The Adaptive Immune System

25

Our **adaptive immune system** saves us from certain death by infection. An infant born with a severely defective adaptive immune system will soon die unless extraordinary measures are taken to isolate it from a host of infectious agents, including bacteria, viruses, fungi, and parasites. All multicellular organisms need to defend themselves against infection by such potentially harmful invaders, collectively called **pathogens**. Invertebrates use relatively simple defense strategies that rely chiefly on protective barriers, toxic molecules, and phagocytic cells that ingest and destroy invading microorganisms *(microbes)* and larger parasites (such as worms). Vertebrates, too, depend on such **innate immune responses** as a first line of defense (discussed in Chapter 24), but they can also mount much more sophisticated defenses, called **adaptive immune responses**. In vertebrates, the innate responses call the adaptive immune responses into play, and both work together to eliminate the pathogens (**Figure 25–1**).

Whereas the innate immune responses are general defense reactions, the adaptive responses are highly specific to the particular pathogen that induced them, and they provide long-lasting protection. A person who recovers from measles, for example, is protected for life against measles by the adaptive immune system, although not against other common viruses, such as those that cause mumps or chickenpox. In this chapter, we focus on adaptive immune responses, and, unless we indicate otherwise, we use the term "immune responses" to refer to them.

Adaptive immune responses eliminate or destroy invading pathogens and any toxic molecules they produce. Because these responses are destructive, it is important that they are directed only against foreign molecules and not against molecules of the host itself. The adaptive immune system uses multiple mechanisms to avoid damaging responses against self molecules. Occasionally, however, these mechanisms fail, and the system turns against the host, causing *autoimmune diseases*, which can be fatal.

Many harmless foreign molecules enter the body, and it would be pointless and potentially dangerous to mount adaptive immune responses against them. Allergic conditions such as hayfever and allergic asthma are examples of deleterious adaptive immune responses against apparently harmless foreign molecules. An individual normally avoids such inappropriate responses because the innate immune system only calls adaptive immune responses into play when it recognizes conserved patterns of molecules that are specifically expressed by invading pathogens. The innate immune system can even distinguish between different classes of pathogens and recruit the most effective form of adaptive immune response to eliminate them.

Any substance capable of eliciting an adaptive immune response is referred to as an **antigen** (*anti*body *gen*erator). Most of what we know about such responses has come from studies in which an experimenter tricks the adaptive immune system of a laboratory animal (usually a mouse) into responding to a harmless foreign molecule, such as a foreign protein. The trick involves injecting the harmless molecule together with immunostimulants (usually microbial in origin) called *adjuvants*, which activate the innate immune system. This trick is called **immunization**. If administered in this way, almost any macromolecule, as long as it is foreign to the recipient, can induce an adaptive immune response

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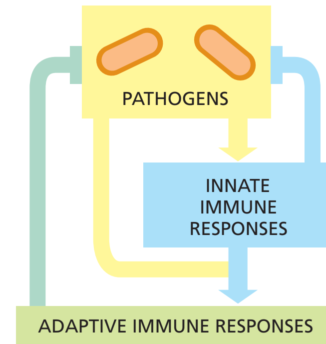


Figure 25–1 Innate and adaptive immune responses. Innate immune responses are activated directly by pathogens and defend all multicellular organisms against infection. In vertebrates, pathogens, together with the innate immune responses they activate, stimulate adaptive immune responses, which then work together with innate immune responses to help fight the infection.

that is specific to that macromolecule. Remarkably, the adaptive immune system can distinguish between antigens that are very similar—such as between two proteins that differ in only a single amino acid, or between two optical isomers of the same molecule. Thus, the adaptive immune system recognizes the fine molecular details of macromolecules.

Adaptive immune responses are carried out by white blood cells called **lymphocytes**. There are two broad classes of such responses—*antibody responses* and *T-cell-mediated immune responses*—and different classes of lymphocytes, called B cells and T cells, respectively, carry them out. In **antibody responses**, B cells are activated to secrete antibodies, which are proteins called *immunoglobulins*. The antibodies circulate in the bloodstream and permeate the other body fluids, where they bind specifically to the foreign antigen that stimulated their production (**Figure 25–2**). Binding of antibody inactivates viruses and microbial toxins (such as tetanus toxin or diphtheria toxin) by blocking their ability to bind to receptors on host cells. Antibody binding also marks invading pathogens for destruction, mainly by making it easier for phagocytic cells of the innate immune system to ingest them.

In **T-cell-mediated immune responses**, the second class of adaptive immune responses, activated T cells react directly against a foreign antigen that is presented to them on the surface of a host cell, which is therefore referred to as an *antigen-presenting cell*. Remarkably, T cells can detect microbes hiding inside host cells and either kill the infected cells or help the infected cells or other cells to eliminate the microbes. The T cell, for example, might kill a virus-infected host cell that has viral antigens on its surface, thereby eliminating the infected cell before the virus has had a chance to replicate (see Figure 25–2). In other cases, the T cell produces signal molecules that either activate macrophages to destroy the microbes that they have phagocytosed or help activate B cells to make antibodies against the microbes.

We begin this chapter by discussing the general properties of lymphocytes. We then consider the functional and structural features of antibodies that enable them to recognize and neutralize extracellular microbes and the toxins they make. Next, we discuss how B cells can produce a virtually unlimited number of different antibody molecules. Finally, we consider the special features of T cells and the immune responses they mediate.

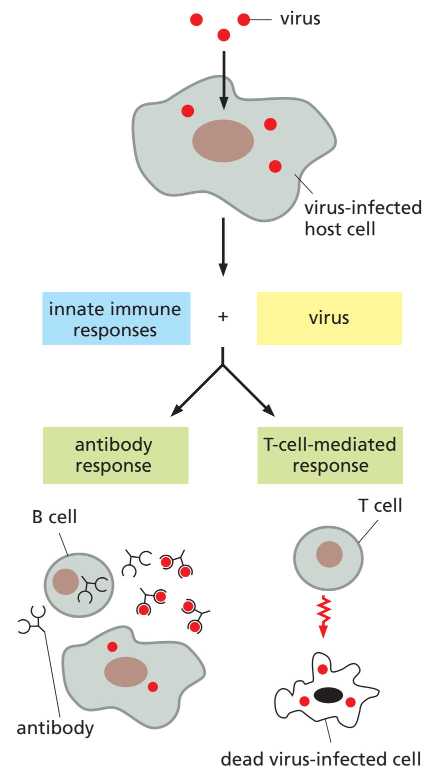


Figure 25–2 The two main classes of adaptive immune responses. Lymphocytes carry out both classes of responses. Here, the lymphocytes are responding to a viral infection. In one class of adaptive response, B cells secrete antibodies that neutralize the virus. In the other, a T-cell-mediated response, T cells kill the virus-infected cells. In both cases, innate immune responses help activate the adaptive immune responses through pathways that are not shown.

