoproteins.

Mechanisms of Immune Evasion by HIV

HIV is the prototype of an infectious pathogen that evades host defenses by destroying the immune system. In addition, several features of HIV may help the virus to evade host immunity.

HIV has an extremely high mutation rate because of error-prone reverse transcription, and in this way it may evade detection by antibodies or T cells generated in response to viral proteins. It has been estimated that in an infected person, every possible point mutation in the viral genome occurs every day. A region of the gp120 molecule, called the V3 loop, is one of the most antigenically variable components of the virus; it varies in HIV isolates taken from the same individual at different times. Furthermore, the regions of the V3 loop that are critical for viral entry and therefore are less frequently mutated are not readily exposed to the humoral immune system.

HIV-infected cells may evade CTLs through down-regulation of class I MHC molecule expression. The HIV Nef protein inhibits expression of class I MHC molecules, mainly by promoting internalization of these molecules. Other mechanisms of inhibiting cell-mediated immunity have been demonstrated in some cases. These include a preferential inhibition of T_H1 cytokines, activation of regulatory T cells, and suppression of dendritic cell functions. The mechanisms of these actions of the virus as well as their pathogenic significance are not established.

Elite Controllers and Long-term Nonprogressors: A Possible Role for Host Genes

Although most individuals infected with HIV eventually develop AIDS, approximately 1% of individuals who are infected do not develop disease. Such individuals have high $CD4^+$ and $CD8^+$ T cell counts, do not require therapy, and have persistent viremia but no disease for at least 10 to 15 years. On the basis of the degree of viremia, this group can be divided into two subsets: long-term nonprogressors have detectable viremia of around 5000 copies of HIV-1 RNA per milliliter of blood; and a much smaller subset of "elite controllers" present with viral

loads of about 50 copies or less of HIV-1 RNA per milliliter of blood. There is considerable interest in understanding the genetic basis of HIV control by examining these cohorts of individuals in detail. So far, a strong role for the MHC locus in protecting individuals and preventing progression has been suggested by genetic association studies. Specific HLA class I and even some HLA class II loci have been linked to the absence of disease progression. We have previously mentioned the importance of the inheritance of the CCR5 homozygous 32-bp deletion in protection from infection, and other genetic factors contributing to resistance are likely to be revealed in the coming years.

Treatment and Prevention of AIDS and Vaccine Development

Active research efforts have been aimed at developing reagents that interfere with the viral life cycle. Treatment of HIV infection and AIDS now includes the administration of three classes of antiviral drugs, used in combination, that target viral molecules for which no human homologues exist. The first antiretroviral drugs to be widely used were nucleoside analogues that inhibit reverse transcriptase activity. These drugs include deoxythymidine nucleoside analogues such as 3′-azido-3′-deoxythymidine (AZT), deoxycytidine nucleoside analogues, and deoxyadenosine analogues. When these drugs are used alone, they are often effective in significantly reducing plasma HIV RNA levels for several months to years, but they usually do not halt progression of HIV-induced disease, largely because of the evolution of virus with mutated forms of reverse transcriptase that are resistant to the drugs. Non-nucleoside reverse transcriptase inhibitors directly bind to the enzyme and inhibit its function. Viral protease inhibitors have been developed that block the processing of precursor proteins into mature viral capsid and core proteins. When these protease inhibitors are used alone, mutant viruses resistant to their effects rapidly emerge. However, protease inhibitors are now being used in combination with two different reverse transcriptase inhibitors. This new triple-drug therapy, commonly referred to as HAART (highly active antiretroviral therapy) or ART (antiretroviral therapy), has proved to be effective in reducing plasma viral RNA to undetectable levels in most treated patients for up to 3 years. An integrase inhibitor is also now used as part of antiviral therapy. “Entry inhibitors,” which prevent viral entry by targeting either CD4 or CCR5 on the host cell or gp41 or gp120 on the virus, are another novel category of therapeutics. Maraviroc is an entry inhibitor that blocks CCR5 and thus prevents viral entry. Drugs that target gp41 include compounds such as enfuvirtide that prevent fusion of the viral envelope with the host cell plasma membrane. Although antiretroviral therapy has reduced viral titers to below detection for up to 10 years in some patients, it is unlikely that such treatment can eliminate the virus from all reservoirs (especially long-lived infected cells), and resistance to the drugs may ultimately develop. Other formidable problems associated with these new drug therapies, which will impair their effective use in many parts of the world, include high expense, complicated administration schedules, and significant adverse effects.

The individual infections experienced by patients with AIDS are treated with the appropriate prophylaxis, antibiotics, and supportive measures. More aggressive antibiotic therapy is often required than for similar infections in less compromised hosts.

Efforts at prevention of HIV infection are extremely important and potentially effective in controlling the HIV epidemic. In the United States, the routine screening of blood products for evidence of donor HIV infection has already reduced the risk of this mode of transmission to negligible levels. Various public health measures to increase condom use and to reduce the use of contaminated needles by intravenous drug users are now widespread. Perhaps the most effective efforts at prevention are campaigns to increase public awareness of HIV.

The development of an effective vaccine against HIV is a priority for biomedical research institutions worldwide. The task has been complicated by the ability of the virus to mutate and vary many of its immunogenic antigens. It is likely that an effective vaccine will have to stimulate both humoral and cell-mediated responses to viral antigens that are critical for the viral life cycle. To achieve this goal, several approaches are being tried for HIV vaccine development. Much of the preliminary work has involved simian immunodeficiency virus (SIV) infection of macaques, and effective vaccines against SIV have already been developed. This success is encouraging because SIV is molecularly closely related to HIV and causes a disease in macaques that is similar to AIDS in humans. Various live virus vaccines have been tested in the hope that they will induce strong CTL responses. Such vaccines include nonvirulent recombinant hybrid viruses composed of part SIV and part HIV sequences or viruses that have been attenuated by deletions in one or more parts of the viral genome, such as the *nef* gene. One concern with live virus vaccines is their potential to cause disease if they are not completely attenuated and possibly to recombine with wild-type HIV to produce a pathogenic variant. Another approach that avoids this safety concern but retains efficacy in inducing CTL-mediated immunity is the use of live recombinant non-HIV viral vectors carrying HIV genes. Preliminary trials in human volunteers have already shown that canarypox vaccines expressing several HIV-1 genes can induce strong CTL responses to the HIV antigens. Many DNA vaccines have also been studied; these vaccines are composed of combinations of structural and regulatory genes of SIV or HIV packaged in mammalian DNA expression vectors. Combinations of vaccines, such as initial immunization with a DNA vaccine followed by boosting with a canarypox vector expressing HIV genes, have yielded some of the most promising results to date. Recombinant protein or peptide subunit vaccines that elicit antibodies have so far been of limited value because the antibodies induced by these vaccines typically do not neutralize clinical isolates of HIV. Attempts are currently under way to generate vaccines that can induce neutralizing antibodies to HIV.

SUMMARY

- Immunodeficiency diseases are caused by congenital or acquired defects in lymphocytes, phagocytes, and other mediators of adaptive and innate immunity. These diseases are associated with an increased susceptibility to infection, the nature and severity of which depend largely on which component of the immune system is abnormal and the extent of the abnormality.
- Disorders of innate immunity include defects in microbial killing by phagocytes (e.g., CGD or Chédiak-Higashi syndrome), leukocyte migration and adhesion (e.g., leukocyte adhesion deficiency), TLR signaling, and complement (Chapter 12).
- Severe combined immunodeficiencies include defects in lymphocyte development that affect both T and B cells and are caused by defective cytokine signaling, abnormal purine metabolism, defective V(D)J recombination, and mutations that affect T cell maturation.
- Antibody immunodeficiencies include diseases caused by defective B cell maturation or activation and defects in T cell–B cell collaboration (X-linked hyper-IgM syndrome).
- T cell immunodeficiencies include diseases in which the expression of MHC molecules is defective, T cell signaling disorders, and rare diseases involving CTL and NK cell functions.
- Treatment of congenital immunodeficiencies involves transfusions of antibodies, bone marrow or stem cell transplantation, or enzyme replacement. Gene therapy may offer improved treatments in the future.
- Acquired immunodeficiencies are caused by infections, malnutrition, disseminated cancer, and immunosuppressive therapy for transplant rejection or autoimmune diseases.
- AIDS is a severe immunodeficiency caused by infection with HIV. This RNA virus infects $CD4^+$ T lymphocytes, macrophages, and dendritic cells and causes progressive dysfunction of the immune system. Most of the immunodeficiency in AIDS can be attributed to the depletion of $CD4^+$ T cells.
- HIV enters cells by binding to both the CD4 molecule and a coreceptor of the chemokine receptor family. Once it is inside the cell, the viral genome is reverse-transcribed into DNA and incorporated into the cellular genome. Viral gene transcription and viral reproduction are stimulated by signals that normally activate the host cell. Production of virus is accompanied by death of infected cells.
- The acute phase of infection is characterized by death of memory $CD4^+$ T cells in mucosal tissues and dissemination of the virus to lymph nodes. In the subsequent latent phase, there is low-level virus replication in lymphoid tissues and slow, progressive loss of T cells. Persistent activation of T cells promotes their death, leading to rapid loss and immune deficiency in the chronic phase of the infection.
- $CD4^+$ T cell depletion in HIV-infected individuals is due to direct cytopathic effects of the virus, toxic effects of viral products such as shed gp120, and indirect effects such as activation-induced cell death or CTL killing of infected $CD4^+$ cells.
- Several reservoirs of HIV exist in infected individuals, including short-lived activated $CD4^+$ T cells, longer-lived macrophages, and very long-lived, latently infected memory T cells.
- HIV-induced depletion of $CD4^+$ T cells results in increased susceptibility to infection by a number of opportunistic microorganisms. In addition, HIV-infected patients have an increased incidence of tumors, particularly Kaposi's sarcoma and EBV-associated B cell lymphomas, and encephalopathy. The incidence of these complications has been greatly reduced by antiretroviral therapy.
- HIV has a high mutation rate, which allows the virus to evade host immune responses and become resistant to drug therapies. Genetic variability also poses a problem for the design of an effective vaccine against HIV. HIV infection can be treated by a combination of inhibitors of viral enzymes.

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Appendix

I

GLOSSARY

αβ T cell receptor (αβ TCR) The most common form of TCR, expressed on both $CD4^+$ and $CD8^+$ T cells. The αβ TCR recognizes peptide antigen bound to an MHC molecule. Both α and β chains contain highly variable (V) regions that together form the antigen-binding site as well as constant (C) regions. TCR V and C regions are structurally homologous to the V and C regions of Ig molecules.

ABO blood group antigens Carbohydrate antigens attached mainly to cell surface proteins (and a small fraction to lipids) that are present on many cell types, including red blood cells. These antigens differ between different individuals, depending on inherited alleles encoding the enzymes required for synthesis of the carbohydrate antigens. The ABO antigens act as alloantigens responsible for blood transfusion reactions and hyperacute rejection of allografts.

Acquired immunodeficiency A deficiency in the immune system that is acquired after birth, usually because of infection (e.g., AIDS), and that is not related to a genetic defect. Synonymous with **Secondary immunodeficiency.**

Acquired immunodeficiency syndrome (AIDS) A disease caused by human immunodeficiency virus (HIV) infection that is characterized by depletion of $CD4^+$ T cells, leading to a profound defect in cell-mediated immunity. Clinically, AIDS includes opportunistic infections, malignant tumors, wasting, and encephalopathy.

Activation-induced cell death (AICD) Apoptosis of activated lymphocytes, generally used for T cells; considered Fas-mediated apoptosis by some, but this is not the only mechanism of AICD.

Activation-induced (cytidine) deaminase (AID) An enzyme expressed in B cells that catalyzes the conversion of cytidine into uridine in DNA molecules and that is required for somatic hypermutation and Ig class switch recombination.

Activation protein 1 (AP-1) A family of DNA-binding transcription factors composed of dimers of two proteins that bind to one another through a shared structural motif called a leucine zipper. The best-characterized AP-1 factor is composed of the proteins Fos and Jun. AP-1 is involved in transcriptional regulation of many different genes important in the immune system, such as cytokine genes.

Active immunity The form of adaptive immunity that is induced by exposure to a foreign antigen and activation of lymphocytes and in which the immunized individual plays an active role in responding to the antigen. This type contrasts with passive immunity, in which an individual receives antibodies or lymphocytes from another individual who was previously actively immunized.

Acute-phase reactants Proteins, mostly synthesized in the liver, whose plasma concentrations increase shortly after infection as part of the systemic inflammatory response syndrome. Examples include C-reactive protein, fibrinogen, and serum amyloid A protein. Hepatic synthesis of these molecules is upregulated by inflammatory cytokines, especially IL-6 and TNF. The acute-phase reactants play various roles in the innate immune response to microbes.

Acute-phase response The increase in plasma concentrations of several proteins, called acute-phase reactants, that occurs as part of the early innate immune response to infections.

Acute rejection A form of graft rejection involving vascular and parenchymal injury mediated by T cells, macrophages, and antibodies that usually occurs days or weeks after transplantation, but may occur later if immunosuppression becomes inadequate.

Adaptive immunity The form of immunity that is mediated by lymphocytes and stimulated by exposure to infectious agents. In contrast to innate immunity, adaptive immunity is characterized by exquisite specificity for distinct macromolecules and memory, which is the ability to respond more vigorously to repeated exposure to the same microbe. Adaptive immunity is also called specific, or acquired, immunity.

Adaptor protein Proteins involved in intracellular signal transduction pathways by serving as bridge molecules or scaffolds for the recruitment of other signaling molecules. During lymphocyte antigen receptor or cytokine receptor signaling, adaptor molecules may be phosphorylated on tyrosine residues to enable them to bind other proteins containing Src homology 2 (SH2) domains. Adaptor molecules

involved in T cell activation include LAT, SLP-76, and Grb-2.

Addressin Molecules expressed on endothelial cells in different anatomic sites that bind to counterreceptors on lymphocytes called homing receptors and that direct organ-specific lymphocyte homing. Mucosal addressin cell adhesion molecule 1 (MadCAM-1) is an example of an addressin expressed in Peyer's patches in the intestinal wall that binds to the integrin $\alpha_4\beta_7$ on gut-homing T cells.

Adhesion molecule A cell surface molecule whose function is to promote adhesive interactions with other cells or the extracellular matrix. Leukocytes express various types of adhesion molecules, such as selectins, integrins, and members of the Ig superfamily, and these molecules play crucial roles in cell migration and cellular activation in innate and adaptive immune responses.

Adjuvant A substance, distinct from antigen, that enhances T and B cell activation mainly by promoting the accumulation and activation of antigen-presenting cells (APCs) at the site of antigen exposure. Adjuvants stimulate expression of T cell–activating costimulators and cytokines by APCs and may also prolong the expression of peptide-MHC complexes on the surface of APCs.

Adoptive transfer The process of transferring lymphocytes from one, usually immunized, individual into another individual. Adoptive transfer is used in research to define the role of a particular cell population (e.g., T cells) in an immune response. Clinically, adoptive transfer of tumor-reactive T lymphocytes is used in experimental cancer therapy.

Affinity The strength of the binding between a single binding site of a molecule (e.g., an antibody) and a ligand (e.g., an antigen). The affinity of a molecule X for a ligand Y is represented by the dissociation constant (K_d), which is the concentration of Y that is required to occupy the combining sites of half the X molecules present in a solution. A smaller K_d indicates a stronger or higher affinity interaction, and a lower concentration of ligand is needed to occupy the sites.

Affinity maturation The process that leads to increased affinity of antibodies for a particular antigen as a humoral response progresses. Affinity maturation takes place in germinal centers of lymphoid tissues and is the result of somatic mutation of Ig genes, followed by selective survival of the B cells producing the highest affinity antibodies.

Allele One of different forms of a gene present at a particular chromosomal locus. An individual who is heterozygous at a locus has two different alleles, each on a different member of a pair of chromosomes, one inherited from the mother and one from the father. If a particular gene in a population has many different alleles, the gene or locus is said to be polymorphic. The MHC locus is extremely polymorphic.

Allelic exclusion The exclusive expression of only one of two inherited alleles encoding Ig heavy and light chains and TCR β chains. Allelic exclusion occurs when the protein product of one productively recombined antigen receptor locus on one chromosome blocks rearrangement of the corresponding locus on the other chromosome. This property ensures that each lymphocyte will express a single antigen receptor and all the antigen receptors expressed by one clone of lymphocytes will have the identical specificity. Because the TCR α chain locus does not show allelic exclusion, some T cells do express two different types of TCR.

Allergen An antigen that elicits an immediate hypersensitivity (allergic) reaction. Allergens are proteins or chemicals bound to proteins that induce IgE antibody responses in atopic individuals.

Allergy A disorder caused by an immediate hypersensitivity reaction, often referring to the type of antigen that elicits the disease, such as food allergy, bee sting allergy, and penicillin allergy. All these conditions are the result of antigen-induced T_H2 generation and IgE production, and mast cell or basophil activation.

Alloantibody An antibody specific for an alloantigen (i.e., an antigen present in some individuals of a species but not in others).

Alloantigen A cell or tissue antigen that is present in some members of a species but not in others and that is recognized as foreign on an allograft. Alloantigens are usually products of polymorphic genes.

Alloantiserum The alloantibody-containing serum of an individual who has previously been exposed to one or more alloantigens.

Allogeneic graft An organ or tissue graft from a donor who is of the same species but genetically nonidentical to the recipient (also called an allograft).

Alloreactive Reactive to alloantigens; describes T cells or antibodies from one individual that will recognize antigens on cells or tissues of another genetically nonidentical individual.

Allotype The property of a group of antibody molecules defined by their sharing of a particular antigenic determinant found on the antibodies of some individuals but not others. Such determinants are called allotopes. Antibodies that share a particular allotope belong to the same allotype. Allotype is also often used synonymously with allotope.

Alternative macrophage activation Macrophage activation by IL-4 and IL-13 leading to an anti-inflammatory and tissue reparative phenotype, in contrast to classical macrophage activation by interferon-γ and TLR ligands.

Alternative pathway of complement activation An antibody-independent pathway of activation of the complement system that occurs when the C3b protein binds to microbial cell surfaces. The alternative pathway is a component of the innate immune system and mediates inflammatory responses to infection as well as direct lysis of microbes.

Anaphylactic shock Cardiovascular collapse occurring in the setting of a systemic immediate hypersensitivity reaction.

Anaphylatoxins The C5a, C4a, and C3a complement fragments that are generated during complement activation. The anaphylatoxins bind specific cell surface receptors and promote acute inflammation by stimulating neutrophil chemotaxis and activating mast cells.

Anaphylaxis A severe systemic form of immediate hypersensitivity in which mast cell or basophil mediators cause bronchial constriction, tissue edema, and cardiovascular collapse.

Anchor residues The amino acid residues of a peptide whose side chains fit into pockets in the peptide-binding cleft of an MHC molecule. The side chains bind to complementary amino acids in the MHC molecule and therefore serve to anchor the peptide in the cleft of the MHC molecule.

Anergy A state of unresponsiveness to antigenic stimulation. Lymphocyte anergy (also called clonal anergy) is the failure of clones of T or B cells to react to antigen and is a mechanism of maintaining immunologic tolerance to self. Clinically, anergy describes the lack of T cell–dependent cutaneous delayed-type hypersensitivity reactions to common antigens.

Angiogenesis New blood vessel formation regulated by a variety of protein factors elaborated by cells of the innate and adaptive immune systems and often accompanying chronic inflammation.

Antibody A type of glycoprotein molecule, also called immunoglobulin (Ig), produced by B lymphocytes that binds antigens, often with a high degree of specificity and affinity. The basic structural unit of an antibody is composed of two identical heavy chains and two identical light chains. N-terminal variable regions of the heavy and light chains form the antigen-binding sites, whereas the C-terminal constant regions of the heavy chains functionally interact with other molecules in the immune system. Every individual has millions of different antibodies, each with a unique antigen-binding site. Secreted antibodies perform various effector functions, including neutralizing antigens, activating complement, and promoting leukocyte-dependent destruction of microbes.

Antibody-dependent cell-mediated cytotoxicity (ADCC) A process by which NK cells are targeted to IgG-coated cells, resulting in lysis of the antibody-coated cells. A specific receptor for the constant region of IgG, called FcγRIII (CD16), is expressed on the NK cell membrane and mediates binding to the IgG.

Antibody feedback The downregulation of antibody production by secreted IgG antibodies that occurs when antigen-antibody complexes simultaneously engage B cell membrane Ig and Fcγ receptors (FcγRII). Under these conditions, the cytoplasmic tails of the Fcγ receptors transduce inhibitory signals inside the B cell.

Antibody repertoire The collection of different antibody specificities expressed in an individual.

Antibody-secreting cell A B lymphocyte that has undergone differentiation and produces the secretory form of Ig. Antibody-secreting cells are produced in response to antigen and reside in the spleen and lymph nodes as well as in the bone marrow. Often used synonymously with plasma cells.

Antigen A molecule that binds to an antibody or a TCR. Antigens that bind to antibodies include all classes of molecules. TCRs bind only peptide fragments of proteins complexed with MHC molecules; both the peptide ligand and the native protein from which it is derived are called T cell antigens.

Antigen presentation The display of peptides bound by MHC molecules on the surface of an APC that permits specific recognition by TCRs and activation of T cells.

Antigen-presenting cell (APC) A cell that displays peptide fragments of protein antigens, in association with MHC molecules, on its surface and activates antigen-specific T cells. In addition to displaying peptide-MHC complexes, APCs also express costimulatory molecules to activate T lymphocytes optimally.

Antigen processing The intracellular conversion of protein antigens derived from the extracellular space or the cytosol into peptides and loading of these peptides onto MHC molecules for display to T lymphocytes.

Antiserum Serum from an individual previously immunized with an antigen that contains antibody specific for that antigen.

Apoptosis A process of cell death characterized by DNA cleavage, nuclear condensation and fragmentation, and plasma membrane blebbing that leads to phagocytosis of cell fragments without inducing an inflammatory response. This type of cell death is important in lymphocyte development, regulation of lymphocyte responses to foreign antigens, and maintenance of tolerance to self antigens.

Antiretroviral therapy (ART) Combination chemotherapy for HIV infection consisting of reverse transcriptase inhibitors and a viral protease inhibitor. ART can reduce plasma virus titers to below detectable levels for more than 1 year and slow the progression of HIV disease. Also called **highly active antiretroviral therapy (HAART).**

Arthus reaction A localized form of experimental immune complex–mediated vasculitis induced by injection of an antigen subcutaneously into a previously immunized animal or into an animal that has been given intravenous antibody specific for the antigen. Circulating antibodies bind to the injected antigen and form immune complexes that are deposited in the walls of small arteries at the injection site and give rise to a local cutaneous vasculitis with necrosis.

Atopy The propensity of an individual to produce IgE antibodies in response to various environmental antigens and to develop strong immediate hypersensitivity (allergic) responses. People who have allergies to environmental antigens, such as pollen or house dust, are said to be atopic.

Autoantibody An antibody produced in an individual that is specific for a self antigen. Autoantibodies can cause damage to cells and tissues and are produced in excess in systemic autoimmune diseases, such as systemic lupus erythematosus.

Autocrine factor A molecule that acts on the same cell that produces the factor. For example, IL-2 is an autocrine T cell growth factor that stimulates mitotic activity of the T cell that produces it.

Autoimmune disease A disease caused by a breakdown of self-tolerance such that the adaptive immune system responds to self antigens and mediates cell and tissue damage. Autoimmune diseases can be organ specific (e.g., thyroiditis or diabetes) or systemic (e.g., systemic lupus erythematosus).

Autoimmune regulator (AIRE) A protein that functions to stimulate expression of peripheral tissue protein antigens in thymic epithelial cells. Mutations in the *AIRE* gene in humans and mice lead to tissue-specific autoimmune disease, because of defective expression of tissue antigens in the thymus and failure to delete T cells specific for these antigens.

Autoimmunity The state of adaptive immune system responsiveness to self antigens that occurs when mechanisms of self-tolerance fail.

Autologous graft A tissue or organ graft in which the donor and recipient are the same individual. Autologous bone marrow and skin grafts are performed in clinical medicine.

Autophagy The process by which a cell degrades its own components by lysosomal catabolism. Polymorphisms of genes that regulate autophagy are linked to risk for some autoimmune diseases.

Avidity The overall strength of interaction between two molecules, such as an antibody and antigen. Avidity depends on both the affinity and the valency of interactions. Therefore, the avidity of a pentameric IgM antibody, with 10 antigen-binding sites, for a multivalent antigen may be much greater than the avidity of a dimeric IgG molecule for the same antigen. Avidity can be used to describe the strength of cell-cell interactions, which are mediated by many binding interactions between cell surface molecules.

B-1 B lymphocytes A subset of B lymphocytes that develop earlier during ontogeny than do conventional B cells, express a limited repertoire of V genes with little junctional diversity, and secrete IgM antibodies that bind T-independent antigens. Many B-1 cells express the CD5 (Ly-1) molecule.

B lymphocyte The only cell type capable of producing antibody molecules and therefore the mediator of humoral immune responses. B lymphocytes, or B cells, develop in the bone marrow, and mature B cells are found mainly in lymphoid follicles in secondary lymphoid tissues, in bone marrow, and in low numbers in the circulation.

Bare lymphocyte syndrome An immunodeficiency disease characterized by a lack of class II MHC molecule expression that leads to defects in antigen presentation and cell-mediated immunity. The disease is caused by mutations in genes encoding factors that regulate class II MHC gene transcription.

Basophil A type of bone marrow–derived circulating granulocyte with structural and functional similarities to mast cells that has granules containing many of the same inflammatory mediators as mast cells and expresses a high-affinity Fc receptor for IgE. Basophils that are recruited into tissue sites where antigen is present may contribute to immediate hypersensitivity reactions.

Bcl-6 A transcriptional repressor that is required for germinal center B cell development and for T_{FH} development.

Bcl-2 family proteins A family of partially homologous cytoplasmic and mitochondrial membrane proteins that regulate apoptosis by influencing mitochondrial outer membrane permeability. Members of this family can be proapoptotic (Bax, Bad, and Bak) or antiapoptotic (Bcl-2 and Bcl-X_L).

BCR (B cell receptor) The cell surface antigen receptor on B lymphocytes, which is a membrane bound immunoglobulin molecule.

BCR (B cell receptor) complex A multiprotein complex expressed on the surface of B lymphocytes that recognizes antigen and transduces activating signals into the cell. The BCR complex includes membrane Ig, which is responsible for binding antigen, and Igα and Igβ proteins, which initiate signaling events.

Biogenic amines Low-molecular-weight, nonlipid compounds, such as histamine, that share the structural feature of an amine group, are stored in and released from the cytoplasmic granules of mast cells, and mediate many of the biologic effects of immediate hypersensitivity (allergic) reactions. (Biogenic amines are sometimes called vasoactive amines.)

Biologic response modifiers Molecules, such as cytokines, used clinically as modulators of inflammation, immunity, and hematopoiesis.

BLIMP-1 A transcriptional repressor that is required for plasma cell generation.

Bone marrow The central cavity of bone that is the site of generation of all circulating blood cells in adults, including immature lymphocytes, and the site of B cell maturation.

Bone marrow transplantation The transplantation of bone marrow, including stem cells that give rise to all mature blood cells and lymphocytes; it is performed clinically to treat hematopoietic or lymphopoietic disorders and malignant diseases and is also used in various immunologic experiments in animals. Used synonymously with **hematopoietic stem cell transplantation.**

Bronchial asthma An inflammatory disease usually caused by repeated immediate hypersensitivity reactions in the lung that leads to intermittent and reversible airway obstruction, chronic bronchial inflammation with eosinophils, and bronchial smooth muscle cell hypertrophy and hyperreactivity.

Bruton's tyrosine kinase (Btk) A tyrosine kinase of the Tec family that plays an essential role in B cell maturation. Mutations in the gene encoding Btk cause X-linked agammaglobulinemia, a disease characterized by failure of B cells to mature beyond the pre-B cell stage.

Burkitt's lymphoma A malignant B cell tumor that is defined by histologic features but almost always carries a reciprocal chromosomal translocation involving Ig gene loci and the cellular *MYC* gene on chromosome 8. Many cases of Burkitt's lymphoma in Africa are associated with Epstein-Barr virus infection.

C (constant region) gene segments The DNA sequences in the Ig and TCR gene loci that encode the nonvariable portions of Ig heavy and light chains and TCR α, β, γ, and δ chains.

C1 A serum complement system protein composed of several polypeptide chains that initiates the classical pathway of complement activation by attaching to the Fc portions of IgG or IgM antibody that has bound antigen.

C1 inhibitor (C1 INH) A plasma protein inhibitor of the classical pathway of complement activation. C1 INH is a serine protease inhibitor (serpin) that mimics the normal substrates of the C1r and C1s components of C1. A genetic deficiency in C1 INH causes the disease hereditary angioneurotic edema.

C3 The central and most abundant complement system protein; it is involved in both the classical and alternative pathway cascades. C3 is proteolytically cleaved during complement activation to generate a C3b fragment, which covalently attaches to cell or microbial surfaces, and a C3a fragment, which has various proinflammatory activities.

C3 convertase A multiprotein enzyme complex generated by the early steps of either the classical or alternative pathway of complement activation. C3 convertase cleaves C3, which gives rise to two proteolytic products called C3a and C3b.

C5 convertase A multiprotein enzyme complex generated by C3b binding to C3 convertase. C5 convertase cleaves C5 and initiates the late steps of complement activation leading to formation of the membrane attack complex and lysis of cells.

Calcineurin A cytoplasmic serine/threonine phosphatase that dephosphorylates and thereby activates the transcription factor NFAT. Calcineurin is activated by calcium signals generated through TCR signaling in response to antigen recognition, and the immunosuppressive drugs cyclosporine and FK506 work by blocking calcineurin activity.

Carcinoembryonic antigen (CEA, CD66) A highly glycosylated membrane protein; increased expression of CEA in many carcinomas of the colon, pancreas, stomach, and breast results in a rise in serum levels. The level of serum CEA is used to monitor the persistence or recurrence of metastatic carcinoma after treatment. Because CEA expression is normally high in many tissues during fetal life but is suppressed in adults except in tumor cells, it is called an oncofetal tumor antigen.

Caspases Intracellular proteases with cysteines in their active sites that cleave substrates at the C-terminal sides of aspartic acid residues. Most are components of enzymatic cascades that cause apoptotic death of cells, but some, like caspase 1, drive inflammation.

Cathelicidins Polypeptides produced by neutrophils and various barrier epithelia that serve various functions in innate immunity, including direct toxicity to microorganisms, activation of leukocytes, and neutralization of lipopolysaccharide.

Cathepsins Thiol and aspartyl proteases with broad substrate specificities. The most abundant proteases of endosomes in APCs, cathepsins play an important role in generating peptide fragments from exogenous protein antigens that bind to class II MHC molecules.

CD molecules Cell surface molecules expressed on various cell types in the immune system that are designated by the "cluster of differentiation" or CD number. See Appendix III for a list of CD molecules.

Cell-mediated immunity (CMI) The form of adaptive immunity that is mediated by T lymphocytes and serves as the defense mechanism against microbes that survive within phagocytes or infect nonphagocytic cells. CMI responses include $CD4^+$ T cell–mediated activation of macrophages that have phagocytosed microbes and $CD8^+$ CTL–mediated killing of infected cells.