LYMPHOCYTES AND THE CELLULAR BASIS OF ADAPTIVE IMMUNITY

Lymphocytes are responsible for the astonishing specificity of adaptive immune responses. They occur in large numbers in the blood and lymph (the colorless fluid in the lymphatic vessels that connect the lymph nodes in the body to each other and to the bloodstream). They are also concentrated in **lymphoid organs**, such as the thymus, lymph nodes (also called lymph glands), spleen, and appendix (**Figure 25–3**). In this section, we discuss the general properties of lymphocytes that apply to both B cells and T cells.

Lymphocytes Are Required for Adaptive Immunity

There are about 2×10^{12} lymphocytes in the human body, making the immune system comparable in cell mass to the liver or the brain. Despite their abundance, their central role in adaptive immunity was not definitively demonstrated until

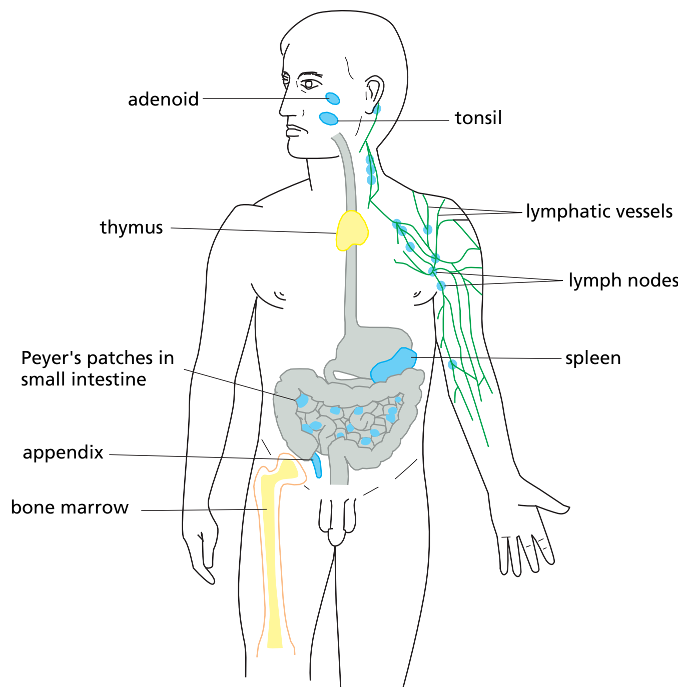


Figure 25–3 Human lymphoid organs. Lymphocytes develop in the thymus and bone marrow *(yellow),* which are therefore called *central* (or *primary*) *lymphoid organs*. The newly formed lymphocytes migrate from these primary organs to *peripheral* (or *secondary*) *lymphoid organs,* where they can react with foreign antigen. Only some of the peripheral lymphoid organs *(blue)* and lymphatic vessels *(green)* are shown; many lymphocytes, for example, are found in the skin and respiratory tract. As we discuss later, the lymphatic vessels ultimately empty into the bloodstream (not shown).

the late 1950s. The crucial experiments were performed in mice and rats that were heavily irradiated to kill most of their white blood cells, including lymphocytes. This treatment makes the animals unable to mount adaptive immune responses. Then, by transferring various types of cells into the animals it was possible to determine which cells reversed the deficiency. Lymphocytes were the only cell type able to restore the adaptive immune responses of irradiated animals, indicating that they are required for these responses (**Figure 25–4**).

The Innate and Adaptive Immune Systems Work Together

As mentioned earlier, lymphocytes usually respond to foreign antigens only if the **innate immune system** is first activated. As discussed in Chapter 24, the rapid innate immune responses to an infection depend largely on **pattern recognition receptors** made by cells of the innate immune system. These receptors recognize

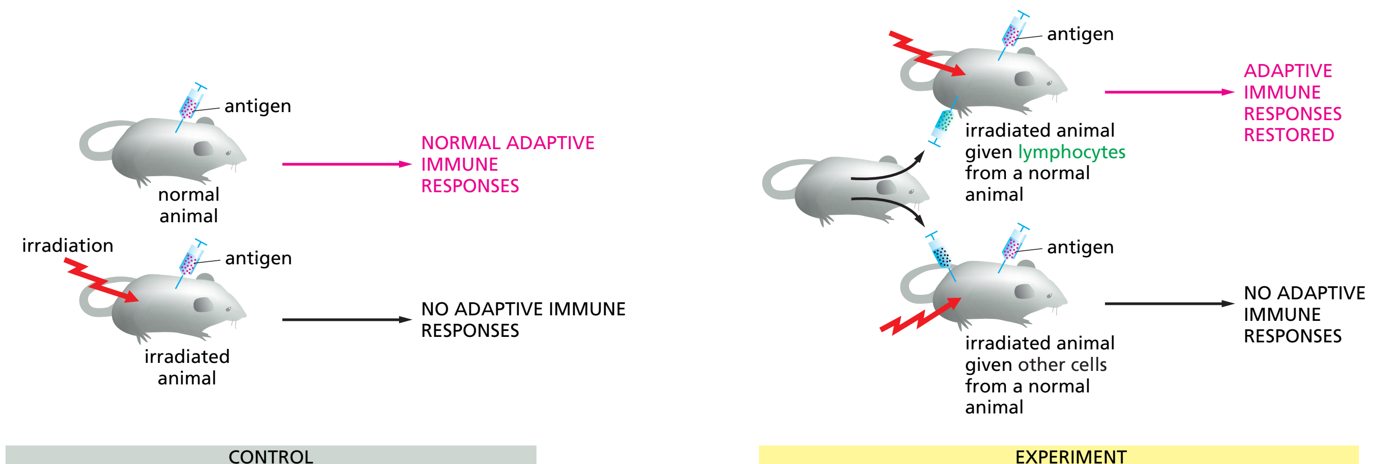


Figure 25–4 A classic experiment showing that lymphocytes are required for adaptive immune responses to foreign antigens. An important requirement of all such cell-transfer experiments is that cells are transferred between animals of the same *inbred strain.* Members of an inbred strain are genetically identical. If lymphocytes are transferred to a genetically different animal that has been irradiated, they react against the "foreign" antigens of the host and can kill the animal. In the experiment shown, the injection of lymphocytes restores both antibody and T-cell-mediated adaptive immune responses, indicating that lymphocytes are required for both types of responses.

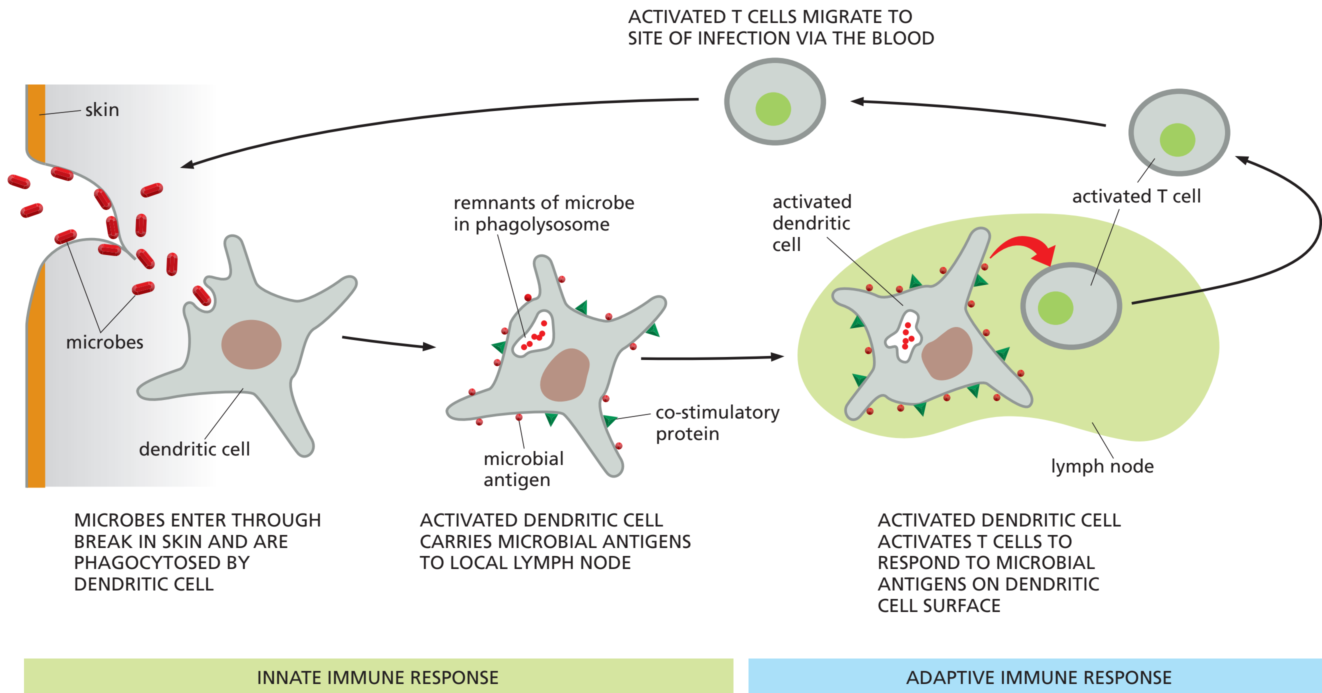


Figure 25–5 How the innate immune system can help activate the adaptive immune system. Dendritic cells ingest invading microbes or their products at the site of an infection. The microbial PAMPs activate the dendritic cells to express *co-stimulatory proteins* on their surface and to migrate in lymphatic vessels to a nearby lymph node. In the lymph node, the activated dendritic cells activate the small fraction of T cells that express a receptor for the microbial antigens displayed on the dendritic cell surface. These T cells proliferate and some then migrate to the site of infection, where they help eliminate the microbes, by either helping to activate macrophages or killing infected cells (not shown).

microbe-associated molecules that are not present in the host organism, called *microbe-associated immunostimulants*. Because they often occur in repeating patterns, they are also called *pathogen-associated molecular patterns (PAMPs)*. PAMPs include repeated patterns of molecular structure in microbial nucleic acids, lipids, polysaccharides, and proteins.

Some of the pattern recognition receptors are present on the surface of professional phagocytic cells (phagocytes) such as macrophages and neutrophils, where they mediate the uptake of pathogens, which are then delivered to lysosomes for destruction. Others are secreted and bind to the surface of pathogens, marking them for destruction by either phagocytes or a system of blood proteins collectively called the *complement system* (discussed in Chapter 24). Still others, including the *Toll-like receptors (TLRs)* discussed in Chapter 24, activate intracellular signaling pathways that lead to the secretion of extracellular signal molecules that promote inflammation and help activate adaptive immune responses.

The cells of the vertebrate innate immune system that respond to PAMPs and activate adaptive immune responses most efficiently are **dendritic cells**. Present in most tissues, dendritic cells express high levels of TLRs and other pattern recognition receptors, and they function by presenting microbial antigens to T cells in peripheral lymphoid organs. In most cases, they recognize and phagocytose invading microbes or their products or fragments of infected cells at a site of infection and then migrate with their prey to a nearby lymph node; in other cases, they pick up microbes or their products directly in a peripheral lymphoid organ such as the spleen. In either case, the microbial PAMPs activate the dendritic cells so that they, in turn, can directly activate the T cells in peripheral lymphoid organs to respond to the microbial antigens displayed on the dendritic cell surface. Once activated, some of the T cells then migrate to the site of infection, where they help destroy the microbes (**Figure 25–5**). Other activated T cells remain in the lymphoid organ, where they help keep the dendritic cells active, help activate other T cells, and help activate B cells to make antibodies against the microbial antigens.

Thus, innate immune responses are activated mainly at sites of infection (or injury), whereas adaptive immune responses are activated mainly in peripheral lymphoid organs such as lymph nodes and spleen. Both types of responses work together to eliminate invading pathogens and foreign macromolecules.

B Lymphocytes Develop in the Bone Marrow; T Lymphocytes Develop in the Thymus

T cells and B cells derive their names from the organs in which they develop. T cells develop in the *thymus*, and B cells, in mammals, develop in the *bone marrow* in adults or the liver in fetuses.

Both T and B cells are thought to develop from the same *common lymphoid progenitor cells*. The common lymphoid progenitor cells themselves derive from multipotential *hemopoietic stem cells*, which give rise to all of the blood cells, including red blood cells, white blood cells, and platelets. These stem cells (discussed in Chapter 23) are located primarily in *hemopoietic* tissues—mainly the liver in fetuses and the bone marrow in adults.

T cells develop in the thymus from common lymphoid progenitor cells that migrate there from the hemopoietic tissues via the blood. In most mammals, including humans and mice, B cells develop from common lymphoid progenitor cells in the hemopoietic tissues themselves (**Figure 25–6**). Because they are sites where lymphocytes develop from precursor cells, the thymus and hemopoietic tissues are referred to as **central** (**primary**) **lymphoid organs** (see Figure 25–3).