Central tolerance A form of self-tolerance induced in generative (central) lymphoid organs as a consequence of immature self-reactive lymphocytes recognizing self antigens and subsequently leading to their death or inactivation. Central tolerance prevents the emergence of lymphocytes with high-affinity receptors for the self antigens that are expressed in the bone marrow or thymus.

Chédiak-Higashi syndrome A rare autosomal recessive immunodeficiency disease caused by a defect in the cytoplasmic granules of various cell types that affects the lysosomes of neutrophils and macrophages as well as the granules of CTLs and NK cells. Patients show reduced resistance to infection with pyogenic bacteria.

Chemokine receptors Cell surface receptors for chemokines that transduce signals stimulating the migration of leukocytes. These receptors are members of the seven-transmembrane α-helical, G protein–coupled receptor family.

Chemokines A large family of structurally homologous, low-molecular-weight cytokines that stimulate leukocyte movement and regulate the migration of leukocytes from the blood to tissues.

Chemotaxis Movement of a cell directed by a chemical concentration gradient. The movement of lymphocytes, polymorphonuclear leukocytes, monocytes, and other leukocytes into various tissues is often directed by gradients of low-molecular-weight cytokines called chemokines.

Chromosomal translocation A chromosomal abnormality in which a segment of one chromosome is transferred to another. Many malignant diseases of lymphocytes are associated with chromosomal translocations involving an Ig or TCR locus and a chromosomal segment containing a cellular oncogene.

Chronic granulomatous disease A rare inherited immunodeficiency disease caused by a defect in the gene encoding a component of the phagocyte oxidase enzyme that is needed for microbial killing by polymorphonuclear leukocytes and macrophages. The disease is characterized by recurrent intracellular bacterial and fungal infections, often accompanied by chronic cell-mediated immune responses and the formation of granulomas.

Chronic rejection A form of allograft rejection characterized by fibrosis with loss of normal organ structures occurring during a prolonged period. In many cases, the major pathologic event in chronic rejection is graft arterial occlusion, which is caused by proliferation of intimal smooth muscle cells and is called graft arteriosclerosis.

c-Kit ligand (stem cell factor) A protein required for hematopoiesis, early steps in T cell development in the thymus, and mast cell development. c-Kit ligand is produced in membrane-bound and soluble forms by stromal cells in the bone marrow and thymus and

binds to the c-Kit tyrosine kinase membrane receptor on pluripotent stem cells.

Class I major histocompatibility complex (MHC) molecule One of two forms of polymorphic, heterodimeric membrane proteins that bind and display peptide fragments of protein antigens on the surface of APCs for recognition by T lymphocytes. Class I MHC molecules usually display peptides derived from the cytoplasm of the cell.

Class II–associated invariant chain peptide (CLIP) A peptide remnant of the invariant chain that sits in the class II MHC peptide-binding cleft and is removed by action of the HLA-DM molecule before the cleft becomes accessible to peptides produced from extracellular protein antigens.

Class II major histocompatibility complex (MHC) molecule One of two major classes of polymorphic, heterodimeric membrane proteins that bind and display peptide fragments of protein antigens on the surface of APCs for recognition by T lymphocytes. Class II MHC molecules usually display peptides derived from extracellular proteins that are internalized into phagocytic or endocytic vesicles.

Class II vesicle (CIIV) A membrane-bound organelle identified in murine B cells that is important in the class II MHC pathway of antigen presentation. The CIIV is similar to the MHC class II compartment (MIIC) identified in other cells and contains all the components required for the formation of complexes of peptide antigens and class II MHC molecules, including the enzymes that degrade protein antigens, class II molecules, invariant chain, and HLA-DM.

Classical macrophage activation Macrophage activation by interferon-γ, T_H1 cells, and TLR ligands, leading to a proinflammatory and microbicidal phenotype. "Classically activated" macrophages are also called M1 macrophages.

Classical pathway of complement activation The pathway of activation of the complement system that is initiated by binding of antigen-antibody complexes to the C1 molecule and induces a proteolytic cascade involving multiple other complement proteins. The classical pathway is an effector arm of the humoral immune system that generates inflammatory mediators, opsonins for phagocytosis of antigens, and lytic complexes that destroy cells.

Clonal anergy A state of antigen unresponsiveness of a clone of T lymphocytes experimentally induced by recognition of antigen in the absence of additional signals (costimulatory signals) required for functional activation. Clonal anergy is considered a model for one mechanism of tolerance to self antigens and may be applicable to B lymphocytes as well.

Clonal deletion A mechanism of lymphocyte tolerance in which an immature T cell in the thymus or an immature B cell in the bone marrow undergoes apoptotic death as a consequence of recognizing an abundant antigen in the generative organ.

Clonal expansion The increase in number of lymphocytes specific for an antigen that results from antigen stimulation and proliferation of naive T cells. Clonal expansion occurs in lymphoid tissues and is required to generate enough antigen-specific effector lymphocytes from rare naive precursors to eradicate infections.

Clonal ignorance A form of lymphocyte unresponsiveness in which self antigens are ignored by the immune system even though lymphocytes specific for those antigens remain viable and functional.

Clonal selection hypothesis A fundamental tenet of the immune system (no longer a hypothesis) stating that every individual possesses numerous clonally derived lymphocytes, each clone having arisen from a single precursor, expresses one antigen receptor, and is capable of recognizing and responding to a distinct antigenic determinant. When an antigen enters, it selects a specific preexisting clone and activates it.

Collectins A family of proteins, including mannose-binding lectin, which are characterized by a collagen-like domain and a lectin (i.e., carbohydrate-binding) domain. Collectins play a role in the innate immune system by acting as microbial pattern recognition receptors, and they may activate the complement system by binding to C1q.

Colony-stimulating factors (CSFs) Cytokines that promote the expansion and differentiation of bone marrow progenitor cells. CSFs are essential for the maturation of red blood cells, granulocytes, monocytes, and lymphocytes. Examples of CSFs include granulocyte-monocyte colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), and IL-3.

Combinatorial diversity Combinatorial diversity describes the many different combinations of variable, diversity, and joining segments that are possible as a result of somatic recombination of DNA in the Ig and TCR loci during B cell or T cell development. Combinatorial diversity is one mechanism for the generation of large numbers of different antigen receptor genes from a limited number of DNA gene segments.

Complement A system of serum and cell surface proteins that interact with one another and with other molecules of the immune system to generate important effectors of innate and adaptive immune responses. The classical, alternative, and lectin pathways of the complement system are activated by antigen-antibody complexes, microbial surfaces, and plasma lectins binding to microbes, respectively, and consist of a cascade of proteolytic enzymes that generate inflammatory mediators and opsonins. All three pathways lead to the formation of a common terminal cell lytic complex that is inserted in cell membranes.

Complement receptor type 1 (CR1) A high-affinity receptor for the C3b and C4b fragments of complement. Phagocytes use CR1 to mediate internalization of C3b- or C4b-coated particles. CR1 on erythrocytes serves in the clearance of immune complexes from the circulation. CR1 is also a regulator of complement activation.

Complement receptor type 2 (CR2) A receptor expressed on B cells and follicular dendritic cells that binds proteolytic fragments of the C3 complement protein, including C3d, C3dg, and iC3b. CR2 functions to stimulate humoral immune responses by enhancing

B cell activation by antigen and by promoting the trapping of antigen-antibody complexes in germinal centers. CR2 is also the receptor for Epstein-Barr virus.

Complementarity-determining region (CDR) Short segments of Ig and TCR proteins that contain most of the sequence differences among different antibodies or TCRs and that make contact with antigen; also called **hypervariable regions.** Three CDRs are present in the variable domain of each antigen receptor polypeptide chain and six CDRs in an intact Ig or TCR molecule. These hypervariable segments assume loop structures that together form a surface complementary to the three-dimensional structure of the bound antigen.

Congenic mouse strains Inbred mouse strains that are identical to one another at every genetic locus except the one for which they are selected to differ; such strains are created by repetitive back-crossbreeding and selection for a particular trait. Congenic strains that differ from one another only at a particular MHC allele have been useful in defining the function of MHC molecules.

Congenital immunodeficiency A genetic defect in which an inherited deficiency in some aspect of the innate or adaptive immune system leads to an increased susceptibility to infections. Congenital immunodeficiency is frequently manifested early in infancy and childhood but is sometimes clinically detected later in life. Synonymous with **Primary immunodeficiency.**

Constant (C) region The portion of Ig or TCR polypeptide chains that does not vary in sequence among different clones and is not involved in antigen binding.

Contact sensitivity The propensity for a T cell–mediated, delayed-type hypersensitivity reaction to develop in the skin on contact with a particular chemical agent. Chemicals that elicit contact hypersensitivity bind to and modify self proteins or molecules on the surfaces of APCs, which are then recognized by $CD4^+$ or $CD8^+$ T cells.

Coreceptor A lymphocyte surface receptor that binds to an antigen complex at the same time that membrane Ig or TCR binds the antigen and delivers signals required for optimal lymphocyte activation. CD4 and CD8 are T cell coreceptors that bind nonpolymorphic parts of an MHC molecule concurrently with the TCR binding to polymorphic residues and the bound peptide. CR2 is a coreceptor on B cells that binds to complement-opsonized antigens at the same time that membrane Ig binds another part of the antigen.

Costimulator A molecule on the surface of or secreted by an APC that provides a stimulus (or second signal) required for the activation of naive T cells, in addition to antigen. The best defined costimulators are the B7 molecules (CD80 and CD86) on APCs that bind to the CD28 molecule on T cells.

CpG nucleotides Unmethylated cytidine-guanine sequences found in microbial DNA that stimulate innate immune responses. CpG nucleotides are recognized by TLR-9, have adjuvant properties in the mammalian immune system, and may be important to the efficacy of DNA vaccines.

C-reactive protein (CRP) A member of the pentraxin family of plasma proteins involved in innate immune responses to bacterial infections. CRP is an acute-phase reactant, and it binds to the capsule of pneumococcal bacteria. CRP also binds to C1q and may thereby activate complement or act as an opsonin by interacting with phagocyte C1q receptors.

Crossmatching A screening test performed to minimize the chance of graft rejection in which a patient in need of an allograft is tested for the presence of preformed antibodies against donor cell surface antigens (usually MHC antigens). The test involves mixing the recipient serum with leukocytes from potential donors, adding complement, and observing whether cell lysis occurs.

Cross-presentation A mechanism by which a dendritic cell activates (or primes) a naive $CD8^+$ CTL specific for the antigens of a third cell (e.g., a virus-infected or tumor cell). Cross-presentation occurs, for example, when an infected (often apoptotic) cell is ingested by a dendritic cell and the microbial antigens are processed and presented in association with class I MHC molecules, unlike the general rule for phagocytosed antigens, which are presented in association with class II MHC molecules. The dendritic cell also provides costimulation for the T cells. Also called **cross-priming.**

C-type lectin A member of a large family of calcium-dependent carbohydrate-binding proteins, many of which play important roles in innate and adaptive immunity. For example, soluble C-type lectins bind to microbial carbohydrate structures and mediate phagocytosis or complement activation (e.g., mannose-binding lectin, dectins, collectins, ficolins).

Cutaneous immune system The components of the innate and adaptive immune system found in the skin that function together in a specialized way to detect and respond to environmental antigens. Components of the cutaneous immune system include keratinocytes, Langerhans cells, intraepithelial lymphocytes, and dermal lymphocytes.

Cyclosporine An immunosuppressive drug used to prevent allograft rejection that functions by blocking T cell cytokine gene transcription. Cyclosporine (also called cyclosporin A) binds to a cytosolic protein called cyclophilin, and cyclosporine-cyclophilin complexes bind to and inhibit calcineurin, thereby inhibiting activation and nuclear translocation of the transcription factor NFAT.

Cytokines Proteins produced by many different cell types that mediate inflammatory and immune reactions. Cytokines are principal mediators of communication between cells of the immune system. (See Appendix II.)

Cytotoxic (or **cytolytic) T lymphocyte (CTL)** A type of T lymphocyte whose major effector function is to recognize and kill host cells infected with viruses or other intracellular microbes. CTLs usually express CD8 and recognize microbial peptides displayed by class I MHC molecules. CTL killing of infected cells involves the release of cytoplasmic granules whose contents include enzymes that initiate apoptosis of the infected

cell and proteins that facilitate entry of these enzymes into the target cells.

Damage-associated molecular patterns (DAMPs) Endogenous molecules that are produced by or released from damaged and dying cells that bind to pattern recognition receptors and stimulate innate immune responses. Examples include high-mobility group box 1 (HMGB1) protein, extracellular ATP, and uric acid.

Dectins Pattern recognition receptors expressed on dendritic cells that recognize fungal cell wall carbohydrates and induce signaling events that promote inflammation and enhance adaptive immune responses.

Defensins Cysteine-rich peptides produced by epithelial barrier cells in the skin, gut, lung, and other tissues and in neutrophil granules that act as broad-spectrum antibiotics to kill a wide variety of bacteria and fungi. The synthesis of defensins is increased in response to stimulation of innate immune system receptors such as Toll-like receptors and inflammatory cytokines such as IL-1 and TNF.

Delayed-type hypersensitivity (DTH) An immune reaction in which T cell–dependent macrophage activation and inflammation cause tissue injury. A DTH reaction to the subcutaneous injection of antigen is often used as an assay for cell-mediated immunity (e.g., the purified protein derivative skin test for immunity to *Mycobacterium tuberculosis*).

Dendritic cells (DCs) Bone marrow–derived cells found in epithelial and lymphoid tissues that are morphologically characterized by thin membranous projections. Many subsets of DCs exist with diverse functions. Activated (mature) DCs function as APCs for naive T lymphocytes and are important for initiation of adaptive immune responses to protein antigen. Immature (resting) DCs are important for induction of tolerance to self antigens.

Desensitization A method of treating immediate hypersensitivity disease (allergies) that involves repetitive administration of low doses of an antigen to which individuals are allergic. This process often prevents severe allergic reactions on subsequent environmental exposure to the antigen, but the mechanisms are not well understood.

Determinant The specific portion of a macromolecular antigen to which an antibody binds. In the case of a protein antigen recognized by a T cell, the determinant is the peptide portion that binds to an MHC molecule for recognition by the TCR. Synonymous with **epitope.**

Diacylglycerol (DAG) A signaling molecule generated by phospholipase C (PLCγ1)–mediated hydrolysis of the plasma membrane phospholipid phosphatidylinositol 4,5-bisphosphate (PIP2) during antigen activation of lymphocytes. The main function of DAG is to activate an enzyme called protein kinase C that participates in the generation of active transcription factors.

DiGeorge syndrome A selective T cell deficiency caused by a congenital malformation that results in defective development of the thymus, parathyroid glands, and other structures that arise from the third and fourth pharyngeal pouches.

Direct antigen presentation (or **direct allorecognition)** Presentation of cell surface allogeneic MHC molecules by graft APCs to a graft recipient's T cells that leads to activation of the alloreactive T cells. In direct recognition of foreign MHC molecules, a TCR that was selected to recognize a self MHC molecule plus foreign peptide cross-reacts with an allogeneic MHC molecule plus peptide. Direct presentation is partly responsible for strong T cell responses to allografts.

Diversity The existence of a large number of lymphocytes with different antigenic specificities in any individual. Diversity is a fundamental property of the adaptive immune system and is the result of variability in the structures of the antigen-binding sites of lymphocyte receptors for antigens (antibodies and TCRs).

Diversity (D) segments Short coding sequences between the variable (V) and constant (C) gene segments in the Ig heavy chain and TCR β and γ loci that together with J segments are somatically recombined with V segments during lymphocyte development. The resulting recombined VDJ DNA codes for the carboxyl-terminal ends of the antigen receptor V regions, including the third hypervariable (CDR) regions. Random use of D segments contributes to the diversity of the antigen receptor repertoire.

DNA vaccine A vaccine composed of a bacterial plasmid containing a complementary DNA encoding a protein antigen. DNA vaccines presumably work because professional APCs are transfected in vivo by the plasmid and express immunogenic peptides that elicit specific responses. Furthermore, the plasmid DNA contains CpG nucleotides that act as potent adjuvants.

Double-negative thymocyte A subset of developing T cells in the thymus (thymocytes) that express neither CD4 nor CD8. Most double-negative thymocytes are at an early developmental stage and do not express antigen receptors. They will later express both CD4 and CD8 during the intermediate double-positive stage before further maturation to single-positive T cells expressing only CD4 or CD8.

Double-positive thymocyte A subset of developing T cells in the thymus (thymocytes) that express both CD4 and CD8 and are at an intermediate developmental stage. Double-positive thymocytes also express TCRs and are subject to selection processes, and they mature to single-positive T cells expressing only CD4 or CD8.

E2A A transcription factor that collaborates with EBF to commit lymphoid precursors to B cell development.

EBF A transcription factor that collaborates with E2A to commit lymphoid precursors to B cell development.

Ectoparasites Parasites that live on the surface of an animal, such as ticks and mites. Both the innate and adaptive immune systems may play a role in protection against ectoparasites, often by destroying the larval stages of these organisms.

Effector cells The cells that perform effector functions during an immune response, such as secreting cytokines (e.g., helper T cells), killing microbes (e.g.,

macrophages), killing microbe-infected host cells (e.g., CTLs), or secreting antibodies (e.g., differentiated B cells).

Effector phase The phase of an immune response in which a foreign antigen is destroyed or inactivated. For example, in a humoral immune response, the effector phase may be characterized by antibody-dependent complement activation and phagocytosis of antibody- and complement-opsonized bacteria.

Endosome An intracellular membrane-bound vesicle into which extracellular proteins are internalized during antigen processing. Endosomes have an acidic pH and contain proteolytic enzymes that degrade proteins into peptides that bind to class II MHC molecules. A subset of class II MHC–rich endosomes, called MIIC, play a special role in antigen processing and presentation by the class II pathway.

Endotoxin A component of the cell wall of gram-negative bacteria, also called **lipopolysaccharide** (LPS), that is released from dying bacteria and stimulates many innate immune responses, including the secretion of cytokines, induction of microbicidal activities of macrophages, and expression of leukocyte adhesion molecules on endothelium. Endotoxin contains both lipid components and carbohydrate (polysaccharide) moieties.

Enhancer A regulatory nucleotide sequence in a gene that is located either upstream or downstream of the promoter, binds transcription factors, and increases the activity of the promoter. In cells of the immune system, enhancers are responsible for integrating cell surface signals that lead to induced transcription of genes encoding many of the effector proteins of an immune response, such as cytokines.

Envelope glycoprotein (Env) A membrane glycoprotein encoded by a retrovirus that is expressed on the plasma membrane of infected cells and on the host cell–derived membrane coat of viral particles. Env proteins are often required for viral infectivity. The Env proteins of HIV include gp41 and gp120, which bind to CD4 and chemokine receptors, respectively, on human T cells and mediate fusion of the viral and T cell membranes.

Enzyme-linked immunosorbent assay (ELISA) A method of quantifying an antigen immobilized on a solid surface by use of a specific antibody with a covalently coupled enzyme. The amount of antibody that binds the antigen is proportional to the amount of antigen present and is determined by spectrophotometrically measuring the conversion of a clear substrate to a colored product by the coupled enzyme. (see Appendix IV).

Eosinophil A bone marrow–derived granulocyte that is abundant in the inflammatory infiltrates of immediate hypersensitivity late-phase reactions and that contributes to many of the pathologic processes in allergic diseases. Eosinophils are important in defense against extracellular parasites, including helminths.

Epitope The specific portion of a macromolecular antigen to which an antibody binds. In the case of a protein antigen recognized by a T cell, an epitope is the peptide portion that binds to an MHC molecule for recognition by the TCR. Synonymous with **determinant.**

Epstein-Barr virus (EBV) A double-stranded DNA virus of the herpesvirus family that is the etiologic agent of infectious mononucleosis and is associated with some B cell malignant tumors and nasopharyngeal carcinoma. EBV infects B lymphocytes and some epithelial cells by specifically binding to CR2 (CD21).

Experimental autoimmune encephalomyelitis (EAE) An animal model of multiple selerosis, an autoimmune demyelinating disease of the central nervous system. EAE is induced in rodents by immunization with components of the myelin sheath (e.g., myelin basic protein) of nerves, mixed with an adjuvant. The disease is mediated in large part by cytokine-secreting $CD4^+$ T cells specific for the myelin sheath proteins.

Fab (fragment, antigen-binding) A proteolytic fragment of an IgG antibody molecule that includes one complete light chain paired with one heavy chain fragment containing the variable domain and only the first constant domain. Fab fragment retains the ability to monovalently bind an antigen but cannot interact with IgG Fc receptors on cells or with complement. Therefore, Fab preparations are used in research and therapeutic applications when antigen binding is desired without activation of effector functions. (The Fab′ fragment retains the hinge region of the heavy chain.)

$F(ab')_2$ fragment A proteolytic fragment of an IgG molecule that includes two complete light chains but only the variable domain, first constant domain, and hinge region of the two heavy chains. $F(ab')_2$ fragments retain the entire bivalent antigen-binding region of an intact IgG molecule but cannot bind complement or IgG Fc receptors. They are used in research and therapeutic applications when antigen binding is desired without antibody effector functions.

Fas (CD95) A death receptor of the TNF receptor family that is expressed on the surface of T cells and many other cell types and initiates a signaling cascade leading to apoptotic death of the cell. The death pathway is initiated when Fas binds to Fas ligand expressed on activated T cells. Fas-mediated killing of lymphocytes is important for the maintenance of self-tolerance. Mutations in the *FAS* gene cause systemic autoimmune disease.

Fas ligand (CD95 ligand) A membrane protein that is a member of the TNF family of proteins expressed on activated T cells. Fas ligand binds to Fas, thereby stimulating a signaling pathway leading to apoptotic cell death of the Fas-expressing cell. Mutations in the Fas ligand gene cause systemic autoimmune disease in mice.

Fc (fragment, crystalline) A proteolytic fragment of IgG that contains only the disulfide-linked carboxyl-terminal regions of the two heavy chains. Fc is also used to describe the corresponding region of an intact Ig molecule that mediates effector functions by binding to cell surface receptors or the C1q complement protein. (Fc fragments are so named because they tend to crystallize out of solution.)

Fc receptor A cell surface receptor specific for the carboxyl-terminal constant region of an Ig molecule. Fc receptors are typically multichain protein complexes that include signaling components and Ig-binding components. Several types of Fc receptors exist, including those specific for different IgG isotypes, IgE, and IgA. Fc receptors mediate many of the cell-dependent effector functions of antibodies, including phagocytosis of antibody-bound antigens, antigen-induced activation of mast cells, and targeting and activation of NK cells.

FcεRI A high-affinity receptor for the carboxyl-terminal constant region of IgE molecules that is expressed on mast cells, basophils, and eosinophils. FcεRI molecules on mast cells are usually occupied by IgE, and antigen-induced cross-linking of these IgE-FcεRI complexes activates the mast cell and initiates immediate hypersensitivity reactions.

Fcγ receptor (FcγR) A specific cell surface receptor for the carboxyl-terminal constant region of IgG molecules. There are several different types of Fcγ receptors, including a high-affinity FcγRI that mediates phagocytosis by macrophages and neutrophils, a low-affinity FcγRIIB that transduces inhibitory signals in B cells, and a low-affinity FcγRIIIA that mediates targeting and activation of NK cells.

Ficolins Pentameric innate immune system plasma proteins, containing collagen-like domains and fibrinogen-like carbohydrate-recognizing domains, which bind to cell wall components of gram-positive bacteria, opsonizing them and activating complement.

First-set rejection Allograft rejection in an individual who has not previously received a graft or otherwise been exposed to tissue alloantigens from the same donor. First-set rejection usually takes about 7 to 14 days.

FK506 An immunosuppressive drug (also known as tacrolimus) used to prevent allograft rejection that functions by blocking T cell cytokine gene transcription, similar to cyclosporine. FK506 binds to a cytosolic protein called FK506-binding protein, and the resulting complex binds to calcineurin, thereby inhibiting activation and nuclear translocation of the transcription factor NFAT.

Flow cytometry A method of analysis of the phenotype of cell populations requiring a specialized instrument (flow cytometer) that can detect fluorescence on individual cells in a suspension and thereby determine the number of cells expressing the molecule to which a fluorescent probe binds. Suspensions of cells are incubated with fluorescently labeled antibodies or other probes, and the amount of probe bound by each cell in the population is measured by passing the cells one at a time through a fluorimeter with a laser-generated incident beam.

Fluorescence-activated cell sorter (FACS) An adaptation of the flow cytometer that is used for the purification of cells from a mixed population according to which and how much fluorescent probe the cells bind. Cells are first stained with fluorescently labeled probe, such as an antibody specific for a surface antigen of a cell population. The cells are then passed one at a time through a fluorimeter with a laser-generated incident beam and are differentially deflected by electromagnetic fields whose strength and direction are varied according to the measured intensity of the fluorescence signal.

Follicle See **Lymphoid follicle.**

Follicular dendritic cells Cells found in lymphoid follicles that express complement receptors, Fc receptors, and CD40 ligand and have long cytoplasmic processes that form a meshwork integral to the architecture of a lymphoid follicle. Follicular dendritic cells display antigens on their surface for B cell recognition and are involved in the activation and selection of B cells expressing high-affinity membrane Ig during the process of affinity maturation.

Follicular helper T cell (T_{FH}) See **T follicular helper (T_{FH}) cells.**

***N*-Formylmethionine** An amino acid that initiates all bacterial proteins and no mammalian proteins (except those synthesized within mitochondria) and serves as a signal to the innate immune system of infection. Specific receptors for *N*-formylmethionine–containing peptides are expressed on neutrophils and mediate activation of the neutrophils.

FoxP3 A forkhead family transcription factor expressed by and required for the development of $CD4^+$ regulatory T cells. Mutations in FoxP3 in mice and humans result in an absence of $CD25^+$ regulatory T cells and multisystem autoimmune disease.

γδ T cell receptor (γδ TCR) A form of TCR that is distinct from the more common αβ TCR and is expressed on a subset of T cells found mostly in epithelial barrier tissues. Although the γδ TCR is structurally similar to the αβ TCR, the forms of antigen recognized by γδ TCRs are poorly understood; they do not recognize peptide complexes bound to polymorphic MHC molecules.

G protein–coupled receptor family A diverse family of receptors for hormones, lipid inflammatory mediators, and chemokines that use associated trimeric G proteins for intracellular signaling.

G proteins Proteins that bind guanyl nucleotides and act as exchange molecules by catalyzing the replacement of bound guanosine diphosphate (GDP) by guanosine triphosphate (GTP). G proteins with bound GTP can activate a variety of cellular enzymes in different signaling cascades. Trimeric GTP-binding proteins are associated with the cytoplasmic portions of many cell surface receptors, such as chemokine receptors. Other small soluble G proteins, such as Ras and Rac, are recruited into signaling pathways by adaptor proteins.

GATA-3 A transcription factor that promotes the differentiation of T_H2 cells from naive T cells.

Generative lymphoid organ An organ in which lymphocytes develop from immature precursors. The bone marrow and thymus are the major generative lymphoid organs in which B cells and T cells develop, respectively.

Germinal centers Specialized structures in lymphoid organs generated during T-dependent humoral immune responses, where extensive B cell proliferation, isotype

switching, somatic mutation, affinity maturation, memory B cell generation, and induction of long-lived plasma cells take place. Germinal centers appear as lightly staining regions within a lymphoid follicle in spleen, lymph node, and mucosal lymphoid tissue.

Germline organization The inherited arrangement of variable, diversity, joining, and constant region gene segments of the antigen receptor loci in nonlymphoid cells or in immature lymphocytes. In developing B or T lymphocytes, the germline organization is modified by somatic recombination to form functional Ig or TCR genes.

Glomerulonephritis Inflammation of the renal glomeruli, often initiated by immunopathologic mechanisms such as deposition of circulating antigen-antibody complexes in the glomerular basement membrane or binding of antibodies to antigens expressed in the glomerulus. The antibodies can activate complement and phagocytes, and the resulting inflammatory response can lead to renal failure.

Graft A tissue or organ that is removed from one site and placed in another site, usually in a different individual.

Graft arteriosclerosis Occlusion of graft arteries caused by proliferation of intimal smooth muscle cells. This process is evident within 6 months to a year after transplantation and is responsible for chronic rejection of vascularized organ grafts. The mechanism is likely to be a chronic immune response to vessel wall alloantigens. Graft arteriosclerosis is also called accelerated arteriosclerosis.

Graft rejection A specific immune response to an organ or tissue graft that leads to inflammation, damage, and possibly graft failure.

Graft-versus-host disease A disease occurring in bone marrow transplant recipients that is caused by the reaction of mature T cells in the marrow graft with alloantigens on host cells. The disease most often affects the skin, liver, and intestines.

Granulocyte colony-stimulating factor (G-CSF) A cytokine made by activated T cells, macrophages, and endothelial cells at sites of infection that acts on bone marrow to increase the production of and mobilize neutrophils to replace those consumed in inflammatory reactions.

Granulocyte-monocyte colony-stimulating factor (GM-CSF) A cytokine made by activated T cells, macrophages, endothelial cells, and stromal fibroblasts that acts on bone marrow to increase the production of neutrophils and monocytes. GM-CSF is also a macrophage-activating factor and promotes the differentiation of Langerhans cells into mature dendritic cells.

Granuloma A nodule of inflammatory tissue composed of clusters of activated macrophages and T lymphocytes, often with associated necrosis and fibrosis. Granulomatous inflammation is a form of chronic delayed-type hypersensitivity, often in response to persistent microbes, such as *Mycobacterium tuberculosis* and some fungi, or in response to particulate antigens that are not readily phagocytosed.

Granzyme A serine protease enzyme found in the granules of CTLs and NK cells that is released by exocytosis, enters target cells, and proteolytically cleaves and activates caspases, which in turn cleave several substrates and induce target cell apoptosis.

Gut-associated lymphoid tissue (GALT) Collections of lymphocytes and APCs within the mucosa of the gastrointestinal tract where adaptive immune responses to intestinal microbial flora and ingested antigens are initiated (see also **Mucosa-associated lymphoid tissues**).

H-2 molecule An MHC molecule in the mouse. The mouse MHC was originally called the H-2 locus.

Haplotype The set of MHC alleles inherited from one parent and therefore on one chromosome.

Hapten A small chemical that can bind to an antibody but must be attached to a macromolecule (carrier) to stimulate an adaptive immune response specific for that chemical. For example, immunization with dinitrophenol (DNP) alone will not stimulate an anti-DNP antibody response, but immunization with a protein with covalently bonded DNP hapten will.

Heavy chain class (isotype) switching The process by which a B lymphocyte changes the class, or isotype, of the antibodies that it produces, from IgM to IgG, IgE, or IgA, without changing the antigen specificity of the antibody. Heavy chain class switching is regulated by helper T cell cytokines and CD40 ligand and involves recombination of B cell VDJ segments with downstream heavy chain gene segments.

Helminth A parasitic worm. Helminthic infections often elicit T_H2-dependent immune responses characterized by eosinophil-rich inflammatory infiltrates and IgE production.

Helper T cells The class of T lymphocytes whose main functions are to activate macrophages in cell-mediated immune responses and to promote B cell antibody production in humoral immune responses. These functions are mediated by secreted cytokines and by T cell CD40 ligand binding to macrophage or B cell CD40. Most helper T cells express the CD4 molecule.

Hematopoiesis The development of mature blood cells, including erythrocytes, leukocytes, and platelets, from pluripotent stem cells in the bone marrow and fetal liver. Hematopoiesis is regulated by several different cytokine growth factors produced by bone marrow stromal cells, T cells, and other cell types.