As we discuss later, most lymphocytes die in the central lymphoid organs soon after they develop, without ever functioning. Others, however, mature and migrate via the blood to the **peripheral** (**secondary**) **lymphoid organs**—mainly, the lymph nodes, spleen, and epithelium-associated lymphoid tissues in the gastrointestinal tract, respiratory tract, and skin (see Figure 25–3). It is in these peripheral lymphoid organs that foreign antigens activate T and B cells (see Figure 25–6).

T and B cells become morphologically distinguishable from each other only after they have been activated by antigen. Resting T and B cells look very similar, even in an electron microscope. Both are small, only marginally bigger than red blood cells, and contain little cytoplasm (**Figure 25–7**A). After activation by an antigen, both proliferate and mature into *effector cells*. Effector B cells secrete antibodies. In their most mature form, called *plasma cells*, they are filled with an extensive rough endoplasmic reticulum that is busily making antibodies (Figure 25–7B). In contrast, effector T cells (Figure 5–7C) contain very little endoplasmic reticulum and do not secrete antibodies; instead, they secrete a variety of signal proteins called **cytokines**, which act as local mediators.

There are three main classes of T cells—cytotoxic T cells, helper T cells, and regulatory (suppressor) T cells. *Cytotoxic T cells* directly kill infected host cells. *Helper T cells* help activate macrophages, dendritic cells, B cells, and cytotoxic T cells by secreting a variety of cytokines and displaying a variety of co-stimulatory proteins on their surface. *Regulatory T cells* are thought to use similar strategies

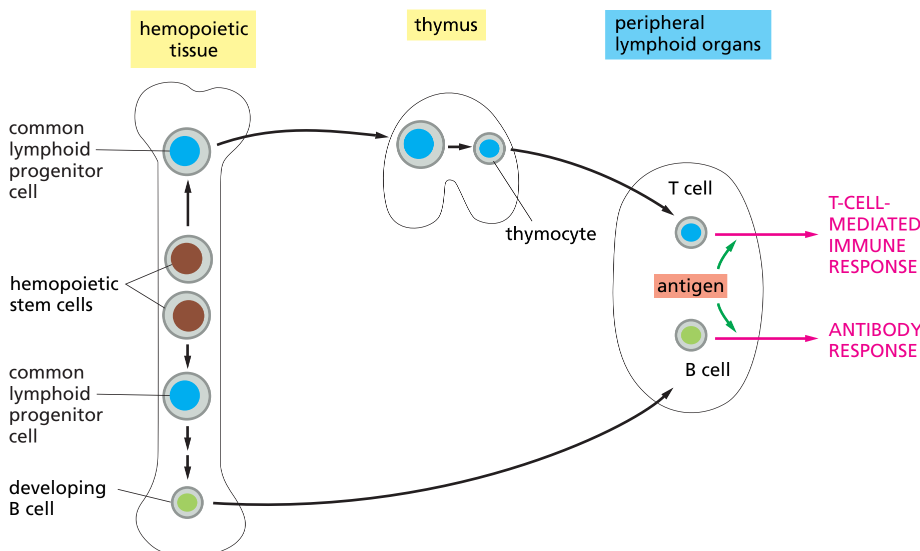


Figure 25–6 The development of T and B cells. The central lymphoid organs, where lymphocytes develop from common lymphoid progenitor cells, are labeled in *yellow boxes*. The common lymphoid progenitor cells develop from multipotent hemopoietic stem cells in the bone marrow. Some of the common lymphoid progenitor cells develop locally in the bone marrow into immature B cells, while others migrate to the thymus (via the bloodstream) where they develop into thymocytes (developing T cells). T cells and B cells are activated by foreign antigens mainly in peripheral lymphoid organs, such as lymph nodes or the spleen.

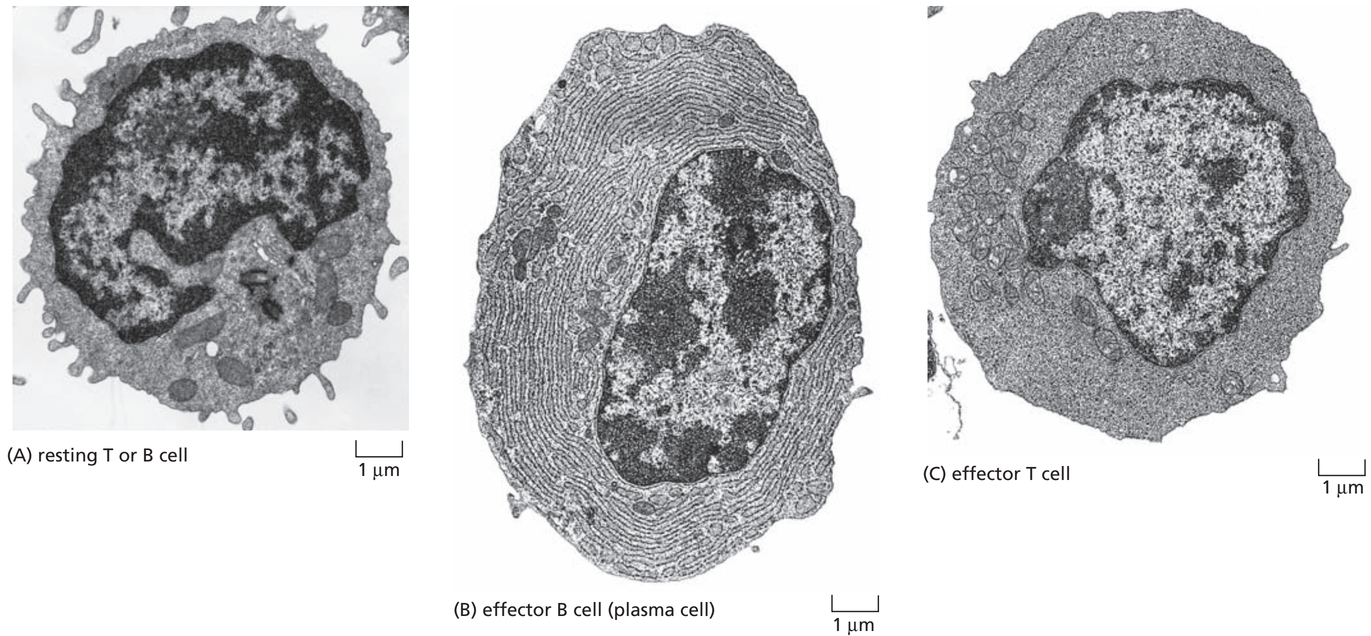


Figure 25–7 Electron micrographs of resting and effector lymphocytes. (A) This resting lymphocyte could be either a T cell or a B cell, as these cells are difficult to distinguish morphologically until they have been activated to become effector cells. (B) An effector B cell (a plasma cell). It is filled with an extensive rough endoplasmic reticulum (ER), which is distended with antibody molecules. (C) An effector T cell, which has relatively little rough ER but is filled with free ribosomes. The three cells are shown at the same magnification. (A, courtesy of Dorothy Zucker-Franklin; B, courtesy of Carlo Grossi; A and B, from D. Zucker-Franklin et al., Atlas of Blood Cells: Function and Pathology, 2nd ed. Milan, Italy: Edi. Ermes, 1988; C, courtesy of Stefanello de Petris.)

to inhibit the function of helper T cells, cytotoxic T cells, and dendritic cells. Thus, whereas B cells can act over long distances by secreting antibodies that are widely distributed by the bloodstream, T cells can migrate to distant sites, but, once there, they act only locally on neighboring cells.