Hematopoietic stem cell An undifferentiated bone marrow cell that divides continuously and gives rise to additional stem cells and cells of multiple different lineages. A hematopoietic stem cell in the bone marrow will give rise to cells of the lymphoid, myeloid, and erythrocytic lineage.

Hematopoietic stem cell transplantation See **Bone marrow transplantation**.

High endothelial venules (HEVs) Specialized venules that are the sites of lymphocyte migration from the blood into the stroma of a peripheral lymph node or mucosal lymphoid tissue. HEVs are lined by plump endothelial cells that protrude into the vessel lumen and express unique adhesion molecules involved in binding naive T cells.

Hinge region A region of Ig heavy chains between the first two constant domains that can assume multiple

conformations, thereby imparting flexibility in the orientation of the two antigen-binding sites. Because of the hinge region, an antibody molecule can simultaneously bind two epitopes that are anywhere within a range of distances from one another.

Histamine A biogenic amine stored in the granules of mast cells that is one of the important mediators of immediate hypersensitivity. Histamine binds to specific receptors in various tissues and causes increased vascular permeability and contraction of bronchial and intestinal smooth muscle.

HLA See **Human leukocyte antigens.**

HLA-DM A peptide exchange molecule that plays a critical role in the class II MHC pathway of antigen presentation. HLA-DM is found in the specialized MIIC endosomal compartment and facilitates removal of the invariant chain–derived CLIP peptide and the binding of other peptides to class II MHC molecules. HLA-DM is encoded by a gene in the MHC and is structurally similar to class II MHC molecules, but it is not polymorphic.

Homeostasis In the adaptive immune system, the maintenance of a constant number and diverse repertoire of lymphocytes, despite the emergence of new lymphocytes and tremendous expansion of individual clones that may occur during responses to immunogenic antigens. Homeostasis is achieved by several regulated pathways of lymphocyte death and inactivation.

Homing receptor Adhesion molecules expressed on the surface of lymphocytes that are responsible for the different pathways of lymphocyte recirculation and tissue homing. Homing receptors bind to ligands (addressins) expressed on endothelial cells in particular vascular beds.

Human immunodeficiency virus (HIV) The etiologic agent of AIDS. HIV is a retrovirus that infects a variety of cell types, including CD4-expressing helper T cells, macrophages, and dendritic cells, and causes chronic progressive destruction of the immune system.

Human leukocyte antigens (HLA) MHC molecules expressed on the surface of human cells. Human MHC molecules were first identified as alloantigens on the surface of white blood cells (leukocytes) that bound serum antibodies from individuals previously exposed to other individuals' cells (e.g., mothers or transfusion recipients).

Humanized antibody A monoclonal antibody encoded by a recombinant hybrid gene and composed of the antigen-binding sites from a murine monoclonal antibody and the constant region of a human antibody. Humanized antibodies are less likely than mouse monoclonal antibodies to induce an anti-antibody response in humans; they are used clinically in the treatment of inflammatory diseases, tumors, and transplant rejection.

Humoral immunity The type of adaptive immune response mediated by antibodies produced by B lymphocytes. Humoral immunity is the principal defense mechanism against extracellular microbes and their toxins.

Hybridoma A cell line derived by fusion, or somatic cell hybridization, between a normal lymphocyte and an immortalized lymphocyte tumor line. B cell hybridomas created by fusion of normal B cells of defined antigen specificity with a myeloma cell line are used to produce monoclonal antibodies. T cell hybridomas created by fusion of a normal T cell of defined specificity with a T cell tumor line are commonly used in research.

Hyperacute rejection A form of allograft or xenograft rejection that begins within minutes to hours after transplantation and that is characterized by thrombotic occlusion of the graft vessels. Hyperacute rejection is mediated by preexisting antibodies in the host circulation that bind to donor endothelial antigens, such as blood group antigens or MHC molecules, and activate the complement system.

Hypersensitivity diseases Disorders caused by immune responses. Hypersensitivity diseases include autoimmune diseases, in which immune responses are directed against self antigens, and diseases that result from uncontrolled or excessive responses against foreign antigens, such as microbes and allergens. The tissue damage that occurs in hypersensitivity diseases is due to the same effector mechanisms used by the immune system to protect against microbes.

Hypervariable loop (hypervariable region) Short segments of about 10 amino acid residues within the variable regions of antibody or TCR proteins that form loop structures that contact antigen. Three hypervariable loops, also called CDRs, are present in each antibody heavy chain and light chain and in each TCR chain. Most of the variability between different antibodies or TCRs is located within these loops.

Idiotype The property of a group of antibodies or TCRs defined by their sharing a particular idiotope; that is, antibodies that share a particular idiotope belong to the same idiotype. *Idiotype* is also used to describe the collection of idiotopes expressed by an Ig molecule, and it is often used synonymously with *idiotope.*

Idiotypic network A network of complementary interactions involving idiotypes and anti-idiotypic antibodies (or T cells) that, according to the network hypothesis, reach a steady state at which the immune system is at homeostasis. Theoretically, when one or a few clones of lymphocytes respond to a foreign antigen, their idiotypes are expanded and anti-idiotype responses are triggered that function to shut off the antigen-specific response.

Igα and Igβ Proteins that are required for surface expression and signaling functions of membrane Ig on B cells. Igα and Igβ pairs are disulfide linked to one another, noncovalently associated with the cytoplasmic tail of membrane Ig, and form the BCR complex. The cytoplasmic domains of Igα and Igβ contain ITAMs that are involved in early signaling events during antigen-induced B cell activation.

IL-1 receptor antagonist (IL-1Ra) A natural inhibitor of IL-1 produced by mononuclear phagocytes that is structurally homologous to IL-1 and binds to the same receptors but is biologically inactive. Attempts to use IL-1 inhibitors to reduce inflammation in diseases such as rheumatoid arthritis are ongoing.

Immature B lymphocyte A membrane IgM^+, IgD^- B cell, recently derived from marrow precursors, that does not proliferate or differentiate in response to antigens but rather may undergo apoptotic death or become functionally unresponsive. This property is important for the negative selection of B cells that are specific for self antigens present in the bone marrow.

Immediate hypersensitivity The type of immune reaction responsible for allergic diseases and dependent on IgE plus antigen-mediated stimulation of tissue mast cells and basophils. The mast cells and basophils release mediators that cause increased vascular permeability, vasodilation, bronchial and visceral smooth muscle contraction, and local inflammation.

Immune complex A multimolecular complex of antibody molecules with bound antigen. Because each antibody molecule has a minimum of two antigen-binding sites and many antigens are multivalent, immune complexes can vary greatly in size. Immune complexes activate effector mechanisms of humoral immunity, such as the classical complement pathway and Fc receptor–mediated phagocyte activation. Deposition of circulating immune complexes in blood vessel walls or renal glomeruli can lead to inflammation and disease.

Immune complex disease An inflammatory disease caused by the deposition of antigen-antibody complexes in blood vessel walls, resulting in local complement activation and phagocyte recruitment. Immune complexes may form because of overproduction of antibodies against microbial antigens or as a result of autoantibody production in the setting of an autoimmune disease such as systemic lupus erythematosus. Immune complex deposition in the specialized capillary basement membranes of renal glomeruli can cause glomerulonephritis and impair renal function. Systemic deposition of immune complexes in arterial walls can cause vasculitis, with thrombosis and ischemic damage to various organs.

Immune deviation The conversion of a T cell response associated with one set of cytokines, such as T_H1 cytokines that stimulate cell-mediated immunity, to a response associated with other cytokines, such as T_H2 cytokines that stimulate the production of selected antibody isotypes.

Immune inflammation Inflammation that is a result of an adaptive immune response to antigen. The cellular infiltrate at the inflammatory site may include cells of the innate immune system such as neutrophils and macrophages, which are recruited as a result of the actions of T cell cytokines.

Immune-mediated inflammatory disease A broad group of disorders in which immune responses, either to self or foreign antigens, and chronic inflammation are major components.

Immune response A collective and coordinated response to the introduction of foreign substances in an individual mediated by the cells and molecules of the immune system.

Immune response (Ir) genes Originally defined as genes in inbred strains of rodents that were inherited in a dominant mendelian manner and that controlled the ability of the animals to make antibodies against simple synthetic polypeptides. We now know that Ir genes are polymorphic genes that encode MHC molecules, which display peptides to T lymphocytes and are therefore required for T cell activation and helper T cell–dependent B cell (antibody) responses to protein antigens.

Immune surveillance The concept that a physiologic function of the immune system is to recognize and destroy clones of transformed cells before they grow into tumors and to kill tumors after they are formed. The term *immune surveillance* is sometimes used in a general sense to describe the function of T lymphocytes to detect and destroy any cell, not necessarily a tumor cell, that is expressing foreign (e.g., microbial) antigens.

Immune system The molecules, cells, tissues, and organs that collectively function to provide immunity, or protection, against foreign organisms.

Immunity Protection against disease, usually infectious disease, mediated by the cells and tissues that are collectively called the immune system. In a broader sense, immunity refers to the ability to respond to foreign substances, including microbes and non-infectious molecules.

Immunoblot An analytical technique in which antibodies are used to detect the presence of an antigen bound to (i.e., blotted on) a solid matrix such as filter paper (also known as a Western blot).

Immunodeficiency See **Acquired immunodeficiency** and **Congenital immunodeficiency**.

Immunodominant epitope The epitope of a protein antigen that elicits most of the response in an individual immunized with the native protein. Immunodominant epitopes correspond to the peptides of the protein that are proteolytically generated within APCs and bind most avidly to MHC molecules, and are most likely to stimulate T cells.

Immunofluorescence A technique in which a molecule is detected by use of an antibody labeled with a fluorescent probe. For example, in immunofluorescence microscopy, cells that express a particular surface antigen can be stained with a fluorescein-conjugated antibody specific for the antigen and then visualized with a fluorescent microscope.

Immunogen An antigen that induces an immune response. Not all antigens are immunogens. For example, small-molecular-weight compounds (haptens) can bind to antibodies but will not stimulate an immune response unless they are linked to macromolecules (carriers).

Immunoglobulin (Ig) Synonymous with antibody (see **Antibody**).

Immunoglobulin domain A three-dimensional globular structural motif found in many proteins in the immune system, including Igs, TCRs, and MHC molecules. Ig domains are about 110 amino acid residues in length, include an internal disulfide bond, and contain two layers of β-pleated sheets, each layer composed of three to five strands of antiparallel polypeptide chain. Ig domains are classified as V-like or C-like

on the basis of closest homology to either the Ig V or C domains.

Immunoglobulin heavy chain One of two types of polypeptide chains in an antibody molecule. The basic structural unit of an antibody includes two identical, disulfide-linked heavy chains and two identical light chains. Each heavy chain is composed of a variable (V) Ig domain and three or four constant (C) Ig domains. The different antibody isotypes, including IgM, IgD, IgG, IgA, and IgE, are distinguished by structural differences in their heavy chain constant regions. The heavy chain constant regions also mediate effector functions, such as complement activation or engagement of phagocytes.

Immunoglobulin light chain One of two types of polypeptide chains in an antibody molecule. The basic structural unit of an antibody includes two identical light chains, each disulfide linked to one of two identical heavy chains. Each light chain is composed of one variable (V) Ig domain and one constant (C) Ig domain. There are two light chain isotypes, called κ and λ, both functionally identical. About 60% of human antibodies have κ light chains and 40% have λ light chains.

Immunoglobulin superfamily A large family of proteins that contain a globular structural motif called an Ig domain, or Ig fold, originally described in antibodies. Many proteins of importance in the immune system, including antibodies, TCRs, MHC molecules, CD4, and CD8, are members of this superfamily.

Immunohistochemistry A technique to detect the presence of an antigen in histologic tissue sections by use of an enzyme-coupled antibody that is specific for the antigen. The enzyme converts a colorless substrate to a colored insoluble substance that precipitates at the site where the antibody and thus the antigen are localized. The position of the colored precipitate, and therefore the antigen, in the tissue section is observed by conventional light microscopy. Immunohistochemistry is a routine technique in diagnostic pathology and various fields of research.

Immunologic tolerance See **Tolerance.**

Immunologically privileged site A site in the body that is inaccessible to or constitutively suppresses immune responses. The anterior chamber of the eye, the testes, and the brain are examples of immunologically privileged sites.

Immunoperoxidase technique A common immunohistochemical technique in which a horseradish peroxidase–coupled antibody is used to identify the presence of an antigen in a tissue section. The peroxidase enzyme converts a colorless substrate to an insoluble brown product that is observable by light microscopy.

Immunoprecipitation A technique for the isolation of a molecule from a solution by binding it to an antibody and then rendering the antigen-antibody complex insoluble, either by precipitation with a second antibody or by coupling the first antibody to an insoluble particle or bead.

Immunoreceptor tyrosine-based activation motif (ITAM) A conserved motif composed of two copies of the sequence tyrosine-x-x-leucine (where x is an unspecified amino acid) found in the cytoplasmic tails of various membrane proteins in the immune system that are involved in signal transduction. ITAMs are present in the ζ and CD3 proteins of the TCR complex, in Igα and Igβ proteins in the BCR complex, and in several Ig Fc receptors. When these receptors bind their ligands, the tyrosine residues of the ITAMs become phosphorylated and form docking sites for other molecules involved in propagating cell-activating signal transduction pathways.

Immunoreceptor tyrosine-based inhibition motif (ITIM) A six–amino acid (isoleucine-x-tyrosine-x-x-leucine) motif found in the cytoplasmic tails of various inhibitory receptors in the immune system, including FcγRIIB on B cells and killer cell Ig-like receptors (KIRs) on NK cells. When these receptors bind their ligands, the ITIMs become phosphorylated on their tyrosine residues and form a docking site for protein tyrosine phosphatases, which in turn function to inhibit other signal transduction pathways.

Immunosuppression Inhibition of one or more components of the adaptive or innate immune system as a result of an underlying disease or intentionally induced by drugs for the purpose of preventing or treating graft rejection or autoimmune disease. A commonly used immunosuppressive drug is cyclosporine, which blocks T cell cytokine production.

Immunotherapy The treatment of a disease with therapeutic agents that promote or inhibit immune responses. Cancer immunotherapy, for example, involves promotion of active immune responses to tumor antigens or administration of anti-tumor antibodies or T cells to establish passive immunity.

Immunotoxins Reagents that may be used in the treatment of cancer and consist of covalent conjugates of a potent cellular toxin, such as ricin or diphtheria toxin, with antibodies specific for antigens expressed on the surface of tumor cells. It is hoped that such reagents can specifically target and kill tumor cells without damaging normal cells, but safe and effective immunotoxins have yet to be developed.

Inbred mouse strain A strain of mice created by repetitive mating of siblings that is characterized by homozygosity at every genetic locus. Every mouse of an inbred strain is genetically identical (syngeneic) to every other mouse of the same strain.

Indirect antigen presentation (or indirect allorecognition) In transplantation immunology, a pathway of presentation of donor (allogeneic) MHC molecules by recipient APCs that involves the same mechanisms used to present microbial proteins. The allogeneic MHC proteins are processed by recipient professional APCs, and peptides derived from the allogeneic MHC molecules are presented, in association with recipient (self) MHC molecules, to host T cells. In contrast to indirect antigen presentation, direct antigen presentation involves recipient T cell recognition of unprocessed allogeneic MHC molecules on the surface of graft cells.

Inflammasome A multiprotein complex in the cytosol of mononuclear phagocytes, dendritic cells, and other cell types that proteolytically generates the active form

of IL-1 from an inactive precursor. The formation of the inflammasome complex, which includes NLRP3 (a NOD-like pattern recognition receptor) and caspase-1, is stimulated by a variety of microbial products, cell damage–associated molecules, and crystals.

Inflammation A complex reaction of vascularized tissue to infection, toxin exposure, or cell injury that involves extravascular accumulation of plasma proteins and leukocytes. Acute inflammation is a common result of innate immune responses, and local adaptive immune responses can also promote inflammation. Although inflammation serves a protective function in controlling infections and promoting tissue repair, it can also cause tissue damage and disease.

Inflammatory bowel disease (IBD) A group of disorders, including ulcerative colitis and Crohn's disease, characterized by chronic inflammation in the gastrointestinal tract. The etiology of IBD is not known, but some evidence indicates that it is caused by inadequate regulation of T cell responses, probably against intestinal commensal bacteria. IBD develops in gene knockout mice lacking IL-2, IL-10, or the TCR α chain.

Innate immunity Protection against infection that relies on mechanisms that exist before infection, are capable of a rapid response to microbes, and react in essentially the same way to repeated infections. The innate immune system includes epithelial barriers, phagocytic cells (neutrophils, macrophages), NK cells, the complement system, and cytokines, largely made by dendritic cells and mononuclear phagocytes, that regulate and coordinate many of the activities of the cells of innate immunity.

Inositol 1,4,5-trisphosphate (IP3) A cytoplasmic signaling molecule generated by phospholipase C (PLCγ1)–mediated hydrolysis of the plasma membrane phospholipid PIP2 during antigen activation of lymphocytes. The main function of IP3 is to stimulate the release of intracellular stores of calcium from membrane-bound compartments such as the endoplasmic reticulum.

Integrins Heterodimeric cell surface proteins whose major functions are to mediate the adhesion of leukocytes to other leukocytes, endothelial cells, and extracellular matrix proteins. Integrins are important for T cell interactions with APCs and for migration of leukocytes from blood into tissues. The ligand-binding affinity of the integrins can be regulated by various stimuli, and the cytoplasmic domains of integrins bind to the cytoskeleton. There are two main subfamilies of integrins; the members of each family express a conserved β chain (β_1, or CD29, and β_2, or CD18) associated with different α chains. VLA-4 (very late antigen 4) is a β_1 integrin and LFA-1 (leukocyte function-associated antigen 1) is a β_2 integrin expressed on T cells and other leukocytes.

Interferon regulatory factors (IRFs) A family of inducibly activated transcription factors that are important in expression of inflammatory and antiviral genes. For example, IRF3 is activated by TLR signals and regulates expression of type I interferons, which are cytokines that protect cells from viral infection.

Interferons A subgroup of cytokines originally named for their ability to interfere with viral infections but that have other important immunomodulatory functions. Type I interferons include interferon-α and interferon-β, whose main function is antiviral; type II interferon, also called interferon-γ, activates macrophages and various other cell types. (See Appendix II.)

Interleukins Any of a large number of cytokines named with a numerical suffix roughly sequentially in order of discovery or molecular characterization (e.g., interleukin-1, interleukin-2). Some cytokines were originally named for their biologic activities and do not have an interleukin designation. (See Appendix II.)

Intracellular bacterium A bacterium that survives or replicates within cells, usually in endosomes. The principal defense against intracellular bacteria, such as *Mycobacterium tuberculosis,* is cell-mediated immunity.

Intraepidermal lymphocytes T lymphocytes found within the epidermal layer of the skin. In the mouse, most of the intraepidermal T cells express the γδ form of TCR (see **Intraepithelial T lymphocytes**).

Intraepithelial T lymphocytes T lymphocytes present in the epidermis of the skin and in mucosal epithelia that typically express a limited diversity of antigen receptors. Some of these lymphocytes may recognize microbial products, such as glycolipids, associated with nonpolymorphic class I MHC–like molecules. Intraepithelial T lymphocytes may be considered effector cells of innate immunity and function in host defense by secreting cytokines and activating phagocytes and by killing infected cells.

Invariant chain (I_i) A nonpolymorphic protein that binds to newly synthesized class II MHC molecules in the endoplasmic reticulum. The invariant chain prevents loading of the class II MHC peptide-binding cleft with peptides present in the endoplasmic reticulum, and such peptides are left to associate with class I molecules. The invariant chain also promotes folding and assembly of class II molecules and directs newly formed class II molecules to the specialized endosomal MIIC compartment, where peptide loading takes place.

Isotype One of five types of antibodies, determined by which of five different forms of heavy chain is present. Antibody isotypes include IgM, IgD, IgG, IgA, and IgE, and each isotype performs a different set of effector functions. Additional structural variations characterize distinct subtypes of IgG and IgA.

J chain A small polypeptide that is disulfide bonded to the tail pieces of multimeric IgM and IgA antibodies and contributes to the transepithelial transport of these immunoglobulins.

JAK-STAT signaling pathway A signaling pathway initiated by cytokine binding to type I and type II cytokine receptors. This pathway sequentially involves activation of receptor-associated Janus kinase (JAK) tyrosine kinases, JAK-mediated tyrosine phosphorylation of the cytoplasmic tails of cytokine receptors, docking of signal transducers and activators of transcription (STATs) to the phosphorylated receptor chains, JAK-mediated tyrosine phosphorylation of the associated STATs, dimerization and nuclear

translocation of the STATs, and STAT binding to regulatory regions of target genes causing transcriptional activation of those genes.

Janus kinases (JAKs) A family of tyrosine kinases that associate with the cytoplasmic tails of several different cytokine receptors, including the receptors for IL-2, IL-3, IL-4, IFN-γ, IL-12, and others. In response to cytokine binding and receptor dimerization, JAKs phosphorylate the cytokine receptors to permit the binding of STATs, and then the JAKs phosphorylate and thereby activate the STATs. Different JAK kinases associate with different cytokine receptors.

Joining (J) chain A polypeptide that links IgA or IgM molecules to form multimers (e.g., dimeric IgA and pentameric IgM).

Joining (J) segments Short coding sequences, between the variable (V) and constant (C) gene segments in all the Ig and TCR loci, which together with D segments are somatically recombined with V segments during lymphocyte development. The resulting recombined VDJ DNA codes for the carboxyl-terminal ends of the antigen receptor V regions, including the third hypervariable (CDR) regions. Random use of different J segments contributes to the diversity of the antigen receptor repertoire.

Junctional diversity The diversity in antibody and TCR repertoires that is attributed to the random addition or removal of nucleotide sequences at junctions between V, D, and J gene segments.

Kaposi's sarcoma A malignant tumor of vascular cells that frequently arises in patients with AIDS. Kaposi's sarcoma is associated with infection by the Kaposi's sarcoma–associated herpesvirus (human herpesvirus 8).

Killer Ig-like receptors (KIRs) Ig superfamily receptors expressed by NK cells that recognize different alleles of HLA-A, HLA-B, and HLA-C molecules. Some KIRs have signaling components with ITIMs in their cytoplasmic tails, and these deliver inhibitory signals to inactivate the NK cells. Some members of the KIR family have short cytoplasmic tails without ITIMs but associate with other ITAM-containing polypeptides and function as activating receptors.

Knockout mouse A mouse with a targeted disruption of one or more genes that is created by homologous recombination techniques. Knockout mice lacking functional genes encoding cytokines, cell surface receptors, signaling molecules, and transcription factors have provided extensive information about the roles of these molecules in the immune system.

Lamina propria A layer of loose connective tissue underlying epithelium in mucosal tissues such as the intestines and airways, where dendritic cells, mast cells, lymphocytes, and macrophages mediate immune responses to invading pathogens.

Langerhans cells Immature dendritic cells found as a meshwork in the epidermal layer of the skin whose major function is to trap microbes and antigens that enter through the skin and transport the antigens to draining lymph nodes. During their migration to the lymph nodes, Langerhans cells mature into lymph node dendritic cells, which can efficiently present antigen to naive T cells.

Large granular lymphocyte Another name for an NK cell based on the morphologic appearance of this cell type in the blood.

Late-phase reaction A component of the immediate hypersensitivity reaction that ensues 2 to 4 hours after mast cell degranulation and that is characterized by an inflammatory infiltrate of eosinophils, basophils, neutrophils, and lymphocytes. Repeated bouts of this late-phase inflammatory reaction can cause tissue damage.

Lck An Src family nonreceptor tyrosine kinase that noncovalently associates with the cytoplasmic tails of CD4 and CD8 molecules in T cells and is involved in the early signaling events of antigen-induced T cell activation. Lck mediates tyrosine phosphorylation of the cytoplasmic tails of CD3 and ζ proteins of the TCR complex.

Lectin pathway of complement activation A pathway of complement activation triggered, in the absence of antibody, by the binding of microbial polysaccharides to circulating lectins such as MBL. MBL is structurally similar to C1q and activates the C1r-C1s enzyme complex (like C1q) or activates another serine esterase, called mannose-binding protein–associated serine esterase. The remaining steps of the lectin pathway, beginning with cleavage of C4, are the same as the classical pathway.

Leishmania An obligate intracellular protozoan parasite that infects macrophages and can cause a chronic inflammatory disease involving many tissues. *Leishmania* infection in mice has served as a model system for study of the effector functions of several cytokines and the helper T cell subsets that produce them. T_H1 responses to *Leishmania major* and associated IFN-γ production control infection, whereas T_H2 responses with IL-4 production lead to disseminated lethal disease.

Lethal hit A term used to describe the events that result in irreversible damage to a target cell when a CTL binds to it. The lethal hit includes CTL granule exocytosis, and perforin-dependent delivery of apoptosis-inducing enzymes (granzymes) into the target cell cytoplasm.

Leukemia A malignant disease of bone marrow precursors of blood cells in which large numbers of leukemic cells usually occupy the bone marrow and often circulate in the blood stream. Lymphocytic leukemias are derived from B or T cell precursors, myelogenous leukemias are derived from granulocyte or monocyte precursors, and erythroid leukemias are derived from red blood cell precursors.

Leukocyte adhesion deficiency (LAD) One of a rare group of immunodeficiency diseases with infectious complications that is caused by defective expression of the leukocyte adhesion molecules required for tissue recruitment of phagocytes and lymphocytes. LAD-1 is due to mutations in the gene encoding the CD18 protein, which is part of β_2 integrins. LAD-2 is caused by mutations in a gene that encodes a fucose transporter involved in the synthesis of leukocyte ligands for endothelial selectins.

Leukotrienes A class of arachidonic acid–derived lipid inflammatory mediators produced by the lipoxygenase pathway in many cell types. Mast cells make abundant leukotriene C_4 (LTC_4) and its degradation products LTD_4 and LTE_4, which bind to specific receptors on smooth muscle cells and cause prolonged bronchoconstriction. Leukotrienes contribute to the pathologic processes of bronchial asthma. Collectively, LTC_4, LTD_4, and LTE_4 constitute what was once called slow-reacting substance of anaphylaxis.

Lipopolysaccharide Synonymous with **endotoxin.**

Live virus vaccine A vaccine composed of a live but nonpathogenic (attenuated) form of a virus. Attenuated viruses carry mutations that interfere with the viral life cycle or pathogenesis. Because live virus vaccines actually infect the recipient cells, they can effectively stimulate immune responses, such as the CTL response, that are optimal for protecting against wild-type viral infection. A commonly used live virus vaccine is the Sabin poliovirus vaccine.

LMP-2 and LMP-7 Two catalytic subunits of the proteasome, the organelle that degrades cytosolic proteins into peptides in the class I MHC pathway of antigen presentation. LMP-2 and LMP-7 are encoded by genes in the MHC, are upregulated by IFN-γ, and are particularly important for generating class I MHC–binding peptides.

Lymph node Small nodular, encapsulated aggregates of lymphocyte-rich tissue situated along lymphatic channels throughout the body where adaptive immune responses to lymph-borne antigens are initiated.

Lymphatic system A system of vessels throughout the body that collects tissue fluid called lymph, originally derived from the blood, and returns it, through the thoracic duct, to the circulation. Lymph nodes are interspersed along these vessels and trap and retain antigens present in the lymph.

Lymphocyte homing The directed migration of subsets of circulating lymphocytes into particular tissue sites. Lymphocyte homing is regulated by the selective expression of endothelial adhesion molecules and chemokines, in different tissues. For example, some lymphocytes preferentially home to the intestinal mucosa, and this directed migration is regulated by the chemokine CCL25 and the endothelial adhesion molecule MadCAM, both expressed in the gut, which bind respectively to the CCR9 chemokine receptor and the $\alpha_4\beta_1$ integrin on gut-homing lymphocytes.

Lymphocyte maturation The process by which pluripotent bone marrow stem cells develop into mature, antigen receptor–expressing naive B or T lymphocytes that populate peripheral lymphoid tissues. This process takes place in the specialized environments of the bone marrow (for B cells) and the thymus (for T cells).

Lymphocyte migration The movement of lymphocytes from the blood stream into peripheral tissues.

Lymphocyte recirculation The continuous movement of lymphocytes through the blood stream and lymphatics, between the lymph nodes or spleen and, if activated, peripheral inflammatory sites.

Lymphocyte repertoire The complete collection of antigen receptors and therefore antigen specificities expressed by the B and T lymphocytes of an individual.

Lymphoid follicle A B cell–rich region of a lymph node or the spleen that is the site of antigen-induced B cell proliferation and differentiation. In T cell–dependent B cell responses to protein antigens, a germinal center forms within the follicles.

Lymphoid tissue inducer cells Hematopoietically derived cells with phenotypic features of both lymphocytes and natural killer cells that stimulate the development of lymph nodes and other secondary lymphoid organs, in part through production of the cytokines lymphotoxin-α (LTα) and lymphotoxin-β (LTβ).

Lymphokine An old name for a cytokine (soluble protein mediator of immune responses) produced by lymphocytes.

Lymphokine-activated killer (LAK) cells NK cells with enhanced cytolytic activity for tumor cells as a result of exposure to high doses of IL-2. LAK cells generated in vitro have been adoptively transferred back into patients with cancer to treat their tumors.

Lymphoma A malignant tumor of B or T lymphocytes usually arising in and spreading between lymphoid tissues but that may spread to other tissues. Lymphomas often express phenotypic characteristics of the normal lymphocytes from which they were derived.

Lymphotoxin (LT, TNF-β) A cytokine produced by T cells that is homologous to and binds to the same receptors as TNF. Like TNF, LT has proinflammatory effects, including endothelial and neutrophil activation. LT is also critical for the normal development of lymphoid organs.

Lysosome A membrane-bound, acidic organelle abundant in phagocytic cells that contains proteolytic enzymes that degrade proteins derived both from the extracellular environment and from within the cell. Lysosomes are involved in the class II MHC pathway of antigen processing.

M cells Specialized epithelial cells overlying Peyer's patches in the gut that play a role in delivery of antigens to Peyer's patches.

M1 macrophages See **Classical macrophage activation.**

M2 macrophages See **Alternative macrophage activation.**

Macrophage A tissue-based phagocytic cell derived from blood monocytes that plays important roles in innate and adaptive immune responses. Macrophages are activated by microbial products such as endotoxin and by T cell cytokines such as IFN-γ. Activated macrophages phagocytose and kill microorganisms, secrete proinflammatory cytokines, and present antigens to helper T cells. Macrophages may assume different morphologic forms in different tissues, including the microglia of the central nervous system, Kupffer cells in the liver, alveolar macrophages in the lung, and osteoclasts in bone.

Major histocompatibility complex (MHC) A large genetic locus (on human chromosome 6 and mouse chromosome 17) that includes the highly polymorphic

genes encoding the peptide-binding molecules recognized by T lymphocytes. The MHC locus also includes genes encoding cytokines, molecules involved in antigen processing, and complement proteins.

Major histocompatibility complex (MHC) molecule A heterodimeric membrane protein encoded in the MHC locus that serves as a peptide display molecule for recognition by T lymphocytes. Two structurally distinct types of MHC molecules exist. Class I MHC molecules are present on most nucleated cells, bind peptides derived from cytosolic proteins, and are recognized by $CD8^+$ T cells. Class II MHC molecules are restricted largely to dendritic cells, macrophages, and B lymphocytes, bind peptides derived from endocytosed proteins, and are recognized by $CD4^+$ T cells.

Mannose-binding lectin (MBL) A plasma protein that binds to mannose residues on bacterial cell walls and acts as an opsonin by promoting phagocytosis of the bacterium by macrophages. Macrophages express a surface receptor for C1q that can also bind MBL and mediate uptake of the opsonized organisms.

Mannose receptor A carbohydrate-binding receptor (lectin) expressed by macrophages that binds mannose and fucose residues on microbial cell walls and mediates phagocytosis of the organisms.

Marginal zone A peripheral region of splenic lymphoid follicles containing macrophages that are particularly efficient at trapping polysaccharide antigens. Such antigens may persist for prolonged periods on the surfaces of marginal zone macrophages, where they are recognized by specific B cells, or they may be transported into follicles.

Marginal zone B lymphocytes A subset of B lymphocytes, found exclusively in the marginal zone of the spleen, that respond rapidly to blood-borne microbial antigens by producing IgM antibodies with limited diversity.

Mast cell The major effector cell of immediate hypersensitivity (allergic) reactions. Mast cells are derived from the marrow, reside in most tissues adjacent to blood vessels, express a high-affinity Fc receptor for IgE, and contain numerous mediator-filled granules. Antigen-induced cross-linking of IgE bound to the mast cell Fc receptors causes release of their granule contents as well as new synthesis and secretion of other mediators, leading to an immediate hypersensitivity reaction.

Mature B cell IgM- and IgD-expressing, functionally competent naive B cells that represent the final stage of B cell maturation in the bone marrow and that populate peripheral lymphoid organs.

Membrane attack complex (MAC) A lytic complex of the terminal components of the complement cascade, including multiple copies of C9, which forms in the membranes of target cells. The MAC causes lethal ionic and osmotic changes in cells.

Memory The property of the adaptive immune system to respond more rapidly, with greater magnitude, and more effectively to a repeated exposure to an antigen compared with the response to the first exposure.

Memory lymphocytes Memory B and T cells are produced by antigen stimulation of naive lymphocytes and survive in a functionally quiescent state for many years after the antigen is eliminated. Memory lymphocytes mediate rapid and enhanced (i.e., memory or recall) responses to second and subsequent exposures to antigens.

MHC class II (MIIC) compartment A subset of endosomes (membrane-bound vesicles involved in cell trafficking pathways) found in macrophages and human B cells that are important in the class II MHC pathway of antigen presentation. The MIIC contains all the components required for formation of peptide–class II MHC molecule complexes, including the enzymes that degrade protein antigens, class II molecules, invariant chain, and HLA-DM.

MHC restriction The characteristic of T lymphocytes that they recognize a foreign peptide antigen only when it is bound to a particular allelic form of an MHC molecule.

MHC tetramer A reagent used to identify and enumerate T cells that specifically recognize a particular MHC-peptide complex. The reagent consists of four recombinant, biotinylated MHC molecules (usually class I) bound to a fluorochrome-labeled avidin molecule and loaded with a peptide. T cells that bind the MHC tetramer can be detected by flow cytometry.

β_2-Microglobulin The light chain of a class I MHC molecule. β_2-Microglobulin is an extracellular protein encoded by a nonpolymorphic gene outside the MHC, is structurally homologous to an Ig domain, and is invariant among all class I molecules.

Mitogen-activated protein (MAP) kinase cascade A signal transduction cascade initiated by the active form of the Ras protein and involving the sequential activation of three serine/threonine kinases, the last one being MAP kinase. MAP kinase in turn phosphorylates and activates other enzymes and transcription factors. The MAP kinase pathway is one of several signal pathways activated by antigen binding to the TCR and BCR.

Mixed leukocyte reaction (MLR) An in vitro reaction of alloreactive T cells from one individual against MHC antigens on blood cells from another individual. The MLR involves proliferation of and cytokine secretion by both $CD4^+$ and $CD8^+$ T cells.

Molecular mimicry A postulated mechanism of autoimmunity triggered by infection with a microbe containing antigens that cross-react with self antigens. Immune responses to the microbe result in reactions against self tissues.

Monoclonal antibody An antibody that is specific for one antigen and is produced by a B cell hybridoma (a cell line derived by the fusion of a single normal B cell and an immortal B cell tumor line). Monoclonal antibodies are widely used in research and clinical diagnosis and therapy.

Monocyte A type of bone marrow–derived circulating blood cell that is the precursor of tissue macrophages. Monocytes are actively recruited into inflammatory sites, where they differentiate into macrophages.

Monocyte colony-stimulating factor (M-CSF) A cytokine made by activated T cells, macrophages, endothelial cells, and stromal fibroblasts that

stimulates the production of monocytes from bone marrow precursor cells.

Monokine An old name for a cytokine produced by mononuclear phagocytes.

Mononuclear phagocytes Cells with a common bone marrow lineage whose primary function is phagocytosis. These cells function as accessory cells in the recognition and activation phases of adaptive immune responses and as effector cells in innate and adaptive immunity. Mononuclear phagocytes circulate in the blood in an incompletely differentiated form called monocytes, and once they settle in tissues, they mature into macrophages.

Mucosa-associated lymphoid tissue (MALT) Collections of lymphocytes, dendritic cells, and other cell types within the mucosa of the gastrointestinal and respiratory tracts that are sites of adaptive immune responses to antigens. Mucosa-associated lymphoid tissues contain intraepithelial lymphocytes, mainly T cells, and organized collections of lymphocytes, often rich in B cells, below mucosal epithelia, such as Peyer's patches in the gut or pharyngeal tonsils.

Mucosal immune system A part of the immune system that responds to and protects against microbes that enter the body through mucosal surfaces, such as the gastrointestinal and respiratory tracts, but also maintains tolerance to commensal organisms that live on the outside of the mucosal epithelium. The mucosal immune system is composed of organized mucosa-associated lymphoid tissues, such as Peyer's patches, as well as diffusely distributed cells within the lamina propria.

Multiple myeloma A malignant tumor of antibody-producing B cells that often secretes Igs or parts of Ig molecules. The monoclonal antibodies produced by multiple myelomas were critical for early biochemical analyses of antibody structure.

Multivalency See **Polyvalency**.

Mycobacterium A genus of aerobic bacteria, many species of which can survive within phagocytes and cause disease. The principal host defense against mycobacteria such as *Mycobacterium tuberculosis* is cell-mediated immunity.

Myeloid-derived suppressor cells A heterogeneous group of immature myeloid precursors found in lymphoid tissues, blood, or tumors of tumor-bearing animals and cancer patients that suppress anti-tumor immune responses. The cells express Ly6C or Ly6G and CD11b in mice and CD33, CD11b, and CD15 in humans.

N nucleotides The name given to nucleotides randomly added to the junctions between V, D, and J gene segments in Ig or TCR genes during lymphocyte development. The addition of up to 20 of these nucleotides, which is mediated by the enzyme terminal deoxyribonucleotidyl transferase, contributes to the diversity of the antibody and TCR repertoires.

Naive lymphocyte A mature B or T lymphocyte that has not previously encountered antigen. When naive lymphocytes are stimulated by antigen, they differentiate into effector lymphocytes, such as antibody-secreting B cells or helper T cells and CTLs. Naive lymphocytes have surface markers and recirculation patterns that are distinct from those of previously activated lymphocytes. ("Naive" also refers to an unimmunized individual.)

Natural antibodies IgM antibodies, largely produced by B-1 cells, specific for bacteria that are common in the environment and gastrointestinal tract. Normal individuals contain natural antibodies without any evidence of infection, and these antibodies serve as a preformed defense mechanism against microbes that succeed in penetrating epithelial barriers. Some of these antibodies cross-react with ABO blood group antigens and are responsible for transfusion reactions.

Natural killer (NK) cells A subset of bone marrow–derived lymphocytes, distinct from B or T cells, which function in innate immune responses to kill microbe-infected cells by direct lytic mechanisms and by secreting IFN-γ. NK cells do not express clonally distributed antigen receptors like Ig receptors or TCRs, and their activation is regulated by a combination of cell surface stimulatory and inhibitory receptors, the latter recognizing self MHC molecules.

Natural killer T cells (NKT cells) A numerically small subset of lymphocytes that express T cell receptors and some surface molecules characteristic of NK cells. Some NKT cells, called invariant NKT (iNKT), express αβ T cell antigen receptors with very little diversity, recognize lipid antigens presented by CD1 molecules, and perform various effector functions typical of helper T cells.

Negative selection The process by which developing lymphocytes that express self-reactive antigen receptors are eliminated, thereby contributing to the maintenance of self-tolerance. Negative selection of developing T lymphocytes (thymocytes) is best understood and involves high-avidity binding of a thymocyte to self MHC molecules with bound peptides on thymic APCs, leading to apoptotic death of the thymocyte.

Neonatal Fc receptor (FcRn) An IgG-specific Fc receptor that mediates the transport of maternal IgG across the placenta and the neonatal intestinal epithelium. FcRn resembles a class I MHC molecule. An adult form of this receptor functions to protect plasma IgG antibodies from catabolism.

Neonatal immunity Passive humoral immunity to infections in mammals in the first months of life, before full development of the immune system. Neonatal immunity is mediated by maternally produced antibodies transported across the placenta into the fetal circulation before birth or derived from ingested milk and transported across the gut epithelium.

Neutrophil (also polymorphonuclear leukocyte, PMN) A phagocytic cell characterized by a segmented lobular nucleus and cytoplasmic granules filled with degradative enzymes. PMNs are the most abundant type of circulating white blood cells and are the major cell type mediating acute inflammatory responses to bacterial infections.

Nitric oxide A biologic effector molecule with a broad range of activities that in macrophages functions as a potent microbicidal agent to kill ingested organisms.

Nitric oxide synthase A member of a family of enzymes that synthesize the vasoactive and microbicidal compound nitric oxide from L-arginine. Macrophages express an inducible form of this enzyme on activation by various microbial or cytokine stimuli.

NOD-like receptors (NLRs) A family of cytosolic multidomain proteins that sense cytoplasmic PAMPs and DAMPs and recruit other proteins to form signaling complexes that promote inflammation.

Notch 1 A cell surface signaling receptor that is proteolytically cleaved after ligand binding, and the cleaved intracellular portion translocates to the nucleus and regulates gene expression. Notch 1 signaling is required for commitment of developing T cell precursors to the alpha beta T cell lineage.

Nuclear factor κB (NF-κB) A family of transcription factors composed of homodimers or heterodimers of proteins homologous to the c-Rel protein. NF-κB proteins are required for the inducible transcription of many genes important in both innate and adaptive immune responses.

Nuclear factor of activated T cells (NFAT) A transcription factor required for the expression of IL-2, IL-4, TNF, and other cytokine genes. The four different NFATs are each encoded by separate genes; NFATp and NFATc are found in T cells. Cytoplasmic NFAT is activated by calcium/calmodulin-dependent, calcineurin-mediated dephosphorylation that permits NFAT to translocate into the nucleus and bind to consensus binding sequences in the regulatory regions of IL-2, IL-4, and other cytokine genes, usually in association with other transcription factors such as AP-1.

Nude mouse A strain of mice that lacks development of the thymus, and therefore T lymphocytes, as well as hair follicles. Nude mice have been used experimentally to define the role of T lymphocytes in immunity and disease.

Oncofetal antigen Proteins that are expressed at high levels on some types of cancer cells and in normal developing (fetal) but not adult tissues. Antibodies specific for these proteins are often used in histopathologic identification of tumors or to monitor the progression of tumor growth in patients. CEA (CD66) and α-fetoprotein are two oncofetal antigens commonly expressed by certain carcinomas.

Opsonin A macromolecule that becomes attached to the surface of a microbe and can be recognized by surface receptors of neutrophils and macrophages and that increases the efficiency of phagocytosis of the microbe. Opsonins include IgG antibodies, which are recognized by the Fcγ receptor on phagocytes, and fragments of complement proteins, which are recognized by CR1 (CD35) and by the leukocyte integrin Mac-1.

Opsonization The process of attaching opsonins, such as IgG or complement fragments, to microbial surfaces to target the microbes for phagocytosis.

Oral tolerance The suppression of systemic humoral and cell-mediated immune responses to an antigen after the oral administration of that antigen as a result of anergy of antigen-specific T cells or the production of immunosuppressive cytokines such as transforming growth factor-β. Oral tolerance is a possible mechanism for prevention of immune responses to food antigens and to bacteria that normally reside as commensals in the intestinal lumen.

P nucleotides Short inverted repeat nucleotide sequences in the VDJ junctions of rearranged Ig and TCR genes that are generated by RAG-1– and RAG-2–mediated asymmetric cleavage of hairpin DNA intermediates during somatic recombination events. P nucleotides contribute to the junctional diversity of antigen receptors.

Paracrine factor A molecule that acts on cells in proximity to the cell that produces the factor. Most cytokines act in a paracrine fashion.

Passive immunity The form of immunity to an antigen that is established in one individual by transfer of antibodies or lymphocytes from another individual who is immune to that antigen. The recipient of such a transfer can become immune to the antigen without ever having been exposed to or having responded to the antigen. An example of passive immunity is the transfer of human sera containing antibodies specific for certain microbial toxins or snake venom to a previously unimmunized individual.

Pathogen-associated molecular patterns (PAMPs) Structures produced by microorganisms but not mammalian (host) cells, which are recognized by and stimulate the innate immune system. Examples include bacterial lipopolysaccharide and viral double stranded RNA.

Pathogenicity The ability of a microorganism to cause disease. Multiple mechanisms may contribute to pathogenicity, including production of toxins, stimulation of host inflammatory responses, and perturbation of host cell metabolism.

Pattern recognition receptors Signaling receptors of the innate immune system that recognize PAMPs and DAMPs, and thereby activate innate immune responses. Examples include Toll-like receptors (TLRs) and Nod-like receptors (NLRs).

Pentraxins A family of plasma proteins that contain five identical globular subunits; includes the acute-phase reactant C-reactive protein.

Peptide-binding cleft The portion of an MHC molecule that binds peptides for display to T cells. The cleft is composed of paired α helices resting on a floor made up of an eight-stranded β-pleated sheet. The polymorphic residues, which are the amino acids that vary among different MHC alleles, are located in and around this cleft.

Perforin A protein that is homologous to the C9 complement protein and is present in the granules of CTLs and NK cells. When perforin is released from the granules of activated CTLs or NK cells, it promotes entry of granzymes into the target cell, leading to apoptotic death of the cell.

Periarteriolar lymphoid sheath (PALS) A cuff of lymphocytes surrounding small arterioles in the spleen, adjacent to lymphoid follicles. A PALS contains mainly T lymphocytes, about two thirds of which are $CD4^+$ and one third $CD8^+$. In humoral immune responses to protein antigens, B lymphocytes are activated at the

interface between the PALS and follicles and then migrate into the follicles to form germinal centers.

Peripheral lymphoid organs and tissues Organized collections of lymphocytes and accessory cells, including the spleen, lymph nodes, and mucosa-associated lymphoid tissues, in which adaptive immune responses are initiated.

Peripheral tolerance Unresponsiveness to self antigens that are present in peripheral tissues and not usually in the generative lymphoid organs. Peripheral tolerance is induced by the recognition of antigens without adequate levels of the costimulators required for lymphocyte activation or by persistent and repeated stimulation by these self antigens.

Peyer's patches Organized lymphoid tissue in the lamina propria of the small intestine in which immune responses to ingested antigens may be initiated. Peyer's patches are composed mostly of B cells, with smaller numbers of T cells and accessory cells, all arranged in follicles similar to those found in lymph nodes, often with germinal centers.

Phagocytosis The process by which certain cells of the innate immune system, including macrophages and neutrophils, engulf large particles (>0.5 μm in diameter) such as intact microbes. The cell surrounds the particle with extensions of its plasma membrane by an energy- and cytoskeleton-dependent process; this process results in the formation of an intracellular vesicle called a phagosome, which contains the ingested particle.

Phagosome A membrane-bound intracellular vesicle that contains microbes or particulate material from the extracellular environment. Phagosomes are formed during the process of phagocytosis. They fuse with other vesicular structures such as lysosomes, leading to enzymatic degradation of the ingested material.

Phosphatase (protein phosphatase) An enzyme that removes phosphate groups from the side chains of certain amino acid residues of proteins. Protein phosphatases in lymphocytes, such as CD45 or calcineurin, regulate the activity of various signal transduction molecules and transcription factors. Some protein phosphatases may be specific for phosphotyrosine residues and others for phosphoserine and phosphothreonine residues.

Phospholipase Cγ (PLCγ) An enzyme that catalyzes hydrolysis of the plasma membrane phospholipid PIP2 to generate two signaling molecules, IP3 and DAG. PLCγ becomes activated in lymphocytes by antigen binding to the antigen receptor.

Phytohemagglutinin (PHA) A carbohydrate-binding protein, or lectin, produced by plants that cross-links human T cell surface molecules, including the T cell receptor, thereby inducing polyclonal activation and agglutination of T cells. PHA is frequently used in experimental immunology to study T cell activation. In clinical medicine, PHA is used to assess whether a patient's T cells are functional or to induce T cell mitosis for the purpose of generating karyotypic data.

Plasmablast Circulating antibody-secreting cells that may be precursors of the plasma cells that reside in the bone marrow and other tissues.

Plasma cell A terminally differentiated antibody-secreting B lymphocyte with a characteristic histologic appearance, including an oval shape, eccentric nucleus, and perinuclear halo.

Platelet-activating factor (PAF) A lipid mediator derived from membrane phospholipids in several cell types, including mast cells and endothelial cells. PAF can cause bronchoconstriction and vascular dilation and leak and may be an important mediator in asthma.

Polyclonal activators Agents that are capable of activating many clones of lymphocytes, regardless of their antigen specificities. Examples of polyclonal activators include anti-IgM antibodies for B cells and anti-CD3 antibodies, bacterial superantigens, and PHA for T cells.

Poly-Ig receptor An Fc receptor expressed by mucosal epithelial cells that mediates the transport of IgA and IgM through the epithelial cells into the intestinal lumen.

Polymerase chain reaction (PCR) A rapid method of copying and amplifying specific DNA sequences up to about 1 kb in length that is widely used as a preparative and analytical technique in all branches of molecular biology. The method relies on the use of short oligonucleotide primers complementary to the sequences at the ends of the DNA to be amplified and involves repetitive cycles of melting, annealing, and synthesis of DNA.

Polymorphism The existence of two or more alternative forms, or variants, of a gene that are present at stable frequencies in a population. Each common variant of a polymorphic gene is called an allele, and one individual may carry two different alleles of a gene, each inherited from a different parent. The MHC genes are the most polymorphic genes in the mammalian genome.

Polyvalency The presence of multiple identical copies of an epitope on a single antigen molecule, cell surface, or particle. Polyvalent antigens, such as bacterial capsular polysaccharides, are often capable of activating B lymphocytes independent of helper T cells. Used synonymously with **Multivalency**.

Positive selection The process by which developing T cells in the thymus (thymocytes) whose TCRs bind to self MHC molecules are rescued from programmed cell death, whereas thymocytes whose receptors do not recognize self MHC molecules die by default. Positive selection ensures that mature T cells are self MHC restricted and that $CD8^+$ T cells are specific for complexes of peptides with class I MHC molecules and $CD4^+$ T cells for complexes of peptides with class II MHC molecules.

Pre-B cell A developing B cell present only in hematopoietic tissues that is at a maturational stage characterized by expression of cytoplasmic Ig μ heavy chains and surrogate light chains but not Ig light chains. Pre-B cell receptors composed of μ chains and surrogate light chains deliver signals that stimulate further maturation of the pre-B cell into an immature B cell.

Pre-B cell receptor A receptor expressed on developing B lymphocytes at the pre-B cell stage that is

composed of an Ig μ heavy chain and an invariant surrogate light chain. The surrogate light chain is composed of two proteins, including the λ5 protein, which is homologous to the λ light chain C domain, and the V pre-B protein, which is homologous to a V domain. The pre-B cell receptor associates with the Igα and Igβ signal transduction proteins to form the pre-B cell receptor complex. Pre-B cell receptors are required for stimulating the proliferation and continued maturation of the developing B cell. It is not known whether the pre-B cell receptor binds a specific ligand.

Pre-T cell A developing T lymphocyte in the thymus at a maturational stage characterized by expression of the TCR β chain but not the α chain or CD4 or CD8. In pre-T cells, the TCR β chain is found on the cell surface as part of the pre-T cell receptor.

Pre-T cell receptor A receptor expressed on the surface of pre-T cells that is composed of the TCR β chain and an invariant pre-Tα protein. This receptor associates with CD3 and ζ molecules to form the pre-T cell receptor complex. The function of this complex is similar to that of the pre-B cell receptor in B cell development, namely, the delivery of signals that stimulate further proliferation, antigen receptor gene rearrangements, and other maturational events. It is not known whether the pre-T cell receptor binds a specific ligand.

Pre-Tα An invariant transmembrane protein with a single extracellular Ig-like domain that associates with the TCR β chain in pre-T cells to form the pre-T cell receptor.

Primary immune response An adaptive immune response that occurs after the first exposure of an individual to a foreign antigen. Primary responses are characterized by relatively slow kinetics and small magnitude compared with the responses after a second or subsequent exposure.

Primary immunodeficiency See **Congenital immunodeficiency.**

Pro-B cell A developing B cell in the bone marrow that is the earliest cell committed to the B lymphocyte lineage. Pro-B cells do not produce Ig, but they can be distinguished from other immature cells by the expression of B lineage–restricted surface molecules such as CD19 and CD10.

Pro-T cell A developing T cell in the thymic cortex that is a recent arrival from the bone marrow and does not express TCRs, CD3, ζ chains, or CD4 or CD8 molecules. Pro-T cells are also called double-negative thymocytes.

Professional antigen-presenting cells (professional APCs) A term sometimes used to refer to APCs that activate T lymphocytes; includes dendritic cells, mononuclear phagocytes, and B lymphocytes, all of which are capable of expressing class II MHC molecules and costimulators. The most important professional APCs for initiation of primary T cell responses are dendritic cells.

Programmed cell death A pathway of cell death by apoptosis that occurs in lymphocytes deprived of necessary survival stimuli, such as growth factors or costimulators. Programmed cell death is caused by the release of mitochondrial cytochrome *c* into the cytoplasm, activation of caspase-9, and initiation of the apoptotic pathway.

Promoter A DNA sequence immediately 5′ to the transcription start site of a gene where the proteins that initiate transcription bind. The term *promoter* is often used to mean the entire 5′ regulatory region of a gene, including enhancers, which are additional sequences that bind transcription factors and interact with the basal transcription complex to increase the rate of transcriptional initiation. Other enhancers may be located at a significant distance from the promoter, either 5′ of the gene, in introns, or 3′ of the gene.

Prostaglandins A class of lipid inflammatory mediators derived from arachidonic acid in many cell types through the cyclooxygenase pathway, which have vasodilator, bronchoconstrictor, and chemotactic activities. Prostaglandins made by mast cells are important mediators of allergic reactions.

Proteasome A large multiprotein enzyme complex with a broad range of proteolytic activity that is found in the cytoplasm of most cells and generates from cytosolic proteins the peptides that bind to class I MHC molecules. Proteins are targeted for proteasomal degradation by covalent linkage of ubiquitin molecules.

Protein kinase C (PKC) Any of several isoforms of an enzyme that mediates the phosphorylation of serine and threonine residues in many different protein substrates and thereby serves to propagate various signal transduction pathways leading to transcription factor activation. In T and B lymphocytes, PKC is activated by DAG, which is generated in response to antigen receptor ligation.

Protein tyrosine kinases (PTKs) Enzymes that mediate the phosphorylation of tyrosine residues in proteins and thereby promote phosphotyrosine-dependent protein-protein interactions. PTKs are involved in numerous signal transduction pathways in cells of the immune system.

Protozoa Single-celled eukaryotic organisms, many of which are human parasites and cause diseases. Examples of pathogenic protozoa include *Entamoeba histolytica,* which causes amebic dysentery; *Plasmodium,* which causes malaria; and *Leishmania,* which causes leishmaniasis. Protozoa stimulate both innate and adaptive immune responses. It has proved difficult to develop effective vaccines against many of these organisms.

Provirus A DNA copy of the genome of a retrovirus that is integrated into the host cell genome and from which viral genes are transcribed and the viral genome is reproduced. HIV proviruses can remain inactive for long periods and thereby represent a latent form of HIV infection that is not accessible to immune defense.

Purified antigen (subunit) vaccine A vaccine composed of purified antigens or subunits of microbes. Examples of this type of vaccine include diphtheria and tetanus toxoids, pneumococcus and *Haemophilus influenzae* polysaccharide vaccines, and purified polypeptide vaccines against hepatitis B and influenza virus. Purified antigen vaccines may stimulate antibody and helper T cell responses, but they typically do not generate CTL responses.

Pyogenic bacteria Bacteria, such as the gram-positive staphylococci and streptococci, that induce inflammatory responses rich in polymorphonuclear leukocytes (giving rise to pus). Antibody responses to these bacteria greatly enhance the efficacy of innate immune effector mechanisms to clear infections.

Rac A small guanine nucleotide–binding protein that is activated by the GDP-GTP exchange factor Vav during the early events of T cell activation. GTP·Rac triggers a three-step protein kinase cascade that culminates in activation of the stress-activated protein (SAP) kinase, c-Jun N-terminal kinase (JNK), and p38 kinase, which are similar to the MAP kinases.

Radioimmunoassay A highly sensitive and specific immunologic method of quantifying the concentration of an antigen in a solution that relies on a radioactively labeled antibody specific for the antigen. Usually, two antibodies specific for the antigen are used. The first antibody is unlabeled but attached to a solid support, where it binds and immobilizes the antigen whose concentration is being determined. The amount of the second, labeled antibody that binds to the immobilized antigen, as determined by radioactive decay detectors, is proportional to the concentration of antigen in the test solution.

Rapamycin An immunosuppressive drug (also called sirolimus) used clinically to prevent allograft rejection. Rapamycin inhibits the activation of a protein called molecular target of rapamycin (mTOR), which is a key signaling molecule in a variety of metabolic and cell growth pathways including the pathway required for interleukin-2–mediated T cell proliferation.

Ras A member of a family of 21-kD guanine nucleotide–binding proteins with intrinsic GTPase activity that are involved in many different signal transduction pathways in diverse cell types. Mutated *ras* genes are associated with neoplastic transformation. In T cell activation, Ras is recruited to the plasma membrane by tyrosine-phosphorylated adaptor proteins, where it is activated by GDP-GTP exchange factors. GTP·Ras then initiates the MAP kinase cascade, which leads to expression of the *fos* gene and assembly of the AP-1 transcription factor.

Reactive oxygen species (ROS) Highly reactive metabolites of oxygen, including superoxide anion, hydroxyl radical, and hydrogen peroxide, that are produced by activated phagocytes. Reactive oxygen species are used by the phagocytes to form oxyhalides that damage ingested bacteria. They may also be released from cells and promote inflammatory responses or cause tissue damage.

Reagin IgE antibody that mediates an immediate hypersensitivity reaction.

Receptor editing A process by which some immature B cells that recognize self antigens in the bone marrow may be induced to change their Ig specificities. Receptor editing involves reactivation of the *RAG* genes, additional light chain VJ recombinations, and new Ig light chain production, which allows the cell to express a different Ig receptor that is not self-reactive.

Recombination-activating genes 1 and 2 (*RAG1* and *RAG2*) The genes encoding RAG-1 and RAG-2 proteins, which make up the V(D)J recombinase and are expressed in developing B and T cells. RAG proteins bind to recombination signal sequences and are critical for DNA recombination events that form functional Ig and TCR genes. Therefore, RAG proteins are required for expression of antigen receptors and for the maturation of B and T lymphocytes.

Recombination signal sequences Specific DNA sequences found adjacent to the V, D, and J segments in the antigen receptor loci and recognized by the RAG-1/RAG-2 complex during V(D)J recombination. The recognition sequences consist of a highly conserved stretch of 7 nucleotides, called the heptamer, located adjacent to the V, D, or J coding sequence, followed by a spacer of exactly 12 or 23 nonconserved nucleotides and a highly conserved stretch of 9 nucleotides, called the nonamer.

Red pulp An anatomic and functional compartment of the spleen composed of vascular sinusoids, scattered among which are large numbers of erythrocytes, macrophages, dendritic cells, sparse lymphocytes, and plasma cells. Red pulp macrophages clear the blood of microbes, other foreign particles, and damaged red blood cells.

Regulatory T cells A population of T cells that regulates the activation of other T cells and is necessary to maintain peripheral tolerance to self antigens. Most regulatory T cells are $CD4^+$ and constitutively express CD25, the α chain of the IL-2 receptor, and the transcription factor FoxP3.

Respiratory burst The process by which reactive oxygen intermediates such as superoxide anion, hydroxyl radical, and hydrogen peroxide are produced in macrophages and polymorphonuclear leukocytes. The respiratory burst is mediated by the enzyme phagocyte oxidase and is usually triggered by inflammatory mediators, such as LTB_4, PAF, and TNF, or by bacterial products, such as *N*-formylmethionyl peptides.

Reverse transcriptase An enzyme encoded by retroviruses, such as HIV, that synthesizes a DNA copy of the viral genome from the RNA genomic template. Purified reverse transcriptase is used widely in molecular biology research for purposes of cloning complementary DNAs encoding a gene of interest from messenger RNA. Reverse transcriptase inhibitors are used as drugs to treat HIV-1 infection.

Rh blood group antigens A complex system of protein alloantigens expressed on red blood cell membranes that are the cause of transfusion reactions and hemolytic disease of the newborn. The most clinically important Rh antigen is designated D.

Rheumatoid arthritis An autoimmune disease characterized primarily by inflammatory damage to joints and sometimes inflammation of blood vessels, lungs, and other tissues. $CD4^+$ T cells, activated B lymphocytes, and plasma cells are found in the inflamed joint lining (synovium), and numerous proinflammatory cytokines, including IL-1 and TNF, are present in the synovial (joint) fluid.

RIG-like receptors (RLRs) Cytosolic receptors of the innate immune system that recognize viral RNA and

induce production of type I interferons. The two best characterized RLRs are RIG-I (retinoic acid–inducible gene I) and MDA5 (melanoma differentiation-associated gene 5).

RORγT (retinoid-related orphan receptor γ T) A transcription factor expressed in and required for differentiation of T_H17 cells and lymphoid tissue inducer (LTi) cells.

Scavenger receptors A family of cell surface receptors expressed on macrophages, originally defined as receptors that mediate endocytosis of oxidized or acetylated low-density lipoprotein particles but that also bind and mediate the phagocytosis of a variety of microbes.

SCID mouse A mouse strain in which B and T cells are absent because of an early block in maturation from bone marrow precursors. SCID mice carry a mutation in a component of the enzyme DNA-dependent protein kinase, which is required for double-stranded DNA break repair. Deficiency of this enzyme results in abnormal joining of Ig and TCR gene segments during recombination and therefore failure to express antigen receptors.

Secondary immune response An adaptive immune response that occurs on second exposure to an antigen. A secondary response is characterized by more rapid kinetics and greater magnitude relative to the primary immune response, which occurs on first exposure.