The Adaptive Immune System Works by Clonal Selection

The most remarkable feature of the adaptive immune system is that it can respond to millions of different foreign antigens in a highly specific way. Human B cells, for example, can make more than 10^{12} different antibody molecules that react specifically with the antigen that induced their production. How do B cells produce such a diversity of specific antibodies? The answer began to emerge in the 1950s with the formulation of the **clonal selection theory**. According to this theory, an animal first randomly generates a vast diversity of lymphocytes and then selects for activation those lymphocytes that can react against the foreign antigens that the animal actually encounters. As each lymphocyte develops in a central lymphoid organ, it becomes committed to react with a particular antigen before ever being exposed to the antigen. It expresses this commitment in the form of cell-surface receptor proteins that specifically bind the antigen. When a lymphocyte encounters its antigen in a peripheral lymphoid organ, the binding of the antigen to the receptors activates the lymphocyte, causing it to proliferate, thereby producing many more cells with the same receptor—a process called *clonal expansion* (as cells derived from a common ancestor cell are referred to as a *clone*). The encounter with antigen also causes the cells to differentiate into *effector cells*. An antigen therefore selectively stimulates those cells that express complementary antigen-specific receptors and are thus already committed to respond to it (**Figure 25–8**). This arrangement is what makes adaptive immune responses antigen-specific.

Compelling evidence supports the main tenets of the clonal selection theory. But how can the adaptive immune system produce lymphocytes that collectively display such an enormous diversity of receptors, including ones that recognize synthetic molecules that never occur in nature? We shall see later that, in humans, the antigen-specific receptors on both T and B cells are encoded by genes that are assembled from a series of gene segments by a special form of genetic recombination that occurs early in a lymphocyte's development, before it has encountered antigen. This assembly process generates an enormous diversity of receptors and lymphocytes, thereby enabling the immune system to respond to an almost unlimited variety of antigens.

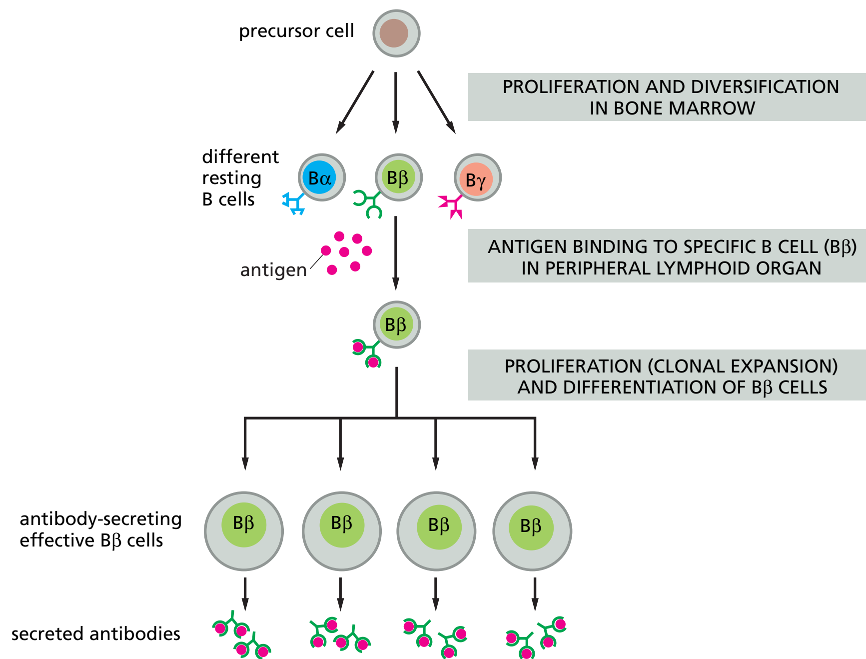


Figure 25–8 The clonal selection theory. An antigen activates only those lymphocytes that are already committed to respond to it. A cell committed to respond to a particular antigen displays cell-surface receptors that specifically recognize the antigen. The human immune system is thought to consist of many millions of different lymphocyte clones, with cells within a clone expressing the same unique receptor. Before their first encounter with antigen, a clone would usually contain only one or a small number of cells. A particular antigen may activate hundreds of different clones. Although only B cells are shown here, T cells operate in a similar way. Note that the receptors on B cells are antibody molecules and that those on the B cells labeled "Bβ" in this diagram bind the same antigen as do the antibodies secreted by the effector "Bβ" cells.

Most Antigens Activate Many Different Lymphocyte Clones

Most large molecules, including virtually all proteins and many polysaccharides, can act as antigens. Those parts of an antigen that bind to the antigen-binding site on either an antibody molecule or a lymphocyte receptor are called **antigenic determinants** (or *epitopes*). Most antigens have a variety of antigenic determinants that can stimulate the production of antibodies, specific T cell responses, or both. Some determinants of an antigen produce a greater response than others, so that the reaction to them may dominate the overall response. Such determinants are called *immunodominant.*

Any antigenic determinant is likely to activate many lymphocyte clones, each of which produces an antigen-binding site with its own characteristic affinity for the determinant. Even a relatively simple structure, like the *dinitrophenyl (DNP)* group in **Figure 25–9**, can be "looked at" in many ways. When it is coupled to a protein, as shown in the figure, it usually stimulates the production of hundreds of species of anti-DNP antibodies, each made by a different B cell clone. Such responses are said to be *polyclonal.* In *oligoclonal* responses, only a few clones are activated, and in *monoclonal* responses only a single B or T cell clone is activated. Monoclonal antibodies are widely used as tools in biology and medicine, but they have to be produced in a special way (see Figure 8–8), as the responses to most antigens are polyclonal.