Secondary immunodeficiency. See **Acquired immunodeficiency.**

Second-set rejection Allograft rejection in an individual who has previously been sensitized to the donor's tissue alloantigens by having received another graft or transfusion from that donor. In contrast to first-set rejection, which occurs in an individual who has not previously been sensitized to the donor alloantigens, second-set rejection is rapid and occurs in 3 to 7 days as a result of immunologic memory.

Secretory component The proteolytically cleaved portion of the extracellular domain of the poly-Ig receptor that remains bound to an IgA molecule in mucosal secretions.

Selectin Any one of three separate but closely related carbohydrate-binding proteins that mediate adhesion of leukocytes to endothelial cells. Each of the selectin molecules is a single-chain transmembrane glycoprotein with a similar modular structure, including an extracellular calcium-dependent lectin domain. The selectins include L-selectin (CD62L), expressed on leukocytes; P-selectin (CD62P), expressed on platelets and activated endothelium; and E-selectin (CD62E), expressed on activated endothelium.

Selective immunoglobulin deficiency Immunodeficiencies characterized by a lack of only one or a few Ig classes or subclasses. IgA deficiency is the most common selective Ig deficiency, followed by IgG3 and IgG2 deficiencies. Patients with these disorders may be at increased risk for bacterial infections, but many are normal.

Self MHC restriction The limitation (or restriction) of T cells to recognize antigens displayed by MHC molecules that the T cell encountered during maturation in the thymus (and thus sees as self).

Self-tolerance Unresponsiveness of the adaptive immune system to self antigens, largely as a result of inactivation or death of self-reactive lymphocytes induced by exposure to these antigens. Self-tolerance is a cardinal feature of the normal immune system, and failure of self-tolerance leads to autoimmune diseases.

Septic shock A severe complication of bacterial infections that spread to the blood stream (sepsis), and is characterized by vascular collapse, disseminated intravascular coagulation, and metabolic disturbances. This syndrome is due to the effects of bacterial cell wall components, such as LPS or peptidoglycan, which bind to TLRs on various cell types and induce expression of inflammatory cytokines, including TNF and IL-12.

Seroconversion The production of detectable antibodies in the serum specific for a microorganism during the course of an infection or in response to immunization.

Serology The study of blood (serum) antibodies and their reactions with antigens. The term *serology* is often used to refer to the diagnosis of infectious diseases by detection of microbe-specific antibodies in the serum.

Serotype An antigenically distinct subset of a species of an infectious organism that is distinguished from other subsets by serologic (i.e., serum antibody) tests. Humoral immune responses to one serotype of microbes (e.g., influenza virus) may not be protective against another serotype.

Serum The cell-free fluid that remains when blood or plasma forms a clot. Blood antibodies are found in the serum fraction.

Serum amyloid A (SAA) An acute-phase protein whose serum concentration rises significantly in the setting of infection and inflammation, mainly because of IL-1– and TNF-induced synthesis by the liver. SAA activates leukocyte chemotaxis, phagocytosis, and adhesion to endothelial cells.

Serum sickness A disease caused by the injection of large doses of a protein antigen into the blood and characterized by the deposition of antigen-antibody (immune) complexes in blood vessel walls, especially in the kidneys and joints. Immune complex deposition leads to complement fixation and leukocyte recruitment and subsequently to glomerulonephritis and arthritis. Serum sickness was originally described as a disorder that occurred in patients receiving injections of serum containing antitoxin antibodies to prevent diphtheria.

Severe combined immunodeficiency (SCID) Immunodeficiency diseases in which both B and T lymphocytes do not develop or do not function properly, and therefore both humoral immunity and cell-mediated immunity are impaired. Children with SCID usually have infections during the first year of life and succumb to these infections unless the immunodeficiency is treated. SCID has several different genetic causes.

Shwartzman reaction An experimental model of the pathologic effects of bacterial LPS and TNF in which two intravenous injections of LPS are administered to a rabbit 24 hours apart. After the second injection, the

rabbit suffers disseminated intravascular coagulation and neutrophil and platelet plugging of small blood vessels.

Signal transducer and activator of transcription (STAT) A member of a family of proteins that function as signaling molecules and transcription factors in response to binding of cytokines to type I and type II cytokine receptors. STATs are present as inactive monomers in the cytoplasm of cells and are recruited to the cytoplasmic tails of cross-linked cytokine receptors, where they are tyrosine phosphorylated by JAKs. The phosphorylated STAT proteins dimerize and move to the nucleus, where they bind to specific sequences in the promoter regions of various genes and stimulate their transcription. Different STATs are activated by different cytokines.

Simian immunodeficiency virus A lentivirus closely related to HIV-1 that causes disease similar to AIDS in monkeys.

Single-positive thymocyte A maturing T cell precursor in the thymus that expresses CD4 or CD8 molecules but not both. Single-positive thymocytes are found mainly in the medulla and have matured from the double-positive stage, during which thymocytes express both CD4 and CD8 molecules.

Smallpox A disease caused by variola virus. Smallpox was the first infectious disease shown to be preventable by vaccination and the first disease to be completely eradicated by a worldwide vaccination program.

Somatic hypermutation High-frequency point mutations in Ig heavy and light chains that occur in germinal center B cells. Mutations that result in increased affinity of antibodies for antigen impart a selective survival advantage to the B cells producing those antibodies and lead to affinity maturation of a humoral immune response.

Somatic recombination The process of DNA recombination by which the functional genes encoding the variable regions of antigen receptors are formed during lymphocyte development. A relatively limited set of inherited, or germline, DNA sequences that are initially separated from one another are brought together by enzymatic deletion of intervening sequences and re-ligation. This process occurs only in developing B or T lymphocytes. This process is also called somatic rearrangement.

Specificity A cardinal feature of the adaptive immune system, namely, that immune responses are directed toward and able to distinguish between distinct antigens or small parts of macromolecular antigens. This fine specificity is attributed to lymphocyte antigen receptors that may bind to one molecule but not to another, even closely related, molecule.

Spleen A secondary lymphoid organ in the left upper quadrant of the abdomen. The spleen is the major site of adaptive immune responses to blood-borne antigens. The red pulp of the spleen is composed of blood-filled vascular sinusoids lined by active phagocytes that ingest opsonized antigens and damaged red blood cells. The white pulp of the spleen contains lymphocytes and lymphoid follicles where B cells are activated.

Src homology 2 (SH2) domain A three-dimensional domain structure of about 100 amino acid residues present in many signaling proteins that permits specific noncovalent interactions with other proteins by binding to phosphotyrosines. Each SH2 domain has a unique binding specificity that is determined by the amino acid residues adjacent to the phosphotyrosine on the target protein. Several proteins involved in early signaling events in T and B lymphocytes interact with one another through SH2 domains.

Src homology 3 (SH3) domain A three-dimensional domain structure of about 60 amino acid residues present in many signaling proteins that mediates protein-protein binding. SH3 domains bind to proline residues and function cooperatively with the SH2 domains of the same protein. For instance, SOS, the guanine nucleotide exchange factor for Ras, contains both SH2 and SH3 domains, and both are involved in SOS binding to the adaptor protein Grb-2.

Stem cell An undifferentiated cell that divides continuously and gives rise to additional stem cells and to cells of multiple different lineages. For example, all blood cells arise from a common hematopoietic stem cell.

Superantigens Proteins that bind to and activate all the T cells in an individual that express a particular set or family of V_β TCR genes. Superantigens are presented to T cells by binding to nonpolymorphic regions of class II MHC molecules on APCs, and they interact with conserved regions of TCR V_β domains. Several staphylococcal enterotoxins are superantigens. Their importance lies in their ability to activate many T cells, which results in large amounts of cytokine production and a clinical syndrome that is similar to septic shock.

Suppressor T cells T cells that block the activation and function of other T lymphocytes. It has been difficult to clearly identify suppressor T cells, and the term is not widely used now. The much better defined T cells that function to control immune responses are **regulatory T cells**.

Surrogate light chain A complex of two nonvariable proteins that associate with Ig μ heavy chains in pre-B cells to form the pre-B cell receptor. The two surrogate light chain proteins include V pre-B protein, which is homologous to a light chain V domain, and λ5, which is covalently attached to the μ heavy chain by a disulfide bond.

Switch recombination The molecular mechanism underlying Ig isotype switching in which a rearranged VDJ gene segment in an antibody-producing B cell recombines with a downstream C gene and the intervening C gene or genes are deleted. DNA recombination events in switch recombination are triggered by CD40 and cytokines and involve nucleotide sequences called switch regions located in the introns at the 5′ end of each C_H locus.

Syk A cytoplasmic protein tyrosine kinase, similar to ZAP-70 in T cells, that is critical for early signaling steps in antigen-induced B cell activation. Syk binds to phosphorylated tyrosines in the cytoplasmic tails of the Igα and Igβ chains of the BCR complex and in turn phosphorylates adaptor proteins that recruit other components of the signaling cascade.

Syngeneic Genetically identical. All animals of an inbred strain and monozygotic twins are syngeneic.

Syngeneic graft A graft from a donor who is genetically identical to the recipient. Syngeneic grafts are not rejected.

Synthetic vaccine Vaccines composed of recombinant DNA–derived antigens. Synthetic vaccines for hepatitis B virus and herpes simplex virus are now in use.

Systemic inflammatory response syndrome (SIRS) The systemic changes observed in patients who have disseminated bacterial infections. In its mild form, SIRS consists of neutrophilia, fever, and a rise in acute-phase reactants in the plasma. These changes are stimulated by bacterial products such as LPS and are mediated by cytokines of the innate immune system. In severe cases, SIRS may include disseminated intravascular coagulation, adult respiratory distress syndrome, and septic shock.

Systemic lupus erythematosus (SLE) A chronic systemic autoimmune disease that affects predominantly women and is characterized by rashes, arthritis, glomerulonephritis, hemolytic anemia, thrombocytopenia, and central nervous system involvement. Many different autoantibodies are found in patients with SLE, particularly anti-DNA antibodies. Many of the manifestations of SLE are due to the formation of immune complexes composed of autoantibodies and their specific antigens, with deposition of these complexes in small blood vessels in various tissues. The underlying mechanism for the breakdown of self-tolerance in SLE is not understood.

T cell receptor (TCR) The clonally distributed antigen receptor on $CD4^+$ and $CD8^+$ T lymphocytes that recognizes complexes of foreign peptides bound to self MHC molecules on the surface of APCs. The most common form of TCR is composed of a heterodimer of two disulfide-linked transmembrane polypeptide chains, designated α and β, each containing one N-terminal Ig-like variable (V) domain, one Ig-like constant (C) domain, a hydrophobic transmembrane region, and a short cytoplasmic region. (Another less common type of TCR, composed of γ and δ chains, is found on a small subset of T cells and recognizes different forms of antigen.)

T cell receptor (TCR) transgenic mouse A mouse in a genetically engineered strain that expresses transgenically encoded functional TCR α and β genes encoding a TCR of a single defined specificity. Because of allelic exclusion of endogenous TCR genes, most or all of the T cells in a TCR transgenic mouse have the same antigen specificity, which is a useful property for various research purposes.

T follicular helper (T_{FH}) cells A heterogeneous subset of $CD4^+$ helper T cells present within lymphoid follicles that are critical in providing signals to B cells in the germinal center reaction. T_{FH} cells express CXCR5, ICOS, IL-21, and Bcl-6.

T lymphocyte The key component of cell-mediated immune responses in the adaptive immune system. T lymphocytes mature in the thymus, circulate in the blood, populate secondary lymphoid tissues, and are recruited to peripheral sites of antigen exposure. They express antigen receptors (TCRs) that recognize peptide fragments of foreign proteins bound to self MHC molecules. Functional subsets of T lymphocytes include $CD4^+$ helper T cells and $CD8^+$ CTLs.

T-bet A T-box family transcription factor that promotes the differentiation of T_H1 cells from naive T cells.

T-dependent antigen An antigen that requires both B cells and helper T cells to stimulate an antibody response. T-dependent antigens are protein antigens that contain some epitopes recognized by T cells and other epitopes recognized by B cells. Helper T cells produce cytokines and cell surface molecules that stimulate B cell growth and differentiation into antibody-secreting cells. Humoral immune responses to T-dependent antigens are characterized by isotype switching, affinity maturation, and memory.

Tertiary lymphoid organ A collection of lymphocytes and antigen-presenting cells organized into B cell follicles and T cell zones that develop in sites of chronic immune-mediated inflammation, such as the joint synovium of rheumatoid arthritis patients.

T-independent antigen Nonprotein antigens, such as polysaccharides and lipids, which can stimulate antibody responses without a requirement for antigen-specific helper T lymphocytes. T-independent antigens usually contain multiple identical epitopes that can cross-link membrane Ig on B cells and thereby activate the cells. Humoral immune responses to T-independent antigens show relatively little heavy chain isotype switching or affinity maturation, two processes that require signals from helper T cells.

T_H1 cells A subset of $CD4^+$ helper T cells that secrete a particular set of cytokines, including IFN-γ, and whose principal function is to stimulate phagocyte-mediated defense against infections, especially with intracellular microbes.

T_H2 cells A functional subset of $CD4^+$ helper T cells that secrete a particular set of cytokines, including IL-4, IL-5, and IL-3 and whose principal function is to stimulate IgE and eosinophil/mast cell–mediated immune reactions.

T_H17 cells A functional subset of $CD4^+$ helper T cells that secrete a particular set of inflammatory cytokines, including IL-17, which are protective against bacterial and fungal infections and also mediate inflammatory reactions in autoimmune and other inflammatory diseases.

Thymic epithelial cells Epithelial cells abundant in the cortical and medullary stroma of the thymus that play a critical role in T cell development. In the process of positive selection, maturing T cells that weakly recognize self peptides bound to MHC molecules on the surface of thymic epithelial cells are rescued from programmed cell death.

Thymocyte A precursor of a mature T lymphocyte present in the thymus.

Thymus A bilobed organ situated in the anterior mediastinum that is the site of maturation of T lymphocytes from bone marrow–derived precursors. Thymic tissue is divided into an outer cortex and an inner medulla and contains stromal thymic epithelial cells, macrophages, dendritic cells, and numerous

T cell precursors (thymocytes) at various stages of maturation.

Tissue typing The determination of the particular MHC alleles expressed by an individual for the purpose of matching allograft donors and recipients. Tissue typing, also called HLA typing, is usually done by molecular (PCR-based) sequencing of HLA alleles, or by serologic methods (lysis of individual's cells by panels of anti-HLA antibodies).

TNF receptor–associated factors (TRAFs) A family of adaptor molecules that interact with the cytoplasmic domains of various receptors in the TNF receptor family, including TNF-RII, lymphotoxin (LT)-β receptor, and CD40. Each of these receptors contains a cytoplasmic motif that binds different TRAFs, which in turn engage other signaling molecules leading to activation of the transcription factors AP-1 and NF-κB.

Tolerance Unresponsiveness of the adaptive immune system to antigens, as a result of inactivation or death of antigen-specific lymphocytes, induced by exposure to the antigens. Tolerance to self antigens is a normal feature of the adaptive immune system, but tolerance to foreign antigens may be induced under certain conditions of antigen exposure.

Tolerogen An antigen that induces immunologic tolerance, in contrast to an immunogen, which induces an immune response. Many antigens can be either tolerogens or immunogens, depending on how they are administered. Tolerogenic forms of antigens include large doses of the proteins administered without adjuvants and orally administered antigens.

Toll-like receptors A family of pattern recognition receptors of the innate immune system, expressed on the surface and in endosomes of many cell types, that recognize microbial structures such as endotoxin and viral RNA and transduce signals that lead to the expression of inflammatory and antiviral genes.

Toxic shock syndrome An acute illness characterized by shock, skin exfoliation, conjunctivitis, and diarrhea that is associated with tampon use and caused by a *Staphylococcus aureus* superantigen.

Transfusion Transplantation of circulating blood cells, platelets, or plasma from one individual to another. Transfusions are performed to treat blood loss from hemorrhage or to treat a deficiency in one or more blood cell types resulting from inadequate production or excess destruction.

Transfusion reactions An immunologic reaction against transfused blood products, usually mediated by preformed antibodies in the recipient that bind to donor blood cell antigens, such as ABO blood group antigens or histocompatibility antigens. Transfusion reactions can lead to intravascular lysis of red blood cells and, in severe cases, kidney damage, fever, shock, and disseminated intravascular coagulation.

Transgenic mouse A mouse that expresses an exogenous gene that has been introduced into the genome by injection of a specific DNA sequence into the pronuclei of fertilized mouse eggs. Transgenes insert randomly at chromosomal break points and are subsequently inherited as simple mendelian traits. By the design of transgenes with tissue-specific regulatory sequences, mice can be produced that express a particular gene only in certain tissues. Transgenic mice are used extensively in immunology research to study the functions of various cytokines, cell surface molecules, and intracellular signaling molecules.

Transplantation The process of transferring cells, tissues, or organs (i.e., grafts) from one individual to another or from one site to another in the same individual. Transplantation is used to treat a variety of diseases in which there is a functional disorder of a tissue or organ. The major barrier to successful transplantation between individuals is immunologic reaction (rejection) to the transplanted graft.

Transporter associated with antigen processing (TAP) An adenosine triphosphate (ATP)-dependent peptide transporter that mediates the active transport of peptides from the cytosol to the site of assembly of class I MHC molecules inside the endoplasmic reticulum. TAP is a heterodimeric molecule composed of TAP-1 and TAP-2 polypeptides, both encoded by genes in the MHC. Because peptides are required for stable assembly of class I MHC molecules, TAP-deficient animals express few cell surface class I MHC molecules, which results in diminished development and activation of $CD8^+$ T cells.

Tumor immunity Protection against the development of tumors by the immune system. Although immune responses to naturally occurring tumors can frequently be demonstrated, true immunity may occur only in the case of a subset of these tumors that express immunogenic antigens (e.g., tumors that are caused by oncogenic viruses and therefore express viral antigens). Research efforts are under way to enhance weak immune responses to other tumors by a variety of approaches.

Tumor-infiltrating lymphocytes (TILs) Lymphocytes isolated from the inflammatory infiltrates present in and around surgical resection samples of solid tumors that are enriched with tumor-specific CTLs and NK cells. In an experimental mode of cancer treatment, TILs are grown in vitro in the presence of high doses of IL-2 and are then adoptively transferred back into patients with the tumor.

Tumor necrosis factor receptor superfamily (TNFRSF) A large family of structurally homologous transmembrane proteins that bind TNFSF proteins and generate signals that regulate proliferation, differentiation, apoptosis, and inflammatory gene expression. (See Appendix II.)

Tumor necrosis factor superfamily (TNFSF) A large family of structurally homologous transmembrane proteins that regulate diverse functions in responding cells, including proliferation, differentiation, apoptosis, and inflammatory gene expression. TNFSF members typically form homotrimers, either within the plasma membrane or after proteolytic release from the membrane, and bind to homotrimeric TNF receptor superfamily (TNFRSF) molecules, which then initiate a variety of signaling pathways. (See Appendix II.)

Tumor-specific antigen An antigen whose expression is restricted to a particular tumor and is not expressed

by normal cells. Tumor-specific antigens may serve as target antigens for anti-tumor immune responses.

Tumor-specific transplantation antigen (TSTA) An antigen expressed on experimental animal tumor cells that can be detected by induction of immunologic rejection of tumor transplants. TSTAs were originally defined on chemically induced rodent sarcomas and shown to stimulate CTL-mediated rejection of transplanted tumors.

Two-signal hypothesis A now proven hypothesis that states that the activation of lymphocytes requires two distinct signals, the first being antigen and the second either microbial products or components of innate immune responses to microbes. The requirement for antigen (so-called signal 1) ensures that the ensuing immune response is specific. The requirement for additional stimuli triggered by microbes or innate immune reactions (signal 2) ensures that immune responses are induced when they are needed, that is, against microbes and other noxious substances and not against harmless substances, including self antigens. Signal 2 is referred to as costimulation and is often mediated by membrane molecules on professional APCs, such as B7 proteins.

Type 1 diabetes mellitus A disease characterized by a lack of insulin that leads to various metabolic and vascular abnormalities. The insulin deficiency results from autoimmune destruction of the insulin-producing β cells of the islets of Langerhans in the pancreas, usually during childhood. $CD4^+$ and $CD8^+$ T cells, antibodies, and cytokines have been implicated in the islet cell damage. Also called insulin-dependent diabetes mellitus.

Ubiquitination Covalent linkage of one or several copies of a small polypeptide called ubiquitin to a protein. Ubiquitination frequently serves to target proteins for proteolytic degradation by proteasomes, a critical step in the class I MHC pathway of antigen processing and presentation.

Urticaria Localized transient swelling and redness of the skin caused by leakage of fluid and plasma proteins from small vessels into the dermis during an immediate hypersensitivity reaction.

V gene segments A DNA sequence that encodes the variable domain of an Ig heavy chain or light chain or a TCR α, β, γ, or δ chain. Each antigen receptor locus contains many different V gene segments, any one of which may recombine with downstream D or J segments during lymphocyte maturation to form functional antigen receptor genes.

V(D)J recombinase The complex of RAG1 and RAG2 proteins that catalyzes lymphocyte antigen receptor gene recombination.

Vaccine A preparation of microbial antigen, often combined with adjuvants, which is administered to individuals to induce protective immunity against microbial infections. The antigen may be in the form of live but avirulent microorganisms, killed microorganisms, purified macromolecular components of a microorganism, or a plasmid that contains a complementary DNA encoding a microbial antigen.

Variable region The extracellular, N-terminal region of an Ig heavy or light chain or a TCR α, β, γ, or δ chain that contains variable amino acid sequences that differ between every clone of lymphocytes and that are responsible for the specificity for antigen. The antigen-binding variable sequences are localized to extended loop structures or hypervariable segments.

Virus A primitive obligate intracellular parasitic organism or infectious particle that consists of a simple nucleic acid genome packaged in a protein capsid, sometimes surrounded by a membrane envelope. Many pathogenic animal viruses cause a wide range of diseases. Humoral immune responses to viruses can be effective in blocking infection of cells, and NK cells and CTLs are necessary to kill cells already infected.

Western blot An immunologic technique to determine the presence of a protein in a biologic sample. The method involves separation of proteins in the sample by electrophoresis, transfer of the protein array from the electrophoresis gel to a support membrane by capillary action (blotting), and finally detection of the protein by binding of an enzymatically or radioactively labeled antibody specific for that protein.

Wheal and flare reaction Local swelling and redness in the skin at a site of an immediate hypersensitivity reaction. The wheal reflects increased vascular permeability, and the flare results from increased local blood flow, both changes resulting from mediators such as histamine released from activated dermal mast cells.

White pulp The part of the spleen that is composed predominantly of lymphocytes, arranged in periarteriolar lymphoid sheaths, and follicles and other leukocytes. The remainder of the spleen contains sinusoids lined with phagocytic cells and filled with blood, called the **red pulp.**

Wiskott-Aldrich syndrome An X-linked disease characterized by eczema, thrombocytopenia (reduced blood platelets), and immunodeficiency manifested as susceptibility to bacterial infections. The defective gene encodes a cytosolic protein involved in signaling cascades and regulation of the actin cytoskeleton.

XBP-1 A transcription factor that is required for plasma cell development.

Xenoantigen An antigen on a graft from another species.

Xenograft (xenogeneic graft) An organ or tissue graft derived from a species different from the recipient. Transplantation of xenogeneic grafts (e.g., from a pig) to humans is not yet practical because of special problems related to immunologic rejection.

Xenoreactive Describing a T cell or antibody that recognizes and responds to an antigen on a graft from another species (a xenoantigen). The T cell may recognize an intact xenogeneic MHC molecule or a peptide derived from a xenogeneic protein bound to a self MHC molecule.

X-linked agammaglobulinemia An immunodeficiency disease, also called Bruton's agammaglobulinemia, characterized by a block in early B cell maturation and absence of serum Ig. Patients suffer from pyogenic bacterial infections. The disease is caused by mutations

or deletions in the gene encoding Btk, an enzyme involved in signal transduction in developing B cells.

X-linked hyper-IgM syndrome A rare immunodeficiency disease caused by mutations in the CD40 ligand gene and characterized by failure of B cell heavy chain isotype switching and cell-mediated immunity. Patients suffer from both pyogenic bacterial and protozoal infections.

ζ Chain A transmembrane protein expressed in T cells as part of the TCR complex that contains ITAMs in its cytoplasmic tail and binds the ZAP-70 protein tyrosine kinase during T cell activation.

Zeta-associated protein of 70 kD (ZAP-70) A cytoplasmic protein tyrosine kinase, similar to Syk in B cells, that is critical for early signaling steps in antigen-induced T cell activation. ZAP-70 binds to phosphorylated tyrosines in the cytoplasmic tails of the ζ chain and CD3 chains of the TCR complex and in turn phosphorylates adaptor proteins that recruit other components of the signaling cascade.

Appendix

II

CYTOKINES

Cytokine and Subunits	Principal Cell Source	Cytokine Receptor and Subunits*	Principal Cellular Targets and Biologic Effects
Type I cytokine family members			
Interleukin-2 (IL-2)	T cells	CD25 (IL-2Rα) CD122 (IL-2Rβ) CD132 (γ_c)	T cells: proliferation and differentiation into effector and memory cells; promotes regulatory T cell development, survival, and function NK cells: proliferation, activation B cells: proliferation, antibody synthesis (in vitro)
Interleukin-3 (IL-3)	T cells	CD123 (IL-3Rα) CD131 (β_c)	Immature hematopoietic progenitors: induced maturation of all hematopoietic lineages
Interleukin-4 (IL-4)	$CD4^+$ T cells (T_H2), mast cells	CD124 (IL-4Rα) CD132 (γ_c)	B cells: isotype switching to IgE T cells: T_H2 differentiation, proliferation Macrophages: alternative activation and inhibition of IFN-γ–mediated classical activation Mast cells: proliferation (in vitro)
Interleukin-5 (IL-5)	$CD4^+$ T cells (T_H2)	CD125 (IL-5Rα) CD131 (β_c)	Eosinophils: activation, increased generation B cells: proliferation, IgA production (in vitro)
Interleukin-6 (IL-6)	Macrophages, endothelial cells, T cells	CD126 (IL-6Rα) CD130 (gp130)	Liver: synthesis of acute-phase protein B cells: proliferation of antibody-producing cells
Interleukin-7 (IL-7)	Fibroblasts, bone marrow stromal cells	CD127 (IL-7R) CD132 (γ_c)	Immature lymphoid progenitors: proliferation of early T and B cell progenitors T lymphocytes: survival of naive and memory cells
Interleukin-9 (IL-9)	$CD4^+$ T cells	CD129 (IL-9R) CD132 (γ_c)	Mast cells, B cells, T cells, and tissue cells: survival and activation
Interleukin-11 (IL-11)		IL-11Rα CD130 (gp130)	Production of platelets
Interleukin-12 (IL-12) IL-12A (p35) IL-12B (p40)	Macrophages, dendritic cells	CD212 (IL-12Rβ1) IL-12Rβ2	T cells: T_H1 differentiation NK cells and T cells: IFN-γ synthesis, increased cytotoxic activity
Interleukin-13 (IL-13)	$CD4^+$ T cells (T_H2), NKT cells, mast cells	CD213a1 (IL-13Rα1) CD213a1 (IL-13Rα2) CD132 (γ_c)	B cells: isotype switching to IgE Epithelial cells: increased mucus production Fibroblasts: increased collagen synthesis Macrophages: alternative activation
Interleukin-15 (IL-15)	Macrophages, others	IL-15Rα CD122 (IL-2Rβ) CD132 (γ_c)	NK cells: proliferation T cells: survival and proliferation of memory $CD8^+$ cells
Interleukin-16 (IL-16)	T cells, mast cells, eosinophils, epithelial cells	CD4	$CD4^+$ T cells, monocytes, and eosinophils: chemoattractant

Continued

Cytokine and Subunits	Principal Cell Source	Cytokine Receptor and Subunits*	Principal Cellular Targets and Biologic Effects
Interleukin-17A (IL-17A) Interleukin-17F (IL-17F)	T cells	CD217 (IL-17RA)	Endothelial cells: increased chemokine production Macrophages: increased chemokine and cytokine production Epithelial cells: GM-CSF and G-CSF production
Interleukin-21 (IL-21)	T_H2 cells, T_H17 cells, T_{FH} cells	IL-21R CD132 (γ_c)	B cells: activation, proliferation, differentiation T_{FH} cells: development T_H17 cells: increased generation? NK cells: functional maturation
Interleukin-23 (IL-23) Heterodimer: IL-23A (p19), IL-12B (p40)	Macrophages, dendritic cells	IL-23R CD212 (IL-12Rβ1)	T cells: maintenance of IL-17–producing T cells
Interleukin-25 (IL-25; IL-17E)	T cells, mast cells, eosinophils, macrophages, mucosal epithelial cells	IL-17BR	T cells and various other cell types: expression of IL-4, IL-5, IL-13
Interleukin-27 (IL-27) IL-27 p28 EBI3 (IL-27B)	Macrophages , dendritic cells	IL-27Rα CD130 (gp130)	T cells: inhibition of T_H1 cells NK cells: IFN-γ synthesis?
Interleukin-31 (IL-31)	T_H2 cells	IL-31RA OSMR CD130 (gp130)	Not established
Stem cell factor (c-Kit ligand)	Bone marrow stromal cells	CD117 (KIT)	Pluripotent hematopoietic stem cells: induced maturation of all hematopoietic lineages
Granulocyte-monocyte CSF (GM-CSF)	T cells, macrophages, endothelial cells, fibroblasts	CD116 (GM-CSFRα) CD131 (β_c)	Immature and committed progenitors, mature macrophages: induced maturation of granulocytes and monocytes, macrophage activation
Monocyte CSF (M-CSF, CSF1)	Macrophages, endothelial cells, bone marrow cells, fibroblasts	CD115 (CSF1R)	Committed hematopoietic progenitors: induced maturation of monocytes
Granulocyte CSF (G-CSF, CSF3)	Macrophages, fibroblasts, endothelial cells	CD114 (CSF3R)	Committed hematopoietic progenitors: induced maturation of granulocytes
Type II cytokine family members			
IFN-α (multiple proteins)	Plasmacytoid dendritic cells, macrophages	IFNAR1 CD118 (IFNAR2)	All cells: antiviral state, increased class I MHC expression NK cells: activation
IFN-β	Fibroblasts, plasmacytoid dendritic cells	IFNAR1 CD118 (IFNAR2)	All cells: antiviral state, increased class I MHC expression NK cells: activation
Interferon-γ (IFN-γ)	T cells (T_H1, $CD8^+$ T cells), NK cells	CD119 (IFNGR1) IFNGR2	Macrophages: classical activation (increased microbicidal functions) B cells: isotype switching to opsonizing and complement-fixing IgG subclasses (established in mice) T cells: T_H1 differentiation Various cells: increased expression of class I and class II MHC molecules, increased antigen processing and presentation to T cells
Interleukin-10 (IL-10)	Macrophages, T cells (mainly regulatory T cells)	CD210 (IL-10R1) CD210B (IL-10R2)	Macrophages, dendritic cells: inhibition of expression of IL-12, costimulators, and class II MHC
Interleukin-19 (IL-19)	Macrophages	IL-20R1 CD210B (IL-10R2)	Macrophages: stimulates IL-1 and TNF secretion Keratinocytes: proliferation
Interleukin-20 (IL-20)	Keratinocytes, monocytes	IL-20R1 IL-20R2 *or* IL-22R1 CD210B (IL-10R2)	Keratinocytes: proliferation and differentiation Hematopoietic stem cells: proliferation

Cytokine and Subunits	Principal Cell Source	Cytokine Receptor and Subunits*	Principal Cellular Targets and Biologic Effects
Interleukin-22 (IL-22)	T_H17 cells	IL-22R1 CD210B (IL-10R2) *or* IL-22BP CD210B (IL-10R2)	Epithelial cells: production of defensins, increased barrier function Hepatocytes: survival
Interleukin-24 (IL-24)	Melanocytes, keratinocytes, monocytes, T cells	IL-20R1 IL-20R2 *or* IL-22R1 IL-20R2	Monocytes: inflammatory cytokine expression Cancer cells: apoptosis
Interleukin-26 (IL-26)	T cells, monocytes	IL-20R1 CD210B (IL-10R2)	Not established
Interferon-λs (type III interferons)	Dendritic cells	IFNLR1 (IL-28Rα) CD210B (IL-10R2)	Epithelial cells: antiviral state
Leukemia inhibitory factor (LIF)	Embryonic trophectoderm Bone marrow stromal cells	CD118 (LIFR) CD130 (gp130)	Stem cells: block in differentiation
Oncostatin M	Bone marrow stromal cells	OSMR CD130 (gp130)	Endothelial cells: regulation of hematopoietic cytokine production Cancer cells: inhibition of proliferation
TNF superfamily cytokines†			
Tumor necrosis factor (TNF, TNFSF1)	Macrophages, NK cells, T cells	CD120a (TNFRSF1) *or* CD120b (TNFRSF2)	Endothelial cells: activation (inflammation, coagulation) Neutrophils: activation Hypothalamus: fever Liver: synthesis of acute-phase proteins Muscle, fat: catabolism (cachexia)
Lymphotoxin-α (LTα, TNFSF1)	T cells, B cells	CD120a (TNFRSF1) *or* CD120b (TNFRSF2)	Same as TNF
Lymphotoxin-αβ (LTαβ)	T cells, NK cells, follicular B cells, lymphoid inducer cells	LTβR *or* HVEM	Lymphoid tissue stromal cells and follicular dendritic cells: chemokine expression and lymphoid organogenesis
BAFF (CD257, TNFSF13B)	Dendritic cells, monocytes, follicular dendritic cells B cells	BAFF-R (TNFRSF13C) *or* TACI (TNFRSF13B) *or* BCMA (TNFRSF17)	B cells: survival, proliferation
APRIL	T cells, dendritic cells, monocytes, follicular dendritic cells	TACI (TNFRSF13B) *or* BCMA (TNFRSF17)	B cells: survival, proliferation
Osteoprotegerin (OPG, TNFRSF11B)	Osteoblasts	RANKL	Osteoclast precursor cells: inhibits osteoclast differentiation
IL-1 family cytokines			
Interleukin-1α (IL-1α)	Macrophages, dendritic cells, fibroblasts, endothelial cells, keratinocytes, hepatocytes	CD121a (IL-1R1) IL-1RAP *or* CD121b (IL-1R2)	Endothelial cells: activation (inflammation, coagulation) Hypothalamus: fever Liver: synthesis of acute-phase proteins
Interleukin-1β (IL-1β)	Macrophages, dendritic cells, fibroblasts, endothelial cells, keratinocytes, hepatocytes	CD121a (IL-1R1) IL-1RAP *or* CD121b (IL-1R2)	Endothelial cells: activation (inflammation, coagulation) Hypothalamus: fever Liver: synthesis of acute-phase proteins
Interleukin-1 receptor antagonist (IL-1Ra)	Macrophages	CD121a (IL-1R1) IL-1RAP	Various cells: competitive antagonist of IL-1

Continued

Cytokine and Subunits	Principal Cell Source	Cytokine Receptor and Subunits*	Principal Cellular Targets and Biologic Effects
Interleukin-18 (IL-18)	Monocytes, macrophages, dendritic cells, Kupffer cells, keratinocytes, chondrocytes, synovial fibroblasts, osteoblasts	CD218a (IL-18R1) CD218b (IL-18RAP)	NK cells and T cells: IFN-γ synthesis Monocytes: expression of GM-CSF, TNF, IL-1β Neutrophils: activation, cytokine release
Other cytokines			
Transforming growth factor-β (TGF-β)	T cells (mainly Tregs), macrophages, other cell types		T cells: inhibition of proliferation and effector functions; differentiation of T_H17 and Treg B cells: inhibition of proliferation; IgA production Macrophages: inhibition of activation; stimulation of angiogenic factors Fibroblasts: increased collagen synthesis

*Most cytokine receptors are dimers or trimers composed of different polypeptide chains, some of which are shared between receptors for different cytokines. The set of polypeptides that compose a functional receptor (cytokine binding plus signaling) for each cytokine is listed. The functions of each subunit polypeptide are not listed.

†All TNF superfamily (TNFSF) members are expressed as cell surface transmembrane proteins, but only the subsets that are predominantly active as proteolytically released soluble cytokines are listed in the table. Other TNFSF members that function predominantly in the membrane-bound form and are not, strictly speaking, cytokines are not listed in the table. These membrane-bound proteins and the TNFRSF receptors they bind to include OX40L (CD252, TNFSF4):OX40 (CD134, TNFRSF4); CD40L (CD154, TNFSF5):CD40 (TNFRSF5); FasL (CD178, TNFSF6):Fas (CD95, TNFRSF6); CD70 (TNFSF7):CD27 (TNFRSF27); CD153 (TNFSF8):CD30 (TNFRSF8); TRAIL (CD253, TNFSF10):TRAIL-R (TNFRSF10A-D); RANKL (TNFSF11):RANK (TNFRSF11); TWEAK (CD257, TNFSF12):TWEAKR (CD266, TNFRSF12); LIGHT (CD258, TNFSF14):HVEM (TNFRSF14); GITRL (TNFSF18):GITR (TNFRSF18); 4-IBBL:4-IBB (CD137).

APRIL, a proliferation-inducing ligand; BAFF, B cell activating factor belonging to the TNF family; BCMA, B cell maturation protein; CSF, colony-stimulating factor; HVEM, herpesvirus entry mediator; IFN, interferon; MHC, major histocompatibility complex; NK cell, natural killer cell; OSMR, oncostatin M receptor; RANK, receptor activator for nuclear factor κB ligand; RANKL, RANK ligand; TACI, transmembrane activator and calcium modulator and cyclophilin ligand interactor; TNF, tumor necrosis factor; TNFSF, TNF superfamily; TNFRSF, TNF receptor superfamily.

Appendix

III

PRINCIPAL FEATURES OF SELECTED CD MOLECULES

The following list includes selected CD molecules that are referred to in the text. Many cytokines and cytokine receptors have been assigned CD numbers, but we refer to these by the more descriptive cytokine designation. A complete and up-to-date listing of CD molecules may be found at *http://www.hlda8.org*.

CD Number	Common Synonyms	Molecular Structure, Family	Main Cellular Expression	Known or Proposed Function(s)
CD1a*	T6	49 kD; class I MHC family; β_2-microglobulin associated	Thymocytes, dendritic cells (including Langerhans cells)	Presentation of nonpeptide (lipid and glycolipid) antigens to some T cells
CD1b	T6	45 kD; class I MHC family; β_2-microglobulin associated	Same as CD1a	Same as CD1a
CD1c	T6	43 kD; class I MHC family; β_2-microglobulin associated	Thymocytes, dendritic cells (including Langerhans cells), some B cells	Same as CD1a
CD1d	—	49 kD; class I MHC family; β_2-microglobulin associated	Thymocytes, dendritic cells (including Langerhans cells), intestinal epithelial cells, some B cells	Same as CD1a
CD1e	—	28 kD; class I MHC family; β_2-microglobulin associated	Dendritic cells	Same as CD1a
CD2	T11; LFA-2; sheep red blood cell receptor	50 kD; Ig superfamily; CD2/CD48/CD58 family	T cells, thymocytes, NK cells	Adhesion molecule (binds CD58); T cell activation; CTL- and NK cell–mediated lysis
CD3γ	T3; Leu-4	25-28 kD; associated with CD3δ and CD3ε in TCR complex; Ig superfamily; ITAM in cytoplasmic tail	T cells, thymocytes	Cell surface expression of and signal transduction by the T cell antigen receptor
CD3δ	T3; Leu-4	20 kD; associated with CD3δ and CD3ε in TCR complex; Ig superfamily; ITAM in cytoplasmic tail (Chapter 7)	T cells, thymocytes	Cell surface expression of and signal transduction by the T cell antigen receptor
CD3ε	T3; Leu-4	20 kD; associated with CD3δ and CD3ε in TCR complex; Ig superfamily; ITAM in cytoplasmic tail	T cells, thymocytes	Required for cell surface expression of and signal transduction by the T cell antigen receptor
CD4	T4; Leu-3; L3T4	55 kD; Ig superfamily; CD2/CD48/CD58 family	Class II MHC–restricted T cells; thymocyte subsets; monocytes and macrophages	Signaling and adhesion coreceptor in class II MHC–restricted antigen-induced T cell activation (binds to class II MHC molecules); thymocyte development; primary receptor for HIV retroviruses

Continued

CD Number	Common Synonyms	Molecular Structure, Family	Main Cellular Expression	Known or Proposed Function(s)
CD5	T1; Ly-1	67 kD; scavenger receptor family	T cells; thymocytes; B cell subset	Signaling molecule; binds CD72
CD8α	T8; Leu2; Lyt2	34 kD; expressed as homodimer or heterodimer with CD8β	Class I MHC–restricted T cells; thymocyte subsets	Signaling and adhesion coreceptor in class I MHC–restricted antigen-induced T cell activation (binds to class I MHC molecules); thymocyte development
CD8β	T8; Leu2; Lyt2	34 kD; expressed as heterodimer with CD8α Ig superfamily	Same as CD8α	Same as CD8α
CD10	Common acute lymphoblastic leukemia antigen (CALLA); neutral endopeptidase; metalloendopeptidase; enkephalinase	100 kD; type II membrane protein	Immature and some mature B cells; lymphoid progenitors, granulocytes	Metalloproteinase; B cell development
CD11a	LFA-1 α chain; α_L integrin subunit	180 kD; noncovalently linked to CD18 to form LFA-1 integrin	Leukocytes	Cell-cell adhesion; binds to ICAM-1 (CD54), ICAM-2 (CD102), and ICAM-3 (CD50)
CD11b	Mac-1; Mo1; CR3 (iC3b receptor) α_M integrin chain	165 kD; noncovalently linked to CD18 to form Mac-1 integrin	Granulocytes, monocytes-macrophages, dendritic cells, NK cells	Phagocytosis of iC3b-coated particles; neutrophil and monocyte adhesion to endothelium (binds CD54) and extracellular matrix proteins
CD11c	p150,95; CR4 α chain; α_X integrin chain	145 kD; noncovalently linked to CD18 to form p150,95 integrin	Monocytes-macrophages, granulocytes, NK cells	Similar functions as CD11b; major CD11CD18 integrin on macrophages
CD14	Mo2; LPS receptor	53 kD; GPI linked	Dendritic cells, monocytes, macrophages, granulocytes	Binds complex of LPS and LPS-binding protein; required for LPS-induced macrophage activation
CD16a	FcγRIIIA	50-70 kD; transmembrane protein; Ig superfamily	NK cells, macrophages	Binds Fc region of IgG; phagocytosis and antibody-dependent cellular cytotoxicity
CD16b	FcγRIIIB	50-70 kD; GPI linked; Ig superfamily	Neutrophils	Binds Fc region of IgG; synergy with FcγRII in immune complex–mediated neutrophil activation
CD18	β chain of LFA-1 family; β_2 integrin subunit	95 kD; noncovalently linked to CD11a, CD11b, or CD11c to form β_2 integrins	Leukocytes	See CD11a, CD11b, CD11c
CD19	B4	95 kD; Ig superfamily	Most B cells	B cell activation; forms a coreceptor complex with CD21 and CD81 that delivers signals that synergize with signals from B cell antigen receptor complex
CD20	B1	35-37 kD; tetraspan (TM4SF) family	Most or all B cells	? Role in B cell activation or regulation; calcium ion channel
CD21	CR2; C3d receptor; B2	145 kD; regulators of complement activation	Mature B cells, follicular dendritic cells	Receptor for complement fragment C3d; forms a coreceptor complex with CD21 and CD81 that delivers activating signals in B cells; Epstein-Barr virus receptor
CD22	SIGLEC-2; BL-CAM	130-140 kD; Ig superfamily; sialadhesin family; ITIM in cytoplasmic tail	B cells	Regulation of B cell activation; adhesion molecule

CD Number	Common Synonyms	Molecular Structure, Family	Main Cellular Expression	Known or Proposed Function(s)
CD23	FcεRIIB; low-affinity IgE receptor	45 kD; C-type lectin	Activated B cells, monocytes, macrophages	Low-affinity Fcε receptor, induced by IL-4; ? regulation of IgE synthesis; ? triggering of monocyte cytokine release
CD25	IL-2 receptor α chain; TAC; p55	55 kD; regulators of complement activation family; noncovalently associates with IL-2Rβ (CD122) and IL-2Rγ (CD132) chains to form high-affinity IL-2 receptor	Activated T and B cells, activated macrophages	Binds IL-2; subunit of IL-2R
CD28	Tp44	Homodimer of 44-kD chains; Ig superfamily	T cells (most CD4, some CD8 cells)	T cell receptor for costimulator molecules CD80 (B7-1) and CD86 (B7-2)
CD29	β chain of VLA antigens; β_1 integrin subunit; platelet gpIIa	130 kD; noncovalently linked with CD49a-d chains to form VLA (β_1) integrins	T cells, B cells, monocytes, granulocytes	Leukocyte adhesion to extracellular matrix proteins and endothelium (see CD49)
CD30	Ki-1	120 kD; TNFR superfamily	Activated T and B cells; NK cells, monocytes, Reed-Sternberg cells in Hodgkin's disease	Role in activation-induced cell death of $CD8^+$ T cells; binds to CD153 (CD30L) on neutrophils, activated T cells, and macrophages
CD31	Platelet/endothelial cell adhesion molecule 1 (PECAM-1); platelet gpIIa	130-140 kD; Ig superfamily	Platelets; monocytes, granulocytes, B cells, endothelial cells	Adhesion molecule involved in leukocyte diapedesis
CD32	FcγRIIA; FcγRIIB; FcγRIIC	40 kD; Ig superfamily; ITIM in cytoplasmic tail; A, B, and C forms are products of different but homologous genes	Macrophages, granulocytes, B cells, eosinophils, platelets	Fc receptor for aggregated IgG; binds C-reactive protein; role in phagocytosis, ADCC; acts as inhibitory receptor that terminates activation signals initiated by the B cell antigen receptor
CD34	gp105-120	105-120 kD; sialomucin	Precursors of hematopoietic cells; endothelial cells in high endothelial venules	Cell-cell adhesion; binds CD62L (L-selectin)
CD35	CR1; C3b receptor	190-285 kD (four products of polymorphic alleles); regulator of complement activation family	Granulocytes, monocytes, erythrocytes, B cells, T cell subsets, follicular dendritic cells	Binds C3b and C4b; promotes phagocytosis of C3b- or C4b-coated particles and immune complexes; regulates complement activation
CD36	Platelet gpIIIb; gpIV	85-90 kD	Platelets, monocytes and macrophages, microvascular endothelial cells	Scavenger receptor for oxidized low-density lipoprotein; platelet adhesion; phagocytosis of apoptotic cells
CD40	TNFRSF5	Homodimer of 44- to 48-kD chains; TNFR superfamily	B cells, macrophages, dendritic cells, endothelial cells	Binds CD154 (CD40 ligand); role in T cell–dependent B cell activation and macrophage, dendritic cell, and endothelial cell activation
CD43	Sialophorin; leukosialin	95-135 kD; sialomucin	Leukocytes (except circulating B cells)	Adhesive and antiadhesive functions
CD44	Pgp-1; Hermes	80->100 kD, highly glycosylated; cartilage link protein family	Leukocytes, erythrocytes	Binds hyaluronan; involved in leukocyte adhesion to endothelial cells and extracellular matrix; leukocyte aggregation
CD45	Leukocyte common antigen (LCA); T200; B220	Multiple isoforms, 180-220 kD (see CD45R); protein tyrosine phosphatase receptor family; fibronectin type III family	Hematopoietic cells	Tyrosine phosphatase that plays critical role in T and B cell antigen receptor–mediated signaling

Continued

CD Number	Common Synonyms	Molecular Structure, Family	Main Cellular Expression	Known or Proposed Function(s)
CD45R	Forms of CD45 with restricted cellular expression	CD45RO:180 kD CD45RA: 220 kD CD45RB: 190-, 205-, and 220-kD isoforms	CD45RO: memory T cells; subset of B cells, monocytes, macrophages CD45RA: naive T cells, B cells, monocytes CD45RB: B cells, subset of T cells	See CD45
CD46	Membrane cofactor protein (MCP)	52-58 kD; regulators of complement activation family	Leukocytes, epithelial cells, fibroblasts	Regulation of complement activation
CD49a	α_1 integrin subunit	210 kD; noncovalently linked to CD29 to form VLA-1 (β_1 integrin)	Activated T cells, monocytes	Leukocyte adhesion to extracellular matrix; binds collagens, laminin
CD49b	α_2 integrin subunit; platelet gpIa	165 kD; noncovalently linked to CD29 to form VLA-2 (β_1 integrin)	Platelets, activated T cells, monocytes, some B cells	Leukocyte adhesion to extracellular matrix; binds collagen, laminin
CD49c	α_3 integrin subunit	Dimer of 130- and 25-kD chains; noncovalently linked to CD29 to form VLA-3 (β_1 integrin)	T cells; some B cells, monocytes	Leukocyte adhesion to extracellular matrix; binds fibronectin, collagens, laminin
CD49d	α_4 integrin subunit	150 kD; noncovalently linked to CD29 to form VLA-4 ($\alpha_4\beta_1$ integrin)	T cells, monocytes, B cells, NK cells, eosinophils, dendritic cells, thymocytes	Leukocyte adhesion to endothelium and extracellular matrix; binds to VCAM-1 and MadCAM-1; binds fibronectin and collagens
CD49e	α_5 integrin subunit	Heterodimer of 135- and 25-kD chains; noncovalently linked to CD29 to form VLA-5 (β_1 integrin)	T cells; few B cells and monocytes, thymocytes	Adhesion to extracellular matrix; binds fibronectin
CD49f	α_6 integrin subunit	Heterodimer of 125- and 25-kD chains; noncovalently linked to CD29 to form VLA-6 (β_1 integrin)	Platelets, megakaryocytes; activated T cells, monocytes	Adhesion to extracellular matrix; binds fibronectin
CD54	ICAM-1	75-114 kD; Ig superfamily	T cells, B cells, monocytes, endothelial cells (cytokine inducible)	Cell-cell adhesion; ligand for CD11aCD18 (LFA-1) and CD11bCD18 (Mac-1); receptor for rhinovirus
CD55	Decay-accelerating factor (DAF)	55-70 kD; GPI linked; regulators of complement activation family	Broad	Regulation of complement activation; binds C3b, C4b
CD58	Leukocyte function-associated antigen 3 (LFA-3)	55-70 kD; GPI-linked or integral membrane protein; CD2/CD48/CD58 family	Broad	Leukocyte adhesion; binds CD2
CD59	Membrane inhibitor of reactive lysis (MIRL)	18-20 kD; GPI linked; Ly-6 superfamily	Broad	Binds C9; inhibits formation of complement membrane attack complex
CD62E	E-selectin; ELAM-1	115 kD; selectin family	Endothelial cells	Leukocyte-endothelial adhesion
CD62L	L-selectin; LAM-1; MEL-14	74-95 kD; selectin family	B cells, T cells, monocytes, granulocytes, some NK cells	Leukocyte-endothelial adhesion; homing of naive T cells to peripheral lymph nodes
CD62P	P-selectin; gmp140; PADGEM (platelet activation–dependent granule–external membrane protein)	140 kD; selectin family	Platelets, endothelial cells; (present in granules, translocated to cell surface on activation)	Leukocyte adhesion to endothelium, platelets; binds CD162 (PSGL-1)
CD64	FcγRI	72 kD; Ig superfamily; noncovalently associated with the common FcR γ chain	Monocytes, macrophages, activated neutrophils	High-affinity Fcγ receptor; role in phagocytosis, ADCC, macrophage activation

CD Number	Common Synonyms	Molecular Structure, Family	Main Cellular Expression	Known or Proposed Function(s)
CD66e	Carcinoembryonic antigen (CEA)	180-220 kD; Ig superfamily; carcinoembryonic antigen (CEA) family	Colonic and other epithelial cells	? Adhesion; clinical marker of carcinoma burden
CD69	CLEC2C; early T cell activation antigen	23 kD; C-type lectin	Activated B cells, T cells, NK cells, neutrophils	Binds to and impairs surface expression of S1PR1, thereby promoting retention of recently activated lymphocytes in lymphoid tissues
CD74	Class II MHC invariant (γ) chain; I_i	33-, 35-, and 41-kD isoforms	B cells, monocytes, macrophages; other class II MHC–expressing cells	Binds to and directs intracellular sorting of newly synthesized class II MHC molecules
CD79a	Igα, MB1	33, 45 kD; forms dimer with CD79b; Ig superfamily; ITAM in cytoplasmic tail	Mature B cells	Required for cell surface expression of and signal transduction by the B cell antigen receptor complex
CD79b	Igβ; B29	37-39 kD; forms dimer with CD79α; Ig superfamily; ITAM in cytoplasmic tail	Mature B cells	Required for cell surface expression of and signal transduction by the B cell antigen receptor complex
CD80	B7-1; BB1	60 kD; Ig superfamily	Dendritic cells, activated B cells and macrophages	Costimulator for T lymphocyte activation; ligand for CD28 and CD152 (CTLA-4)
CD81	Target for antiproliferative antigen 1 (TAPA-1)	26 kD; tetraspan (TM4SF)	T cells, B cells, NK cells, dendritic cells, thymocytes, endothelium	B cell activation; forms a coreceptor complex with CD19 and CD21 that delivers signals that synergize with signals from B cell antigen receptor complex
CD86	B7-2	80 kD; Ig superfamily	B cells, monocytes; dendritic cells; some T cells	Costimulator for T lymphocyte activation; ligand for CD28 and CD152 (CTLA-4)
CD88	C5a receptor	43 kD; G protein–coupled, 7 membrane–spanning receptor family	Granulocytes, monocytes, dendritic cells, mast cells	Receptor for C5a complement fragment; role in complement-induced inflammation
CD89	Fcα receptor (FcαR)	55-75 kD; Ig superfamily; noncovalently associated with the common FcR γ chain	Granulocytes, monocytes, macrophages, T cell subset, B cell subset	Binds IgA; mediates IgA-dependent cellular cytotoxicity
CD90	Thy-1	25-35 kD; GPI linked; Ig superfamily	Thymocytes, peripheral T cells (mice), $CD34^+$ hematopoietic progenitor cells, neurons	Marker for T cells; ? role in T cell activation
CD94	Kp43; KIR	43 kD; C-type lectin; on NK cells, covalently assembles with other C-type lectin molecules (NKG2)	NK cells; subset of $CD8^+$ T cells	CD94/NKG2 complex functions as an NK cell killer inhibitory receptor; binds HLA-E class I MHC molecules
CD95	Fas antigen, APO-1	Homotrimer of 45-kD chains; TNFR superfamily	Multiple cell types	Binds Fas ligand; mediates signals leading to apoptotic death
CD102	ICAM-2	55-65 kD; Ig superfamily	Endothelial cells, lymphocytes, monocytes, platelets	Ligand for CD11aCD18 (LFA-1); cell-cell adhesion
CD103	HML-1; α_E integrin subunit	Dimer of 150- and 25-kD subunits; noncovalently linked to β_7 integrin subunit to form $\alpha_E\beta_7$ integrin	Intraepithelial lymphocytes, other cell types	Role in T cell homing to and retention in mucosa; binds E-cadherin
CD106	Vascular cell adhesion molecule 1 (VCAM-1); INCAM-110	100-110 kD; Ig superfamily	Endothelial cells, macrophages, follicular dendritic cells, marrow stromal cells	Adhesion; receptor for CD49dCD29 (VLA-4) integrin; role in lymphocyte trafficking, activation; role in hematopoiesis

Continued

CD Number	Common Synonyms	Molecular Structure, Family	Main Cellular Expression	Known or Proposed Function(s)
CD150	Signaling lymphocyte activation molecule (SLAM; IPO-3	37 kD; Ig superfamily; CD2/CD48/CD58 family	Thymocytes, activated lymphocytes, dendritic cells, endothelial cells	Regulation of B cell–T cell interactions and proliferative signals in B lymphocytes; binds itself as a self ligand
CD152	Cytotoxic T lymphocyte–associated protein 4 (CTLA-4)	33, 50 kD; Ig superfamily	Activated T lymphocytes	Inhibitory signaling in T cells; binds CD80 (B7-1) and CD86 (B7-2) on antigen-presenting cells
CD153	CD30 ligand (CD30L); TNFSF8	40 kD; TNF superfamily	Activated T cells, resting B cells, granulocytes, macrophages, thymocytes	Role in activation-induced cell death of $CD8^+$ T cells; binds to CD30
CD154	CD40 ligand (CD40L); TNF-related activation protein (TRAP); gp39	Homotrimer of 32- to 39-kD chains; TNFR superfamily	Activated $CD4^+$ T cells	Activates B cells, macrophages, and endothelial cells; ligand for CD40
CD158	Killer inhibitor receptor (KIR)	50, 58 kD; Ig superfamily; killer Ig-like receptor (KIR) family; ITIMs in cytoplasmic tail	NK cells, T cell subset	Inhibition or activation of NK cell on interaction with the appropriate class I HLA molecules
CD159a	NKG2A	43 kD; C-type lectin; forms heterodimer with CD94	NK cells, T cell subset	Inhibition or activation of NK cell on interaction with the appropriate class I HLA molecules
CD159c	NKG2C	40 kD; C-type lectin; forms heterodimer with CD94	NK cells	Activation of NK cell on interaction with the appropriate class I HLA molecules
CD162	P-selectin glycoprotein ligand 1 (PSGL-1)	Homodimer of 120-kD chains; sialomucin	T cells, monocytes, granulocytes, some B cells	Ligand for selectins (CD62P, CD62L); adhesion of leukocytes to endothelium
CD178	Fas ligand; TNFSF6	Homotrimer of 31-kD subunits; TNF superfamily	Activated T cells	Ligand for CD95 (Fas); triggers apoptotic death
CD206	Mannose receptor	166 kD; C-type lectin	Macrophages	Binds high-mannose structures on pathogens; mediates macrophage endocytosis of glycoproteins and phagocytosis of bacteria, fungi, and other pathogens
CD244	2B4; NAIL; natural killer cell–activating ligand	41 kD; Ig superfamily; CD2/CD48/CD58 family; SLAM family	NK cells, CD8 T cells, γδ T cells	Receptor for CD148; modulates NK cell cytolytic activity
CD247	Zeta chain; TCRζ	18 kD; ITAMs in cytoplasmic tail	T cells; NK cells	Signaling chain of TCR and NK cell activating receptors
CD252	OX40L; TNFSF4	21 kD; TNF superfamily	Dendritic cells, macrophages, B cells	Ligand for CD134 (OX40,TNFRSF4); costimulates T cells
CD267	TACI; TNFRSF13B	31 kD; TNFR superfamily	B cells	Receptor of BAFF and APRIL; mediates B cell proliferation, survival, and differentiation
CD268	BAFFR; TNFRSF13C	19 kD; TNFR superfamily	B cells	Receptor of BAFF; mediates B cell proliferation, survival, and differentiation
CD269	BCMA (B cell maturation antigen); TNFRSF17	20 kD; TNFR superfamily	B cells	Receptor of BAFF and APRIL; mediates B cell proliferation, survival, and differentiation
CD270	TNFRSF14; herpesvirus entry mediator (HVEM)	30 kD; TNFR superfamily	T cells, B cells, dendritic cells	Receptor for BTLA4, TNFSF14/LIGHT, lymphotoxin-α; negative regulation of immune responses
CD273	B7-DC; PD-L2	25 kD; Ig superfamily; B7 costimulator family	Dendritic cells, monocytes, macrophages	Binds PD-1; inhibition of T cell activation
CD274	B7-H1; PD-L1	33 kD; Ig superfamily; B7 costimulator family	Leukocytes	Binds PD-1; inhibition of T cell activation

CD Number	Common Synonyms	Molecular Structure, Family	Main Cellular Expression	Known or Proposed Function(s)
CD275	B7-H2; ICOS ligand; B7-RP1	60 kD; Ig superfamily; B7 costimulator family	B cells, dendritic cells, monocytes	Binds ICOS (CD278); T cell costimulation
CD276	B7-H3	40-45 kD; Ig superfamily; B7 costimulator family	Dendritic cells, monocytes, activated T cells	T cell costimulation
CD278	ICOS; AILIM	55-60 kD; Ig superfamily; CD28 costimulator family	Activated T cells	Binds ICOS-L (CD275); T costimulation
CD279	PD1; SLEB2	55 kD; Ig superfamily; CD28 costimulator family	Activated T cells, activated B cells	Binds B7-H1 (CD274) and B7-DC (CD273); regulation of T cell activation
CD281	TLR1	90 kD; Toll-like receptor family	Dendritic cells, monocytes	Pairs with CD82 (TLR2); recognizes several PAMPs, including bacterial lipopeptides; activating pattern recognition receptor of innate immunity
CD282	TLR2	90 kD; Toll-like receptor family	Monocytes, neutrophils, macrophages	Recognizes several PAMPs, including bacterial peptidoglycan and lipoproteins; activating pattern recognition receptor of innate immunity
CD283	TLR3	100 kD; Toll-like receptor family	Dendritic cells, monocytes (endosomal)	Recognizes several PAMPs, including viral dsRNA; activating pattern recognition receptor of innate immunity
CD284	TLR4	100 kD; Toll-like receptor family	Monocytes, macrophages, dendritic cells, endothelial cells	Recognizes several PAMPs, including LPS; activating pattern recognition receptor of innate immunity
CD286	TLR6	92 kD; Toll-like receptor family		Pairs with TLR2; recognizes several PAMPs, including bacterial lipopeptides; activating pattern recognition receptor of innate immunity
CD288	TLR8	120 kD; Toll-like receptor family	Dendritic cells (endosomal)	Recognizes several PAMPs, including viral ssRNA; activating pattern recognition receptor of innate immunity
CD289	TLR9	120 kD; Toll-like receptor family	Dendritic cells (endosomal)	Recognizes several PAMPs, including CpG DNA; activating pattern recognition receptor of innate immunity
CD314	NKG2D; KLR	42 kD; C-type lectin	NK cells, activated $CD8^+$ T cells, NK1.1 T cells, some myeloid cells	Binds MHC class I, MIC-A, MIC-B, Rae1, and ULBP4; NK cell and CTL activation
CD357	TNFRSF18; GITR	26 kD; TNFR superfamily	$CD4^+$ and $CD8^+$ T cells, Treg	Receptor for GITR ligand (TNFSF18); important in T cell tolerance/Treg function
CD363	S1PR1 (sphingosine 1-phosphate receptor 1)	42.8 kD; G protein–coupled, 7 membrane–spanning receptor family	Lymphocytes, endothelial cells	Binds sphingosine 1-phosphate and mediates chemotaxis of lymphocytes out of lymphoid organs

*The lowercase letters affixed to some CD numbers refer to complex CD molecules that are encoded by multiple genes or that belong to families of structurally related proteins. For instance, CD1a, CD1b, and CD1c are structurally related but distinct forms of a β_2-microglobulin–associated nonpolymorphic protein.
ADCC, antibody-dependent cell-mediated cytotoxicity; APRIL, a proliferation-inducing ligand; BAFF, B cell activating factor belonging to the TNF family; CTL, cytotoxic T lymphocyte; gp, glycoprotein; GPI, glycophosphatidylinositol; ICAM, intercellular adhesion molecule; Ig, immunoglobulin; IL, interleukin; ITAM, immunoreceptor tyrosine-based activation motif; ITIM, immunoreceptor tyrosine-based inhibition motif; LFA, lymphocyte function-associated antigen; LPS, lipopolysaccharide; MadCAM, mucosal addressin cell adhesion molecule; MHC, major histocompatibility complex; NK cells, natural killer cells; PAMPs, pathogen-associated molecular patterns; TACI, transmembrane activator and CAML interactor; TCR, T cell receptor; TLR, Toll-like receptor; TNF, tumor necrosis factor; TNFR, TNF receptor; VCAM, vascular cell adhesion molecule; VLA, very late activation.