Immunological Memory Involves Both Clonal Expansion and Lymphocyte Differentiation

The adaptive immune system, like the nervous system, can remember prior experiences. This is why we develop lifelong immunity to many common infectious diseases after our initial exposure to the pathogen, and it is why vaccination works. The same phenomenon can be demonstrated in experimental animals. If an animal is immunized once with antigen A, an immune response (antibody, T-cell-mediated, or both) appears after several days, rises rapidly and

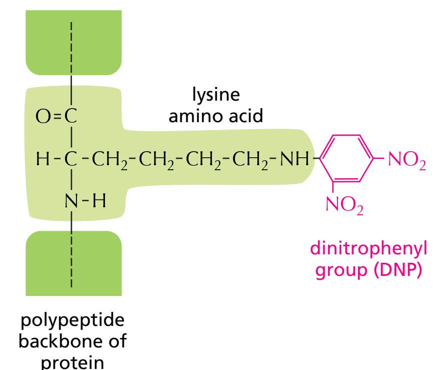


Figure 25–9 The dinitrophenyl (DNP) group. Although it is too small to induce an immune response on its own, when it is coupled covalently to a lysine side chain on a protein, as illustrated, DNP stimulates the production of hundreds of different species of antibodies that all bind specifically to it.

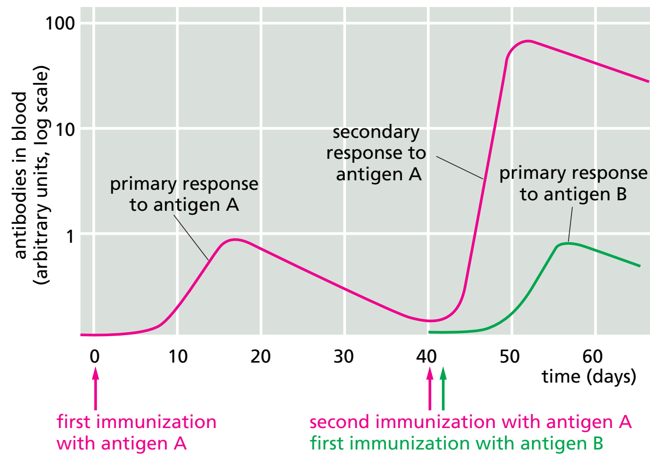


Figure 25–10 Primary and secondary antibody responses. The secondary response induced by a second exposure to antigen A is faster and greater than the primary response and is specific for A, indicating that the adaptive immune system has specifically remembered its previous encounter with antigen A. The same type of immunological memory is observed in T-cell-mediated responses. As we discuss later, the types of antibodies produced in the secondary response are different from those produced in the primary response, and these antibodies bind the antigen more tightly.

exponentially, and then, more gradually, declines. This is the characteristic course of a **primary immune response**, occurring on an animal's first exposure to an antigen. If, after some weeks, months, or even years have elapsed, the animal is immunized again with antigen A, it will usually produce a **secondary immune response** that differs from the primary response: the lag period is shorter, and the response is greater and more efficient. These differences indicate that the animal has "remembered" its first exposure to antigen A. If the animal is given a different antigen (for example, antigen B) instead of a second immunization with antigen A, the response is typical of a primary, and not a secondary, immune response. The secondary response must therefore reflect antigen-specific **immunological memory** for antigen A (**Figure 25–10**).

The clonal selection theory provides a useful conceptual framework for understanding the cellular basis of immunological memory. In an adult animal, the peripheral lymphoid organs contain a mixture of lymphocytes in at least three stages of maturation: *naïve cells*, *effector cells*, and *memory cells*. When **naïve cells** encounter their antigen for the first time, the antigen stimulates some of them to proliferate and differentiate into **effector cells**, which then carry out an immune response (effector B cells secrete antibody, while effector T cells either kill infected cells or influence the response of other cells). Some of the antigen-stimulated naïve cells multiply and differentiate into **memory cells**, which do not themselves carry out immune responses but are more easily and more quickly induced to become effector cells by a later encounter with the same antigen. When they encounter their antigen, memory cells (like naïve cells), give rise to either effector cells or more memory cells (**Figure 25–11**).

Thus, the primary response generates immunological memory because of clonal expansion, whereby the proliferation of antigen-stimulated naïve cells creates many memory cells, as well as because these memory cells are able to respond more sensitively, rapidly, and effectively to the same antigen than do naïve cells. And, unlike most effector cells, which die within days or weeks, memory cells can persist for the lifetime of the animal, even in the absence of their specific antigen, thereby providing lifelong immunological memory.

As we discuss later, memory B cells produce antibodies of different classes and of much higher affinity for antigen than those produced by naïve B cells. This is the main reason that secondary antibody responses are much more effective at eliminating pathogens than are primary antibody responses.

Although most effector T and B cells die after an immune response is over, some survive as effector cells and help provide long-term protection against the

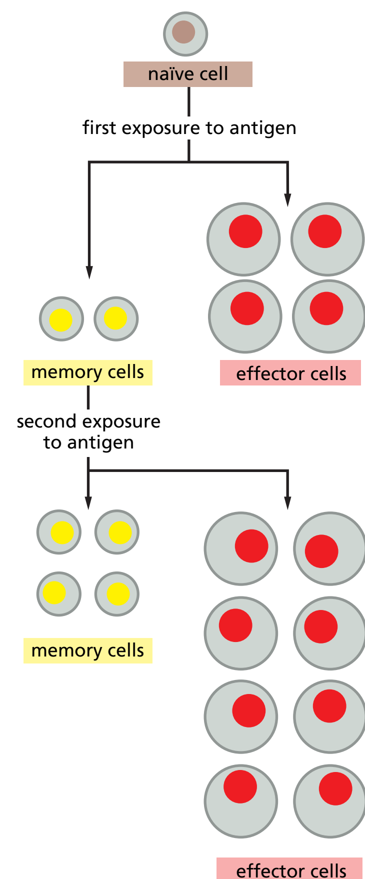


Figure 25–11 A model for the cellular basis of immunological memory. When stimulated by their specific antigen, naïve cells proliferate and differentiate. Most become effector cells, which function and then usually die, while others become memory cells. During a subsequent exposure to the same antigen, the memory cells respond more readily, rapidly, and efficiently than did the naïve cells: they proliferate and give rise to effector cells and to more memory cells. Memory T cells might also develop from effector cells (not shown).

pathogen. A small proportion of the plasma cells produced in a primary B cell response, for example, can survive for many months in the bone marrow, where they continue to secrete their specific antibodies into the bloodstream.

Immunological Tolerance Ensures That Self Antigens Are Not Normally Attacked

As discussed in Chapter 24, cells of the innate immune system use pattern recognition receptors to distinguish pathogens from the normal molecules of the host. The adaptive immune system has a far more difficult recognition task: it must be able to respond specifically to an almost unlimited number of foreign macromolecules, while avoiding responding to the large number of molecules made by the host organism itself. How is this possible? It helps that self molecules do not induce the innate immune reactions required to activate the adaptive immune system. But even when an infection or tissue injury triggers innate reactions, the vast excess of self molecules present normally fail to induce an adaptive immune response. Why not?