Appendix

IV

LABORATORY TECHNIQUES COMMONLY USED IN IMMUNOLOGY

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Many laboratory techniques that are routine in research and clinical settings are based on the use of antibodies. In addition, many of the techniques of modern molecular biology have provided invaluable information about the immune system. We have mentioned these techniques often throughout the book. In this appendix, we describe the principles underlying some of the most commonly used laboratory methods in immunology. In addition, we summarize how B and T lymphocyte responses are studied with use of laboratory techniques. Details of how to carry out various assays may be found in laboratory manuals.

LABORATORY METHODS USING ANTIBODIES

The exquisite specificity of antibodies for particular antigens makes antibodies valuable reagents for detecting, purifying, and quantitating antigens. Because antibodies can be produced against virtually any type of macromolecule and small chemical, antibody-based techniques may be used to study virtually any type of molecule in solution or in cells. The method for producing monoclonal antibodies (see Chapter 5) has greatly increased our ability to generate antibodies of almost any desired specificity. Historically, many of the uses of antibody depended on the ability of antibody and specific antigen to form large immune complexes, either in solution or in gels, that could be detected by various optical methods. These methods were of great importance in early studies but have now been replaced almost entirely by simpler methods based on immobilized antibodies or antigens.

Quantitation of Antigen by Immunoassays

Immunologic methods of quantifying antigen concentration provide exquisite sensitivity and specificity and have become standard techniques for both research and clinical applications. All modern immunochemical methods of quantitation are based on having a pure antigen or antibody whose quantity can be measured by an indicator molecule (or a label). When the antigen or antibody is labeled with a radioisotope, as first introduced by Rosalyn Yalow and colleagues, it may be quantified by instruments that detect radioactive decay events; the assay is called a **radioimmunoassay (RIA)**. When the antigen or antibody is covalently coupled to an enzyme, it may be quantified by determining with a spectrophotometer the rate at which the enzyme converts a clear substrate to a colored product; the assay is called an **enzyme-linked immunosorbent assay (ELISA)**. Several variations of RIA and ELISA exist, but the most commonly used version is the sandwich assay (Fig. A-1). The sandwich assay uses two different antibodies reactive with different epitopes on the antigen whose concentration needs to be determined. A fixed quantity of one

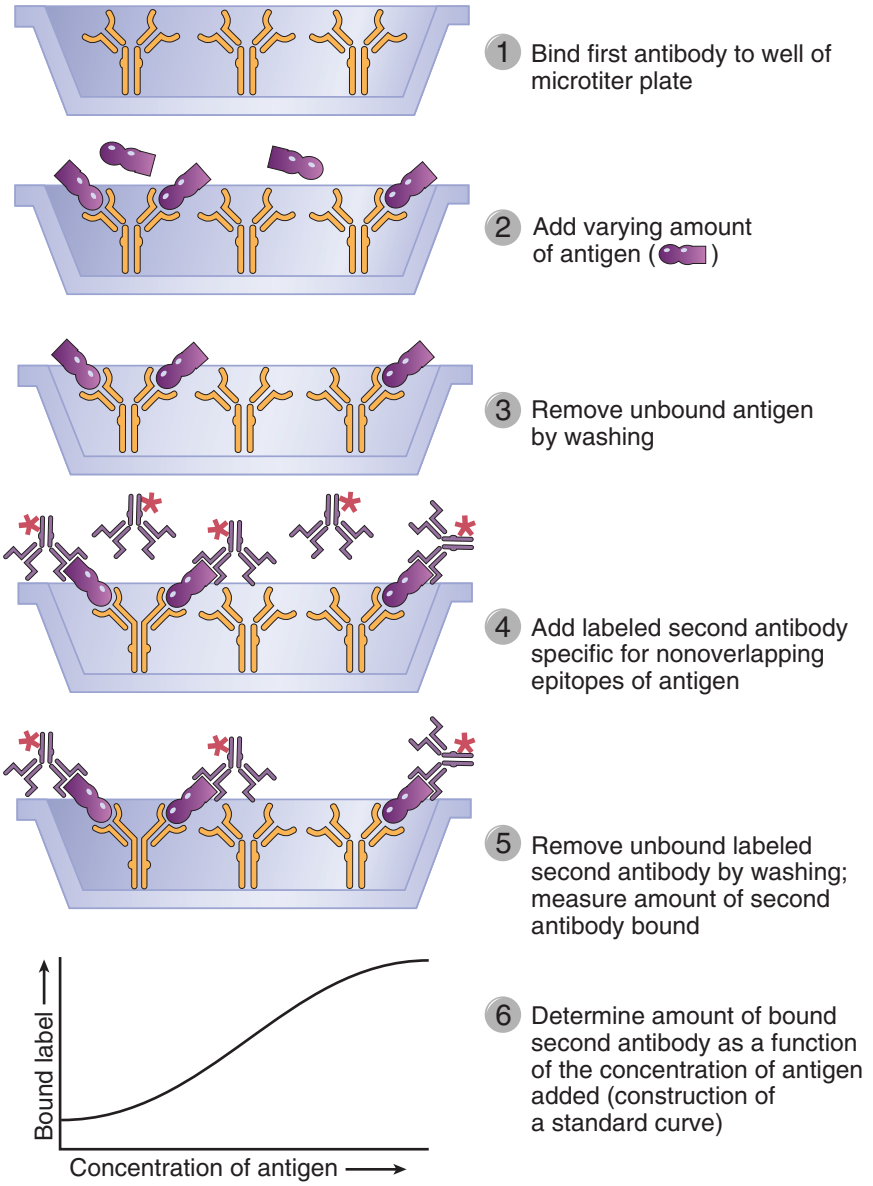


FIGURE A–1 Sandwich enzyme-linked immunosorbent assay or radioimmunoassay. A fixed amount of one immobilized antibody is used to capture an antigen. The binding of a second, labeled antibody that recognizes a nonoverlapping determinant on the antigen will increase as the concentration of antigen increases and thus allow quantification of the antigen.

antibody is attached to a series of replicate solid supports, such as plastic microtiter wells. Test solutions containing antigen at an unknown concentration or a series of standard solutions with known concentrations of antigen are added to the wells and allowed to bind. Unbound antigen is removed by washing, and the second antibody, which is enzyme linked or radiolabeled, is allowed to bind. The antigen serves as a bridge, so the more antigen in the test or standard solutions, the more enzyme-linked or radiolabeled second antibody will bind. The results from the standard solutions are used to construct a binding curve for the second antibody as a function of antigen concentration, from which the quantities of antigen in the test solutions may be inferred. When this test is performed with two monoclonal antibodies, it is essential that these antibodies see nonoverlapping determinants on the antigen; otherwise, the second antibody cannot bind.

In an important clinical variant of immunobinding assays, samples from patients may be tested for the presence of antibodies that are specific for a microbial antigen (e.g., antibodies reactive with proteins from human immunodeficiency virus [HIV] or hepatitis B virus) as indicators of infection. In this case, a saturating quantity of antigen is added to replicate wells containing plate-bound antibody or the antigen is attached directly to the plate, and serial dilutions of the patient's serum are then allowed to bind. The amount of the patient's antibody bound to the immobilized antigen is determined by use of an enzyme-linked or radiolabeled second anti-human immunoglobulin (Ig) antibody.

Identification and Purification of Proteins

Antibodies can be used to identify and characterize proteins and to purify specific proteins from mixtures. Two commonly used methods to identify and purify proteins are immunoprecipitation and immuno–affinity chromatography. Western blotting is a widely used technique to

determine the presence and size of a protein in a biologic sample.

Immunoprecipitation and Immuno–Affinity Chromatography

Immunoprecipitation is a technique in which an antibody specific for one protein antigen in a mixture of proteins is used to identify this specific antigen (Fig. A-2A). The antibody is typically added to a protein mixture (usually a detergent lysate of specific cells), and staphylococcal protein A (or protein G) covalently attached to agarose beads is added to the mixture. The Fab portions of the antibody bind to the target protein, and the Fc portion of the antibody is captured by the protein A or protein G on the beads. Unwanted proteins that do not bind to the antibody are then removed by washing the beads (by repeated detergent addition and centrifugation). The specific protein that is recognized by and now bound to the antibody may be eluted from the beads and dissociated from the antibody by use of a harsh denaturant (such as sodium dodecyl sulfate), and the proteins are separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). Proteins may be detected after electrophoresis by staining the polyacrylamide gel with a protein stain or by a Western blot analysis (described later). If the original mixture contained radioactively labeled proteins, specific proteins immunoprecipitated by the antibody may be revealed by autofluorography or autoradiography, protein bands being captured on x-ray film placed on the dried SDS–polyacrylamide gel containing separated proteins.

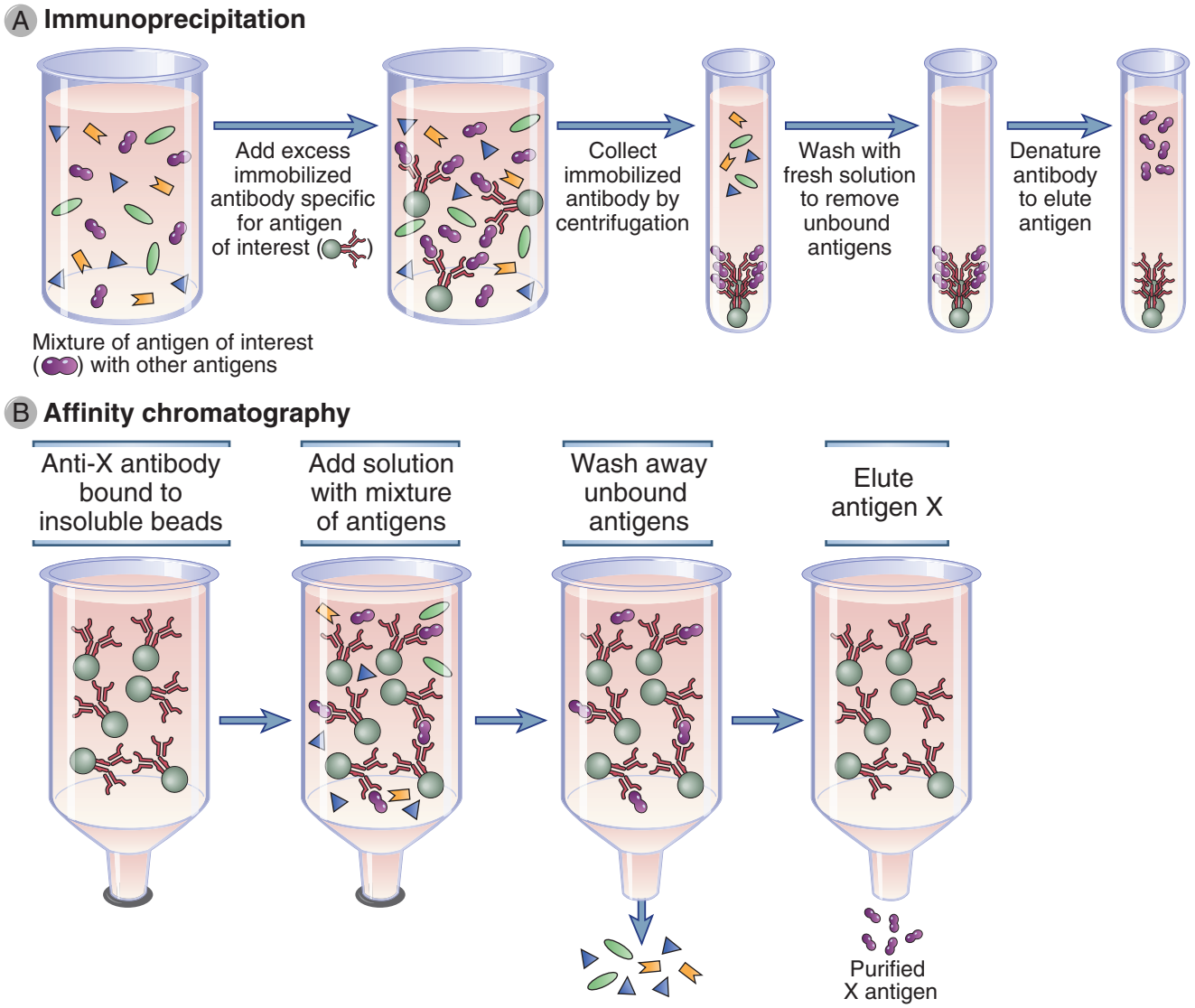


FIGURE A–2 Isolation of an antigen by immunoprecipitation or affinity chromatography. A, A particular antigen can be purified from a mixture of antigens in serum or other solutions by adding antibodies specific to the antigen that are bound to insoluble beads. Unbound antigens are then washed away, and the desired antigen is recovered by changing the pH or ionic strength of the solution so that the affinity of antibody-antigen binding is lowered. Immunoprecipitation can be used as a means of purification, as a means of quantification, or as a means of identification of an antigen. Antigens purified by immunoprecipitation are often analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. **B,** Affinity chromatography is based on the same principle as immunoprecipitation, except that the antibody is fixed to an insoluble matrix or beads, usually in a column. The method is often used to isolate soluble antigens (shown) or antibodies specific for an immobilized antigen.

Immuno–affinity chromatography, a variant of affinity chromatography, is a purification method that relies on antibodies attached to an insoluble support to purify antigens from a solution (Fig. A-2B). Antibodies specific for the desired antigen are typically covalently attached to a solid support, such as agarose beads, and packed into a column. A complex mixture of antigens is passed through the beads to allow the antigen that is recognized by the antibody to bind. Unbound molecules are washed away, and the bound antigen is eluted by changing the pH or by exposure to high salt or other chaotropic conditions that break antigen-antibody interactions. A similar method may be used to purify antibodies from culture supernatants or natural fluids, such as serum, by first attaching the antigen to beads and passing the supernatants or serum through.

Western Blotting

Western blotting (Fig. A-3) is used to identify and determine the relative quantity and molecular weight of a protein within a mixture of proteins or other molecules. The mixture is first subjected to analytical separation, typically by SDS-PAGE, so that the final positions of different proteins in the gel are a function of their molecular size. The array of separated proteins is then transferred from the separating polyacrylamide gel to a support membrane by electrophoresis such that the membrane acquires a replica of the array of separated macromolecules present in the gel. SDS is displaced from the protein during the transfer process, and native antigenic determinants are often regained as the protein refolds. The position of the protein antigen on the membrane can then be detected by binding of an unlabeled antibody specific for that protein (the primary antibody) followed by a labeled second antibody that binds to the primary antibody. This approach provides information about antigen size and quantity. In general, second antibody probes are labeled with enzymes that generate chemiluminescent signals and leave images on photographic film. Near-infrared fluorophores can also be used to label antibodies, and light produced by the excitation of the fluorophore provides more accurate antigen quantitation compared with enzyme-linked second antibodies. The sensitivity and specificity of this technique can be increased by starting with immunoprecipitated proteins instead of crude protein mixtures. This sequential procedure is especially useful for detection of protein-protein interactions. For example, the physical association of two different proteins in the membrane of a lymphocyte can be established by immunoprecipitating a membrane extract by use of an antibody specific for one of the proteins and probing a Western blot of the immunoprecipitate using an antibody specific for the second protein that may have been co-immunoprecipitated along with the first protein. A variation of the Western blot technique is routinely used to detect the presence of anti-HIV antibodies in patients' sera. In this case, a defined mixture of HIV proteins is separated by SDS-PAGE and blotted onto a membrane, and the membrane is incubated with dilutions of the test serum. The blot is then probed with a second labeled anti-human Ig to detect the presence of HIV-specific antibodies that were in the serum and bound to the HIV proteins.

The technique of transferring proteins from a gel to a membrane is called Western blotting as a biochemist's joke. Southern is the last name of the scientist who first blotted DNA from a separating gel to a membrane by capillary transfer, a technique since called Southern blotting. By analogy, Northern blotting was the term applied to the technique of transferring RNA from a gel to a membrane, and Western blotting is the term used to describe the transfer of proteins to a membrane.

Labeling and Detection of Antigens in Cells and Tissues

Antibodies specific for antigens expressed on or in particular cell types are commonly used to identify these cells in tissues or cell suspensions and to separate these cells from mixed populations. In these methods, the antibody can be radiolabeled, enzyme linked, or, most commonly, fluorescently labeled, and a detection system is used that can identify the bound antibody. Antibodies attached to magnetic beads can be used to physically isolate cells expressing specific antigens.

Flow Cytometry and Fluorescence-Activated Cell Sorting

The tissue lineage, maturation stage, or activation status of a cell can often be determined by analyzing the cell surface or intracellular expression of different molecules. This technique is commonly done by staining the cell with fluorescently labeled probes that are specific for those molecules and measuring the quantity of fluorescence emitted by the cell (Fig. A-4). The flow cytometer is a specialized instrument that can detect fluorescence on individual cells in a suspension and thereby determine the number of cells expressing the molecule to which a fluorescent probe binds. Suspensions of cells are incubated with fluorescently labeled probes, and the amount of probe bound by each cell in the population is measured by passing the cells one at a time through a fluorimeter with a laser-generated incident beam. The relative amounts of a particular molecule on different cell populations can be compared by staining each population with the same probe and determining the amount of fluorescence emitted. In preparation for flow cytometric analysis, cell suspensions are stained with the fluorescent probes of choice. Most often, these probes are fluorochrome-labeled antibodies specific for a cell surface molecule. Alternatively, cytoplasmic molecules can be stained by temporarily permeabilizing cells and permitting the labeled antibodies to enter through the plasma membrane. In addition to antibodies, various fluorescent indicators of cytoplasmic ion concentrations and reduction-oxidation potential can be detected by flow cytometry. Cell cycle studies can be performed by flow cytometric analysis of cells stained with fluorescent DNA-binding probes such as propidium iodide. Apoptotic cells can be identified with fluorescent probes, such as annexin V, that bind to abnormally exposed phospholipids on the surface of the dying cells. Modern flow cytometers can routinely detect three or more different-colored

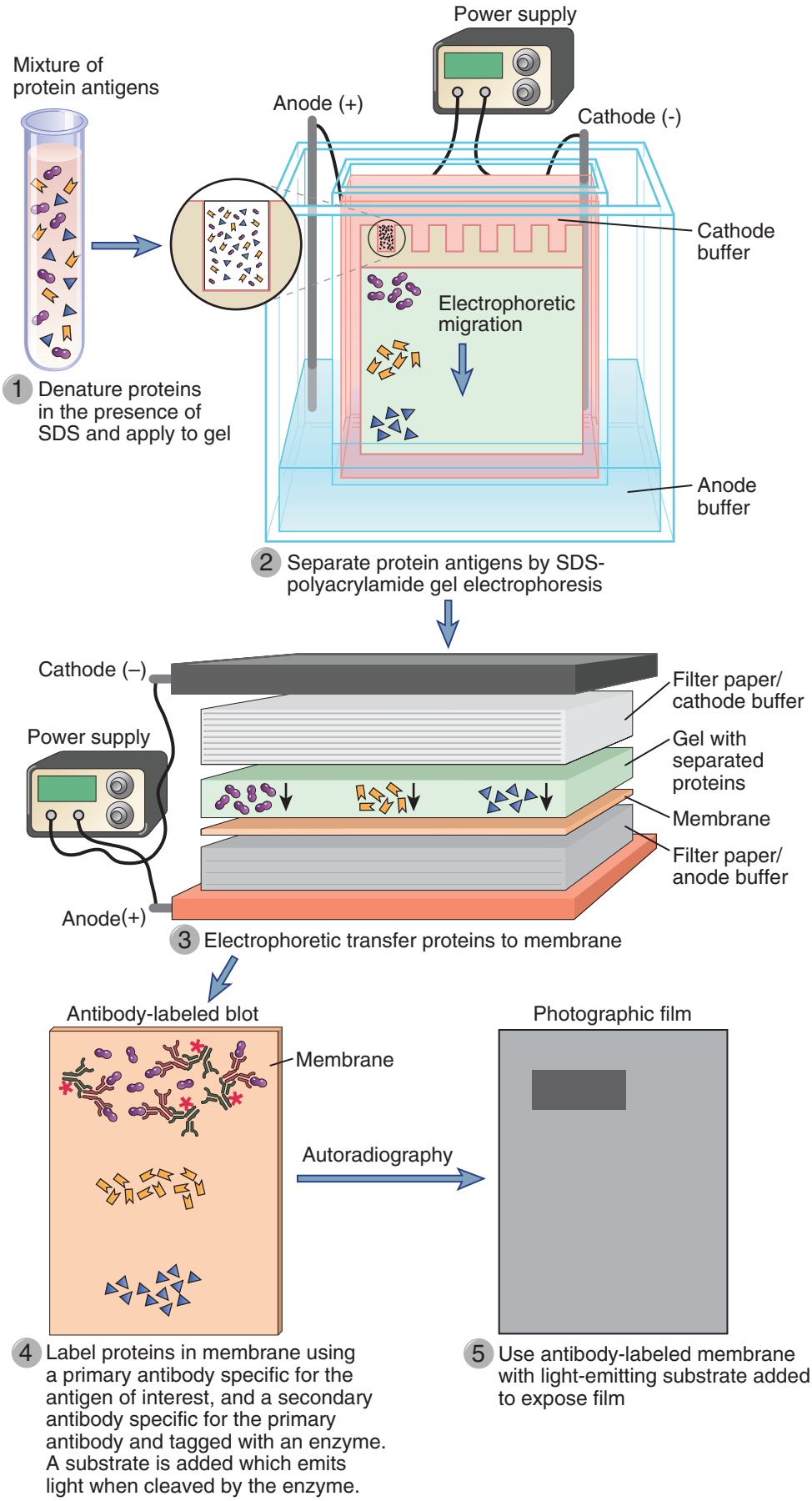


FIGURE A–3 Characterization of antigens by Western blotting. Protein antigens, separated by sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis and transferred to a membrane, can be detected by an antibody that is in turn revealed by a second antibody that may be conjugated to an enzyme such as horseradish peroxidase or to a fluorophore.

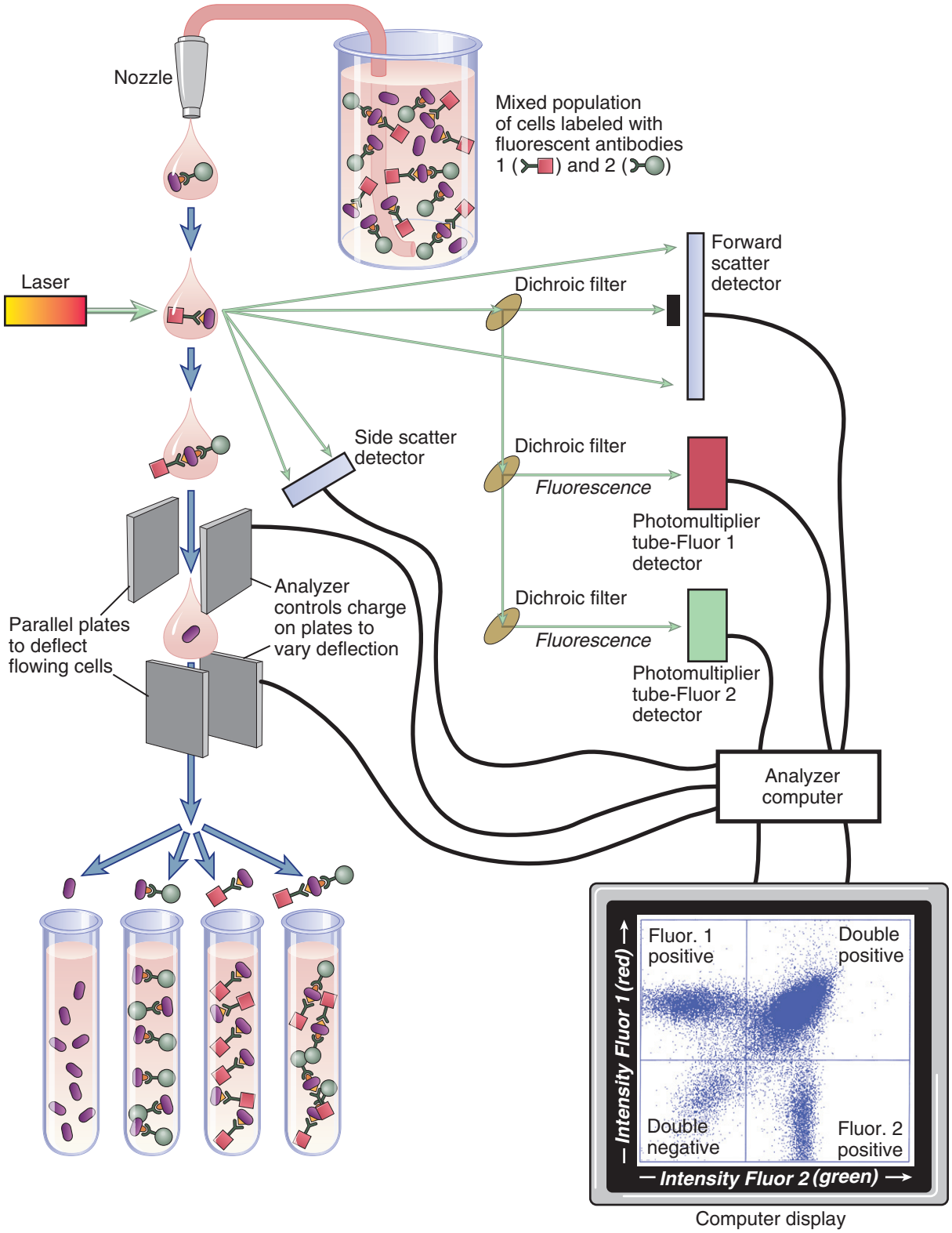


FIGURE A–4 Principle of flow cytometry and fluorescence-activated cell sorting. The incident laser beam is of a designated wavelength, and the light that emerges from the sample is analyzed for forward and side scatter as well as fluorescent light of two or more wavelengths that depend on the fluorochrome labels attached to the antibodies. The separation depicted here is based on two antigenic markers (two-color sorting). Modern instruments can routinely analyze and separate cell populations on the basis of three or more different-colored probes.

fluorescent signals, each attached to a different antibody or other probe. This technique permits simultaneous analysis of the expression of many different combinations of molecules by a cell. In addition to detecting fluorescent signals, flow cytometers also measure the forward and side light-scattering properties of cells, which reflect cell size and internal complexity, respectively. This information is often used to distinguish different cell types. For example, compared with lymphocytes, neutrophils cause greater side scatter because of their cytoplasmic granules, and monocytes cause greater forward scatter because of their size.

Purification of Cells

A fluorescent-activated cell sorter is an adaptation of the flow cytometer that allows one to separate cell populations according to which and how much fluorescent probe they bind. This technique is accomplished by differentially deflecting the cells with electromagnetic fields whose strength and direction are varied according to the measured intensity of the fluorescent signal (see Fig. A-4). The cells may be labeled with fluorescently tagged antibodies ex vivo, or, in the case of experimental animal studies, labeling may be accomplished in vivo by expression of transgenes that encode fluorescent proteins, such as green fluorescent protein. (Transgenic technology is described later in this appendix.)

Another commonly used technique to purify cells with a particular phenotype relies on antibodies that are attached to magnetic beads. These "immunomagnetic reagents" will bind to certain cells, depending on the specificity of the antibody used, and the bound cells can then be pulled out of suspension by a strong magnet.

Immunofluorescence and Immunohistochemistry

Antibodies can be used to identify the anatomic distribution of an antigen within a tissue or within compartments of a cell. To do so, the tissue or cell is incubated with an antibody that is labeled with a fluorochrome or enzyme, and the position of the label, determined with a suitable microscope, is used to infer the position of the antigen. In the earliest version of this method, called immunofluorescence, the antibody was labeled with a fluorescent dye and allowed to bind to a monolayer of cells or to a frozen section of a tissue. The stained cells or tissues were examined with a fluorescence microscope to locate the antibody. Although sensitive, the fluorescence microscope is not an ideal tool to identify the detailed structures of the cell or tissue because of a low signal-to-noise ratio. This problem has been overcome by new technologies including confocal microscopy, which uses optical sectioning technology to filter out unfocused fluorescent light, and two-photon microscopy, which prevents out-of-focus light from forming. Alternatively, antibodies may be coupled to enzymes that convert colorless substrates to colored insoluble substances that precipitate at the position of the enzyme. A conventional light microscope may then be used to localize the antibody in a stained cell or tissue. The most common variant of this method uses the enzyme horseradish peroxidase, and the method is commonly referred to as the immunoperoxidase technique. Another commonly used enzyme is alkaline phosphatase. Different antibodies coupled to different enzymes may be used in conjunction to produce simultaneous two-color localizations of different antigens. In other variations, antibody can be coupled to an electron-dense probe such as colloidal gold, and the location of antibody can be determined subcellularly by means of an electron microscope, a technique called immunoelectron microscopy. Different-sized gold particles have been used for simultaneous localization of different antigens at the ultrastructural level.

In all immunomicroscopic methods, signals may be enhanced by use of sandwich techniques. For example, instead of attaching horseradish peroxidase to a specific mouse antibody directed against the antigen of interest, it can be attached to a second anti-antibody (e.g., rabbit anti-mouse Ig antibody) that is used to bind to the first, unlabeled antibody. When the label is attached directly to the specific, primary antibody, the method is referred to as direct; when the label is attached to a secondary or even tertiary antibody, the method is indirect. In some cases, molecules other than antibody can be used in indirect methods. For example, staphylococcal protein A, which binds to IgG, or avidin, which binds to primary antibodies labeled with biotin, can be coupled to fluorochromes or enzymes.