One answer is that the adaptive immune system has "learned" not to respond to self antigens. Transplantation experiments provide one line of evidence for this learning process. When tissues are transplanted from one individual to another (and the two individuals are not identical twins), the immune system of the recipient usually recognizes the donor cells as foreign and destroys them. (For reasons we discuss later, the foreign antigens on the donor cells are so powerful that they can stimulate adaptive immune responses in the absence of infection, injury, or an adjuvant.) If, however, we introduce cells from one strain of mouse into a newborn mouse of another strain, some of these cells survive for most of the recipient animal's life, and the recipient will now accept a graft from the original donor, even though it rejects "third-party" grafts. Apparently, nonself antigens can, in particular circumstances, induce the immune system to become specifically unresponsive to them. This antigen-specific unresponsiveness to foreign antigens is known as *acquired immunological tolerance* (**Figure 25–12**).

The unresponsiveness of an animal's adaptive immune system to its own macromolecules (*natural immunological tolerance*, or **self-tolerance**) is acquired in the same way. Normal mice, for example, cannot make an immune response against one of their own protein components of the complement system called C5 (discussed in Chapter 24). But, mutant mice that lack the gene encoding C5 (but are otherwise genetically identical to the normal mice) can make a strong immune response to this blood protein when immunized with it. Similarly, humans that lack a normal gene that codes for the clotting protein Factor VIII (and therefore bleed excessively) make antibodies against the protein when it is administered to them to control bleeding.

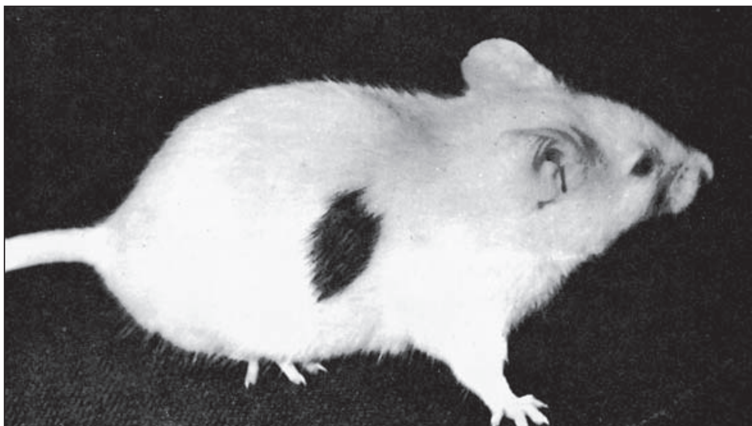


Figure 25–12 Acquired immunological tolerance. The skin graft seen here was transplanted from an adult brown mouse to an adult white mouse. It has survived for many weeks only because the white mouse, at the time of its birth, received an injection of bone marrow cells from the brown mouse and therefore became immunologically tolerant to them. Some of the bone marrow cells (and their progeny) from the brown mouse persist in the adult white mouse and continue to induce tolerance in newly formed lymphocytes that would otherwise react against the brown skin. (Courtesy of Leslie Brent, from I. Roitt, Essential Immunology, 6th ed. Oxford, UK: Blackwell Scientific, 1988.)

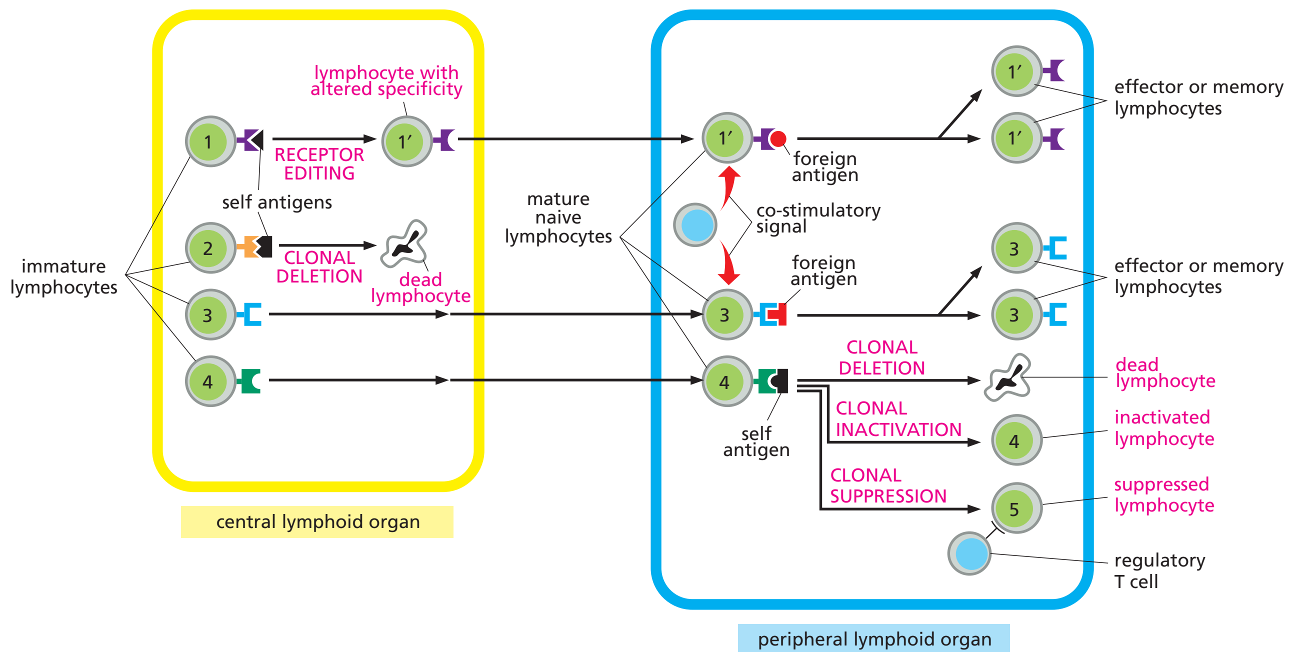


Figure 25–13 Mechanisms of immunological tolerance to self antigens. When a self-reactive immature lymphocyte binds its self antigen in the central lymphoid organ where the cell is produced, it may alter its antigen receptor so that it is no longer self-reactive (cell 1). This process is called receptor editing and is thought to occur mainly in developing B cells. Alternatively, the cell may die by apoptosis, a process called clonal deletion (cell 2). Because these two forms of tolerance (shown on the left) occur in central lymphoid organs, they are called *central tolerance*.