Measurement of Antigen-Antibody Interactions

In many situations, it is important to know the affinity of an antibody for an antigen. For example, the usefulness of a monoclonal antibody as an experimental or therapeutic reagent depends on its affinity. Antibody affinities for antigen can be measured directly for small antigens (e.g., haptens) by a method called **equilibrium dialysis** (Fig. A-5). In this method, a solution of antibody is confined within a "semipermeable" membrane of porous cellulose and immersed in a solution containing

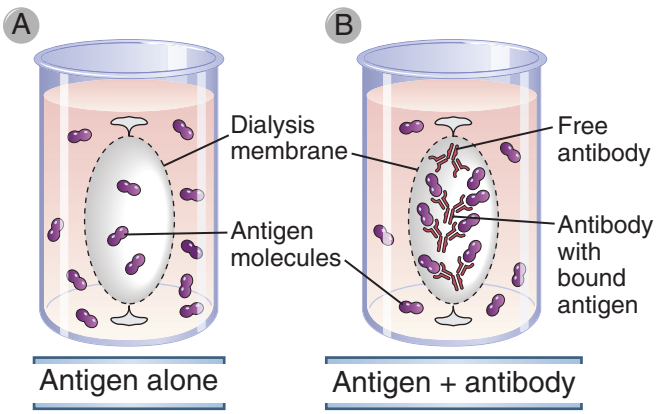


FIGURE A–5 Analysis of antigen-antibody binding by equilibrium dialysis. In the presence of antibody **(B)**, the amount of antigen within the dialysis membrane is increased compared with the absence of antibody **(A)**. As described in the text, this difference, caused by antibody binding of antigen, can be used to measure the affinity of the antibody for the antigen. This experiment can be performed only when the antigen is a small molecule (e.g., a hapten) capable of freely crossing the dialysis membrane.

the antigen. (Semipermeable in this context means that small molecules, such as antigen, can pass freely through the membrane pores but that macromolecules, such as antibody, cannot.) If no antibody is present within the membrane-bound compartment, the antigen in the bathing solution enters until the concentration of antigen within the membrane-bound compartment becomes exactly the same as that outside. Another way to view the system is that at dynamic equilibrium, antigen enters and leaves the membrane-bound compartment at exactly the same rate. However, when antibody is present inside the membrane, the net amount of antigen inside the membrane at equilibrium increases by the quantity that is bound to antibody. This phenomenon occurs because only unbound antigen can diffuse across the membrane, and at equilibrium, it is the unbound concentration of antigen that must be identical inside and outside the membrane. The extent of the increase in antigen inside the membrane depends on the antigen concentration, on the antibody concentration, and on the dissociation constant (K_d) of the binding interaction. By measurement of the antigen and antibody concentrations, by spectroscopy or by other means, K_d can be calculated.

An alternative way to determine K_d is by measurement of the rates of antigen-antibody complex formation and dissociation. These rates depend, in part, on the concentrations of antibody and antigen and on the affinity of the interaction. All parameters except the concentrations can be summarized as rate constants, and both the **on-rate constant** (K_{on}) and the **off-rate constant** (K_{off}) can be calculated experimentally by determining the concentrations and the actual rates of association or dissociation, respectively. The ratio of K_{off}/K_{on} allows one to cancel out all the parameters not related to affinity and is exactly equal to the dissociation constant K_d. Thus, one can measure K_d at equilibrium by equilibrium dialysis or calculate K_d from rate constants measured under non-equilibrium conditions.

Another method, more commonly used today, to measure the kinetics of antigen-antibody interactions depends on **surface plasmon resonance**. In this method, a specialized biosensing instrument (such as the Biacore) uses an optical approach to measure the affinity of an antibody that is passed over an antigen that is immobilized over a metal film. A light source is focused on this film through a prism at a specific angle (resonance), and the reflected light provides a surface plasmon resonance readout. Adsorption of an antibody to the antigen alters the surface plasmon resonance readout, and this alteration can provide information on affinity.

TRANSGENIC MICE AND GENE TARGETING

Three important and related methods for studying the functional effects of specific gene products in vivo are the creation of conventional transgenic mice that ectopically express a particular gene in a defined tissue; the creation of gene "knockout" mice, in which a targeted disruption is used to ablate the function of a particular gene; and the generation of "knockin" mice, in which an existing gene in the germline is replaced with a modified version of the same. A knockin approach could either replace a normal version of a gene with a mutant version or, in principle, "correct" an existing mutant gene with a "normal" version. These techniques involving genetically engineered mice have been widely used to analyze many biologic phenomena, including the development, activation, and tolerance of lymphocytes.

For the creation of conventional transgenic mice, foreign DNA sequences, called transgenes, are introduced into the pronuclei of fertilized mouse eggs, and the eggs are implanted into the oviducts of pseudopregnant females. Usually, if a few hundred copies of a gene are injected into pronuclei, about 25% of the mice that are born are transgenic. One to 50 copies of the transgene insert in tandem into a random site of breakage in a chromosome and are subsequently inherited as a simple mendelian trait. Because integration usually occurs before DNA replication, most (about 75%) of the transgenic pups carry the transgene in all their cells, including germ cells. In most cases, integration of the foreign DNA does not disrupt endogenous gene function. Also, each founder mouse carrying the transgene is a heterozygote, from which homozygous lines can be bred.

The great value of transgenic technology is that it can be used to express genes in particular tissues by attaching coding sequences of the gene to regulatory sequences that normally drive the expression of genes selectively in that tissue. For instance, lymphoid promoters and enhancers can be used to overexpress genes, such as rearranged antigen receptor genes, in lymphocytes, and the insulin promoter can be used to express genes in the β cells of pancreatic islets. Examples of the utility of these methods for study of the immune system are mentioned in many chapters of this book. Transgenes can also be expressed under the control of promoter elements that respond to drugs or hormones, such as tetracycline or estrogens. In these cases, transcription of the transgene can be controlled at will by administration of the inducing agent.

A powerful method for development of animal models of single-gene disorders, and the most definitive way to establish the obligatory function of a gene in vivo, is the creation of knockout mice by targeted mutation or disruption of the gene. This technique relies on the phenomenon of homologous recombination. If an exogenous gene is inserted into a cell, for instance, by electroporation, it can integrate randomly into the cell's genome. However, if the gene contains sequences that are homologous to an endogenous gene, it will preferentially recombine with and replace endogenous sequences. To select for cells that have undergone homologous recombination, a drug-based selection strategy is used. The fragment of homologous DNA to be inserted into a cell is placed in a vector typically containing a neomycin resistance gene and a viral thymidine kinase *(tk)* gene (Fig. A-6A). This targeting vector is constructed in such a way that the neomycin resistance gene is always inserted into the chromosomal DNA, but the *tk* gene is lost whenever homologous recombination (as opposed to random insertion) occurs. The vector is introduced into cells, and the cells are grown in neomycin and ganciclovir, a drug that is metabolized by thymidine kinase to

generate a lethal product. Cells in which the gene is integrated randomly will be resistant to neomycin but will be killed by ganciclovir, whereas cells in which homologous recombination has occurred will be resistant to both drugs because the *tk* gene will not be incorporated. This positive-negative selection ensures that the inserted gene in surviving cells has undergone homologous recombination with endogenous sequences. The presence of the inserted DNA in the middle of an endogenous gene usually disrupts the coding sequences and ablates expression or function of that gene. In addition, targeting vectors can be designed such that homologous recombination will lead to the deletion of one or more exons of the endogenous gene.

To generate a mouse carrying a targeted gene disruption or mutation, a targeting vector is used to first disrupt the gene in a murine embryonic stem (ES) cell line. ES cells are pluripotent cells derived from mouse embryos that can be propagated and induced to differentiate in culture or that can be incorporated into a mouse blastocyst, which may be implanted in a pseudopregnant mother and carried to term. Importantly, the progeny of the ES cells develop normally into mature tissues that will express the exogenous genes that have been transfected into the ES cells. Thus, the targeting vector designed to disrupt a particular gene is inserted into ES cells, and colonies in which homologous recombination has occurred (on one chromosome) are selected with drugs, as described before (Fig. A-6B). The presence of the desired recombination is verified by analysis of DNA with techniques such as Southern blot hybridization or polymerase chain reaction. The selected ES cells are injected into blastocysts, which are implanted into pseudopregnant females. Mice that develop will be chimeric for a heterozygous disruption or mutation, that is, some of the tissues will be derived from the ES cells and others from the remainder of the normal blastocyst. The germ cells are also usually chimeric, but because these cells are haploid, only some will contain the chromosome copy with the disrupted (mutated) gene. If chimeric mice are mated with normal (wild-type) animals and either sperm or eggs containing the chromosome with the mutation fuse with the wild-type partner, all cells in the offspring derived from such a zygote will be heterozygous for the mutation (so-called germline transmission). Such heterozygous mice can be mated to yield animals that will be homozygous for the mutation with a frequency that is predictable by simple mendelian segregation. Such knockout mice are deficient in expression of the targeted gene.

Homologous recombination can also be used to replace a normal gene sequence with a modified version of the same gene (or of another gene), thereby creating a knockin mouse strain. Knockin mice can be used to assess the biologic consequences of a change in a single base, for instance, as opposed to the deletion of a gene. A knockin approach could in principle also be used to replace a defective gene with a normal one. In certain circumstances, a different gene may be placed at a defined site in the genome by use of a knockin strategy rather than in a random site as in conventional transgenic mice. Knockin approaches are used when it is desirable to have the expression of the transgene regulated by certain endogenous DNA sequences, such as a particular enhancer or promoter region. In this case, the targeting vector contains an exogenous gene encoding a desired

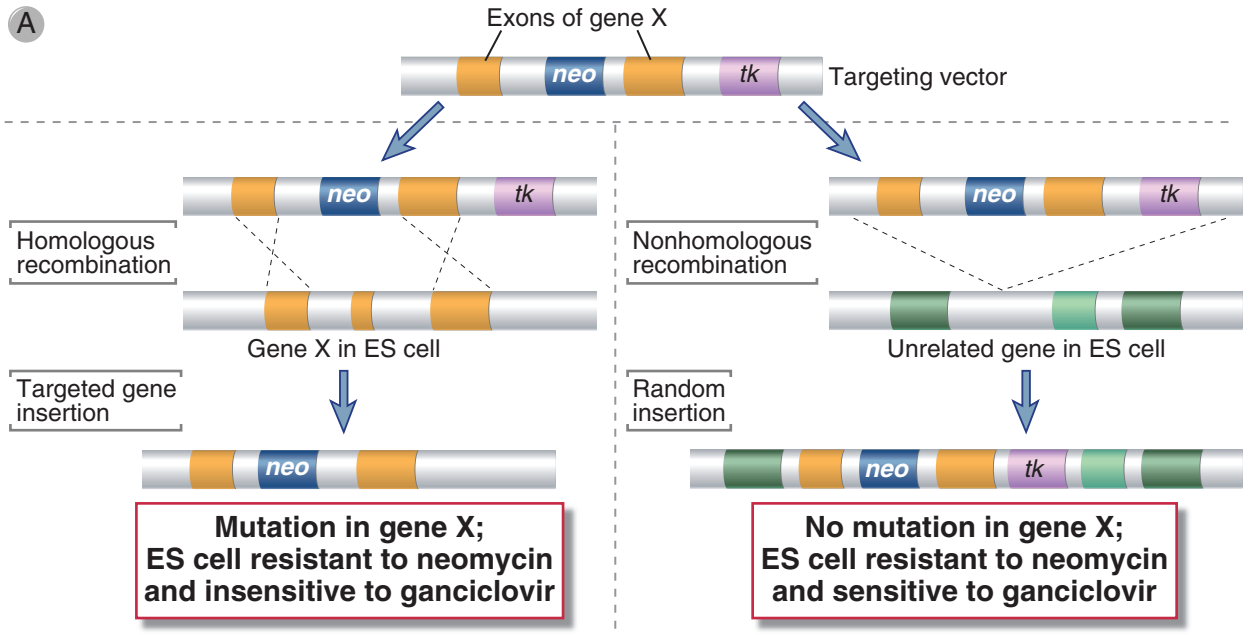


FIGURE A–6 Generation of gene knockout. A, The disruption of gene X in an embryonic stem (ES) cell is accomplished by homologous recombination. A population of ES cells is transfected with a targeting vector that contains sequences homologous to two exons of gene X flanking a neomycin resistance *(neo)* gene. The *neo* gene replaces or disrupts one of the exons of gene X on homologous recombination. The thymidine kinase *(tk)* gene in the vector will be inserted into the genome only if random, nonhomologous recombination occurs. *Continued*

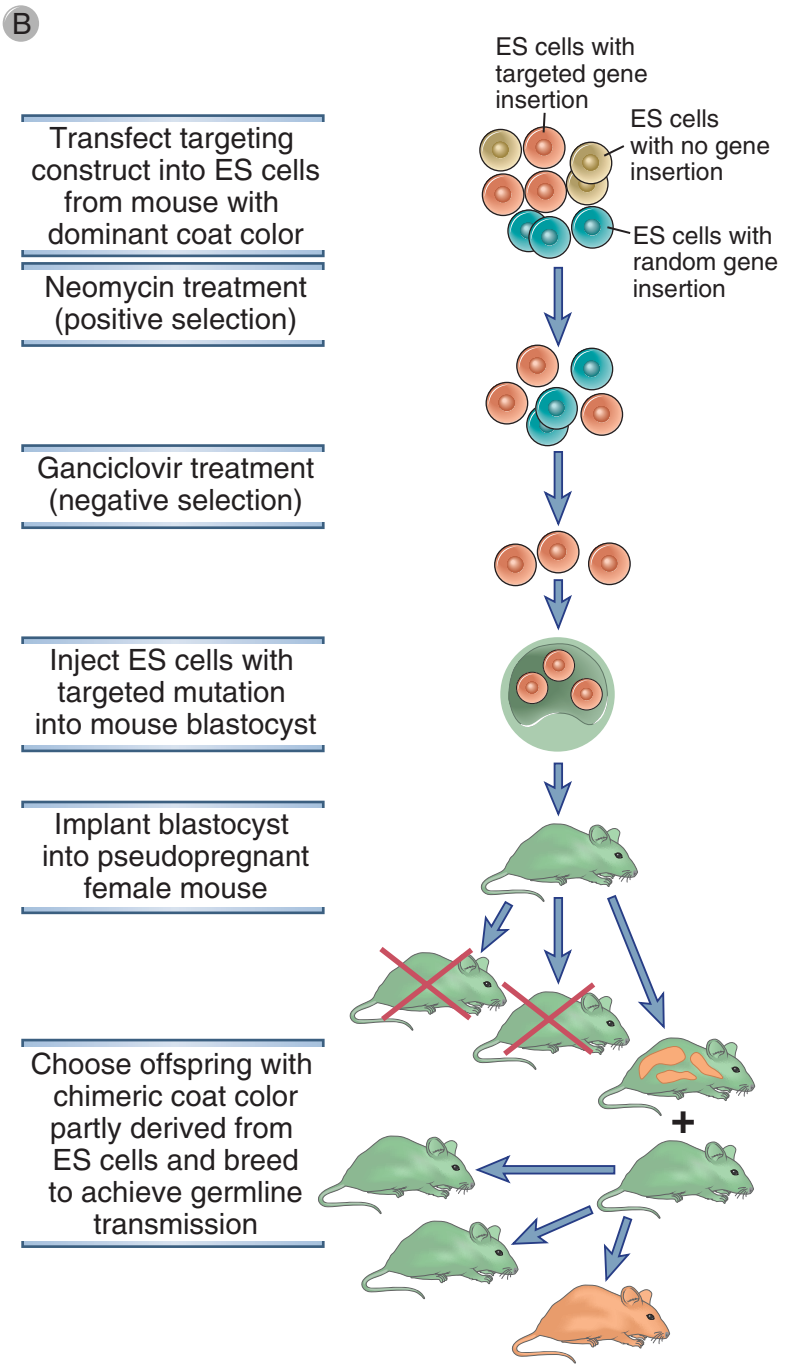


FIGURE A–6, cont'd B, The ES cells that were transfected by the targeting vector are selected by neomycin and ganciclovir so that only those cells with targeted insertion (homologous recombination) survive. These cells are then injected into a blastocyst, which is then implanted into the uterus of a pseudopregnant mouse. A chimeric mouse will develop in which some of the tissues are derived from the ES cell carrying the targeted mutation in gene X. These chimeric mice are identified by a mixed-color coat, including the color of the mouse strain from which the ES cells were derived and the color of the mouse strain from which the blastocyst was derived. If the mutation is present in germ cells, it can be propagated by further breeding.

product as well as sequences homologous to an endogenous gene that are needed to target the site of recombination.

Although the conventional gene-targeting strategy has proved to be of great usefulness in immunology research, the approach has some limitations. First, the mutation of one gene during development may be compensated for by altered expression of other gene products, and therefore the function of the targeted gene may be obscured. Second, in a conventional gene knockout mouse, the importance of a gene in only one tissue or at only one time during development cannot be easily assessed. Third, a functional selection marker gene, such as the neomycin resistance gene, is permanently introduced into the animal genome, and this alteration may have unpredictable results on the phenotype of the animal. An important refinement of gene knockout technology that can overcome many of these drawbacks is a "conditional" targeting approach. A commonly used conditional strategy takes advantage of the bacteriophage-derived Cre/*loxP* recombination system. The Cre enzyme is a DNA recombinase that recognizes a 34-bp sequence motif called *loxP,* and the enzyme mediates the deletion of gene segments flanked by two *loxP* sites in the same orientation. To generate mice with *loxP*-tagged genes, targeting vectors are constructed with one *loxP* site

flanking the neomycin resistance gene at one end and a second *loxP* site flanking the sequences homologous to the target at the other end. These vectors are transfected into ES cells, and mice carrying the *loxP*-flanked but still functional target gene are generated as described for conventional knockout mice. A second strain of mice carrying a *cre* transgene is then bred with the strain carrying the *loxP*-flanked target gene. In the offspring, expression of Cre recombinase will mediate deletion of the target gene. Both the normal gene sequences and the neomycin resistance gene will be deleted. Importantly, expression of the *cre* gene, and therefore deletion of the targeted gene, can be restricted to certain tissues or specified times by the use of *cre* transgene constructs with different promoters. For example, selective deletion of a gene only in helper T cells can be accomplished by using a *cre* transgenic mouse in which *cre* is driven by a CD4 promoter. Alternatively, a steroid-inducible promoter can be used so that Cre expression and subsequent gene deletion occur only after mice are given a dose of dexamethasone. Many other variations on this technology have been devised to create conditional mutants. Cre/*loxP* technology can also be used to create knockin mice. In this case, *loxP* sites are placed in the targeting vector to flank the neomycin resistance gene and the homologous sequences, but they do not flank the replacement (knockin) gene sequences. Therefore, after *cre*-mediated deletion, the exogenous gene remains in the genome at the targeted site.

METHODS FOR STUDYING T LYMPHOCYTE RESPONSES

Our current knowledge of the cellular events in T cell activation is based on a variety of experimental techniques in which different populations of T cells are activated by defined stimuli and functional responses are measured. In vitro experiments have provided a great deal of information on the changes that occur in a T cell when it is stimulated by antigen. More recently, several techniques have been developed to study T cell proliferation, cytokine expression, and anatomic redistribution in response to antigen activation in vivo. The new experimental approaches have been particularly useful for the study of naive T cell activation and the localization of antigen-specific memory T cells after an immune response has waned.

Polyclonal Activation of T Cells

Polyclonal activators of T cells bind to many or all T cell receptor (TCR) complexes regardless of specificity and activate the T cells in ways similar to peptide-MHC complexes on antigen-presenting cells (APCs). Polyclonal activators are mostly used in vitro to activate T cells isolated from human blood or the lymphoid tissues of experimental animals. Polyclonal activators can also be used to activate T cells with unknown antigen specificities, and they can evoke a detectable response from mixed populations of naive T cells, even though the frequency of cells specific for any one antigen would be too low to elicit a detectable response. The polymeric carbohydrate-binding plant proteins called lectins, such as concanavalin-A and phytohemagglutinin, are one commonly used group of polyclonal T cell activator. These lectins bind specifically to certain sugar residues on T cell surface glycoproteins, including the TCR and CD3 proteins, and thereby stimulate the T cells. Antibodies specific for invariant framework epitopes on TCR or CD3 proteins also function as polyclonal activators of T cells. Often, these antibodies need to be immobilized on solid surfaces or beads or cross-linked with secondary anti-antibodies to induce optimal activation responses. Because soluble polyclonal activators do not provide costimulatory signals that are normally provided by APCs, they are often used together with stimulatory antibodies to receptors for costimulators, such as anti-CD28 or anti-CD2. Superantigens, another kind of polyclonal stimulus, bind to and activate all T cells that express particular types of TCR β chain (see Chapter 15, Fig. 15-2). T cells of any antigen specificity can also be stimulated with pharmacologic reagents, such as the combination of the phorbol ester PMA and the calcium ionophore ionomycin, that mimic signals generated by the TCR complex.

Antigen-Induced Activation of Polyclonal T Cell Populations

Polyclonal populations of normal T cells that are enriched for T cells specific for a particular antigen can be derived from the blood and peripheral lymphoid organs of individuals after immunization with the antigen. The immunization serves to expand the number of antigen-specific T cells, which can then be restimulated in vitro by adding antigen and MHC-matched APCs to the T cells. This approach can be used to study antigen-induced activation of a mixed population of previously activated ("primed") T cells expressing many different TCRs, but the method does not permit analysis of responses of naive T cells.

Antigen-Induced Activation of T Cell Populations with a Single Antigen Specificity

Monoclonal populations of T cells, which express identical TCRs, have been useful for functional, biochemical, and molecular analyses. The limitation of these monoclonal populations is that they are maintained as long-term tissue culture lines and therefore may have phenotypically diverged from normal T cells in vivo. One type of monoclonal T cell population that is frequently used in experimental immunology is an antigen-specific T cell clone. Such clones are derived by isolating T cells from immunized individuals, as described for polyclonal T cells, followed by repetitive in vitro stimulation with the immunizing antigen plus MHC-matched APCs and cloning of single antigen–responsive cells in semisolid media or in liquid media by limiting dilution. Antigen-specific responses can easily be measured in these populations because all the cells in a cloned cell line have the same receptors and have been selected for growth in response to a known antigen-MHC complex. Both helper and cytotoxic T lymphocyte clones have been established

from mice and humans. Other monoclonal T cell populations used in the study of T cell activation include antigen-specific T cell hybridomas, which are produced like B cell hybridomas (see Fig. 5-9, Chapter 5), and tumor lines derived from T cells have been established in vitro after removal of malignant T cells from animals or humans with T cell leukemias or lymphomas. Although some tumor-derived lines express functional TCR complexes, their antigen specificities are not known, and the cells are usually stimulated with polyclonal activators for experimental purposes. The Jurkat line, derived from a human T cell leukemia cell, is an example of a tumor line that is widely used as a model to study T cell signal transduction.

TCR transgenic mice are a source of homogeneous, phenotypically normal T cells with identical antigen specificities that are widely used for in vitro and in vivo experimental analyses. If the rearranged α and β chain genes of a single TCR of known specificity are expressed as a transgene in mice, a majority of the mature T cells in the mice will express that TCR. If the TCR transgene is crossed onto a RAG-1– or RAG-2–deficient background, no endogenous TCR gene expression occurs and 100% of the T cells will express only the transgenic TCR. TCR transgenic T cells can be activated in vitro or in vivo with a single peptide antigen, and they can be identified by antibodies specific for the transgenic TCR. One of the unique advantages of TCR transgenic mice is that they permit the isolation of sufficient numbers of naive T cells of defined specificity to allow one to study functional responses to the first exposure to antigen. This advantage has allowed investigators to study the in vitro conditions under which antigen activation of naive T cells leads to differentiation into functional subsets such as T_H1 and T_H2 cells (see Chapter 9). Naive T cells from TCR transgenic mice can also be injected into normal syngeneic recipient mice, where they home to lymphoid tissues. The recipient mouse is then exposed to the antigen for which the transgenic TCR is specific. By use of antibodies that label the TCR transgenic T cells, it is possible to follow their expansion and differentiation in vivo and to isolate them for analysis of recall (secondary) responses to antigen ex vivo.

Methods to Enumerate and Study Functional Responses of T Cells

Proliferation assays for T lymphocytes, like those of other cells, are conducted in vitro by determining the amount of ^3H-labeled thymidine incorporated into the replicating DNA of cultured cells. Thymidine incorporation provides a quantitative measure of the rate of DNA synthesis, which is usually directly proportional to the rate of cell division. Cellular proliferation in vivo can be measured by injecting the thymidine analogue bromodeoxyuridine (BrdU) into animals and staining cells with anti-BrdU antibody to identify and enumerate nuclei that have incorporated BrdU into their DNA during DNA replication.

Fluorescent dyes can be used to study proliferation of T cells in vivo. T cells are first labeled with chemically reactive lipophilic fluorescent esters and then adoptively transferred into experimental animals. The dyes enter cells, form covalent bonds with cytoplasmic proteins, and then cannot leave the cells. One commonly used dye of this type is 5,6-carboxyfluorescein diacetate succinimidyl ester (CFSE), which can be detected in cells by standard flow cytometric techniques. Every time a T cell divides, its dye content is halved, and therefore it is possible to determine whether the adoptively transferred T cells present in lymphoid tissues of the recipient mouse have divided in vivo and to estimate the number of doublings each T cell has gone through.

Peptide-MHC tetramers are used to enumerate T cells with a single antigen specificity isolated from blood or lymphoid tissues of experimental animals or humans. These tetramers contain four of the peptide-MHC complexes that the T cell would normally recognize on the surface of APCs. The tetramer is made by producing a class I MHC molecule to which is attached a small molecule called biotin by use of recombinant DNA technology. Biotin binds with high affinity to a protein called avidin, and each avidin molecule binds four biotin molecules. Thus, avidin forms a substrate for assembly of four biotin-conjugated MHC proteins. The MHC molecules can be loaded with a peptide of interest and thus stabilized, and the avidin molecule is labeled with a fluorochrome, such as FITC. This tetramer binds to T cells specific for the peptide-MHC complex with high enough avidity to label the T cells even in suspension. This method is the only feasible approach for identification of antigen-specific T cells in humans. For instance, it is possible to identify and enumerate circulating HLA-A2–restricted T cells specific for an HIV peptide by staining blood cells with a tetramer of HLA-A2 molecules loaded with the peptide. The same technique is being used to enumerate and isolate T cells specific for self antigens in normal individuals and in patients with autoimmune diseases. Peptide-MHC tetramers that bind to a particular transgenic TCR can also be used to quantify the transgenic T cells in different tissues after adoptive transfer and antigen stimulation. The technique is now widely used with class I MHC molecules; in class I molecules, only one polypeptide is polymorphic, and stable molecules can be produced in vitro. This is more difficult for class II molecules because both chains are polymorphic and required for proper assembly, but class II–peptide tetramers are also being produced.

Cytokine secretion assays can be used to quantify cytokine-secreting effector T cells within lymphoid tissues. The most commonly used methods are cytoplasmic staining of cytokines and single-cell enzyme-linked immunosorbent assays (ELISpot). In these types of studies, antigen-induced activation and differentiation of T cells take place in vivo, and then T cells are isolated and tested for cytokine expression in vitro. Cytoplasmic staining of cytokines requires permeabilizing of the cells so that fluorochrome-labeled antibodies specific for a particular cytokine can gain entry into the cell, and the stained cells are analyzed by flow cytometry. Cytokine expression by T cells specific for a particular antigen can be determined by additionally staining T cells with

peptide-MHC tetramers or, in the case of TCR transgenic T cells, antibodies specific for the transgenic TCR. By use of a combination of CFSE and anticytokine antibodies, it is possible to examine the relationship between cell division and cytokine expression. In the ELISpot assay, T cells freshly isolated from blood or lymphoid tissues are cultured in plastic wells coated with antibody specific for a particular cytokine. As cytokines are secreted from individual T cells, they bind to the antibodies in discrete spots corresponding to the location of individual T cells. The spots are visualized by adding secondary enzyme-linked anti-Ig, as in a standard ELISA (see earlier), and the number of spots is counted to determine the number of cytokine-secreting T cells.

METHODS FOR STUDYING B LYMPHOCYTE RESPONSES

Activation of Polyclonal B Cell Populations

It is technically difficult to study the effects of antigens on normal B cells because, as the clonal selection hypothesis predicted, very few lymphocytes in an individual are specific for any one antigen. An approach to circumventing this problem is to use anti-Ig antibodies as analogues of antigens, with the assumption that anti-Ig will bind to constant (C) regions of membrane Ig molecules on all B cells and will have the same biologic effects as an antigen that binds to the hypervariable regions of membrane Ig molecules on only the antigen-specific B cells. To the extent that precise comparisons are feasible, this assumption appears generally correct, indicating that anti-Ig antibody is a valid model for antigens. Thus, anti-Ig antibody is frequently used as a polyclonal activator of B lymphocytes, similar to the use of anti-CD3 antibodies as polyclonal activators of T lymphocytes discussed earlier.

Antigen-Induced Activation of B Cell Populations with a Single Antigen Specificity

To examine the effects of antigen binding to B cells, investigators have attempted to isolate antigen-specific B cells from complex populations of normal lymphocytes or to produce cloned B cell lines with defined antigenic specificities. These efforts have met with little success. However, transgenic mice have been developed in which virtually all B cells express a transgenic Ig of known specificity, so that most of the B cells in these mice respond to the same antigen. A somewhat more sophisticated approach has been to generate antigen receptor knockin mice, in which rearranged Ig H and L chain genes have been homologously recombined into their endogenous loci. Such knockin animals have proved particularly useful in the examination of receptor editing.

Assays to Measure B Cell Proliferation and Antibody Production

Much of our knowledge of B cell activation is based on in vitro experiments, in which different stimuli are used to activate B cells and their proliferation and differentiation can be measured accurately. The same assays may be done with B cells recovered from mice exposed to different antigens or with homogeneous B cells expressing transgene-encoded antigen receptors.

B cell proliferation is measured by use of CFSE labeling or ^{3}H-labeled thymidine incorporation in vitro and BrdU labeling in vivo, as described for T cell proliferation before.

Antibody production is measured in two different ways: with assays for cumulative Ig secretion, which measure the amount of Ig that accumulates in the supernatant of cultured lymphocytes or in the serum of an immunized individual; and with single-cell assays, which determine the number of cells in an immune population that secrete Ig of a particular specificity or isotype. The most accurate, quantitative, and widely used technique to measure the total amount of Ig in a culture supernatant or serum sample is ELISA. By use of antigens bound to solid supports, it is possible to use ELISA to quantify the amount of antibody in a sample specific for a particular antigen. In addition, the availability of anti-Ig antibodies that detect Igs of different heavy or light chain classes allows measurement of the quantities of different isotypes in a sample. Other techniques to measure antibody levels include hemagglutination for anti-erythrocyte antibodies and complement-dependent lysis for antibodies specific for known cell types. Both assays are based on the demonstration that if the amount of antigen (i.e., cells) is constant, the concentration of antibody determines the amount of antibody bound to cells, and this is reflected in the degree of cell agglutination or subsequent binding of complement and cell lysis. Results from these assays are usually expressed as antibody titers, which are the dilution of the sample giving half-maximal effects or the dilution at which the endpoint of the assay is reached.

A single-cell assay for antibody secretion is the ELISpot assay. In this method, antigen is bound to the bottom of a well, antibody-secreting cells are added, and antibodies that have been secreted and are bound to the antigen are detected by an enzyme-linked anti-Ig antibody, as in an ELISA, in a semisolid medium. Each spot represents the location of an antibody-secreting cell. Single-cell assays provide a measure of the numbers of Ig-secreting cells, but they cannot accurately quantify the amount of Ig secreted by each cell or by the total population. The ELISA and ELISpot techniques can be adapted to assess affinity of antibodies, by the use of antigens with differing numbers of hapten moieties. In this way, affinity maturation can be assessed by testing serum or B cells sampled at different times during an immune response.

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