When a self-reactive naïve lymphocyte escapes tolerance in the central lymphoid organ and binds its self antigen in a peripheral lymphoid organ (cell 4), it will generally not be activated, because the binding usually occurs in the absence of appropriate co-stimulatory signals; instead, the cell may die by apoptosis (often after a period of proliferation), be inactivated, or be suppressed by regulatory T cells (if the self-reactive lymphocyte is an effector T cell). These forms of tolerance shown on the right are called *peripheral tolerance*.

The natural immunological tolerance for a particular self molecule persists only for as long as the molecule remains present in the body. If a self molecule such as C5 is experimentally removed from an adult mouse, the animal gains the ability to respond to it after a few weeks or months. Thus, the immune system is genetically capable of responding to self molecules but learns not to do so.

Self-tolerance depends on a number of distinct mechanisms:

1. In *receptor editing,* developing lymphocytes that recognize self molecules *(self-reactive lymphocytes)* change their antigen receptors so that they no longer recognize self antigens.
2. In *clonal deletion,* self-reactive lymphocytes die by apoptosis when they bind their self antigen.
3. In *clonal inactivation* (also called clonal anergy), self-reactive lymphocytes become functionally inactivated when they encounter their self antigen.
4. In *clonal suppression,* regulatory T cells suppress the activity of self-reactive lymphocytes.

Some of these mechanisms—especially the first two, clonal deletion and receptor editing—operate in central lymphoid organs when newly formed self-reactive lymphocytes first encounter their self antigens, and they are largely responsible for the process of *central tolerance.* Clonal inactivation and clonal suppression, by contrast, operate mainly when lymphocytes encounter their self antigens in peripheral lymphoid organs, and they are responsible for the process of *peripheral tolerance.* Clonal deletion and clonal inactivation, however, are known to operate both centrally and peripherally (**Figure 25–13**).

Why does the binding of a self antigen lead to tolerance rather than activation? The answer is still not completely known. As we discuss later, to activate a lymphocyte in a peripheral lymphoid organ, the cell must do more than bind its antigen: it must also receive membrane-bound and secreted co-stimulatory signals (the secreted signals are various cytokines). Both types of signals are provided by a helper T cell in the case of a B lymphocyte and by an activated dendritic cell in the case of a T lymphocyte. Because the production of these signals is usually triggered by exposure to a pathogen, a self-reactive lymphocyte normally encounters its self antigen in the absence of such signals. Under these conditions, a B cell interacting with its antigen or a T cell interacting with its antigen on the surface of a nonactivated dendritic cell will not only fail to be activated, it will often be rendered tolerant—being either killed, inactivated, or actively suppressed by a regulatory T cell (see Figure 25–13). As we discuss later, in peripheral lymphoid organs, both T cell tolerance and activation usually occur on the surface of a dendritic cell.

The tolerance mechanisms sometimes break down, causing T or B cells (or both) to react against the organism's own tissue antigens. *Myasthenia gravis* is an example of such an **autoimmune disease**. Affected individuals make antibodies against the acetylcholine receptors on their own skeletal muscle cells. These antibodies interfere with the normal functioning of the receptors so that the patients become weak and may die because they cannot breathe. Similarly, in *childhood (type 1) diabetes*, immune reactions against insulin-secreting cells in the pancreas kill these cells, leading to severe insulin deficiency.

For the most part, the mechanisms responsible for the breakdown of tolerance to self antigens in autoimmune diseases are unknown. It is thought, however, that activation of the innate immune system by infection or tissue injury may help trigger anti-self responses in individuals with defects in their self-tolerance mechanisms, leading to autoimmunity.

Lymphocytes Continuously Circulate Through Peripheral Lymphoid Organs

Pathogens generally enter the body through an epithelial surface, usually through the skin, gut, or respiratory tract. To induce an adaptive immune response, microbial antigens must travel from these entry points to a peripheral lymphoid organ, such as a lymph node or the spleen, the sites where lymphocytes are activated (see Figure 25–5). The route and destination depend on the site of entry. Lymphatic vessels (see Figure 25–3) carry antigens that enter through the skin or respiratory tract to local lymph nodes; antigens that enter through the gut end up in gut-associated peripheral lymphoid organs such as Peyer's patches; and the spleen filters out antigens that enter the blood. As discussed earlier, in many cases, dendritic cells will carry the antigen from the site of infection to the peripheral lymphoid organ, where they play a crucial part in activating T cells (see Figure 25–5).

But only a tiny fraction of the total lymphocyte population can recognize a particular microbial antigen in a peripheral lymph organ (estimated to be between 1/10,000 and 1/100,000 of each class of lymphocyte). How do these rare cells find an antigen-presenting cell displaying their antigen? The answer is that the lymphocytes continuously circulate between one peripheral lymphoid organ and another via the lymph and blood. In a lymph node, for example, lymphocytes continually leave the bloodstream by squeezing out between specialized endothelial cells lining small veins called *postcapillary venules*. After percolating through the node, they accumulate in small lymphatic vessels that leave the node and connect with other lymphatic vessels that pass through other lymph nodes downstream (see Figure 25–3). Passing into larger and larger vessels, the lymphocytes eventually enter the main lymphatic vessel (the *thoracic duct*), which carries them back into the blood (**Figure 25–14**).

The continuous recirculation between the blood and lymph ends only if a lymphocyte is activated by its specific antigen in a peripheral lymphoid organ. Now the lymphocyte remains in the peripheral lymphoid organ, where it proliferates and differentiates into either effector cells or memory cells. Many of the effector T cells leave the lymphoid organ via the lymph and migrate through the

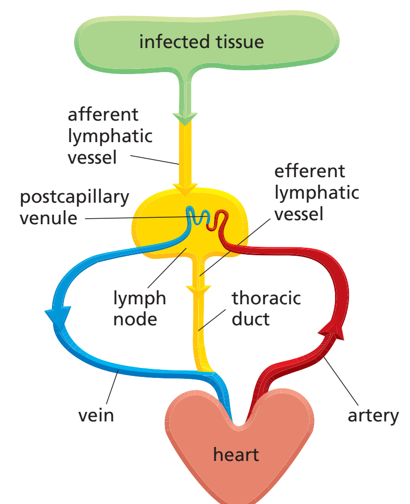


Figure 25–14 The path followed by lymphocytes as they continuously circulate between the lymph and blood. The circulation through a lymph node *(yellow)* is shown here. Microbial antigens are usually carried into the lymph node by dendritic cells (not shown), which enter the node via afferent lymphatic vessels draining an infected tissue *(green)*. T and B cells, by contrast, enter the lymph node via an artery and migrate out of the bloodstream through postcapillary venules. Unless they encounter their antigen, the T and B cells leave the lymph node via efferent lymphatic vessels, which eventually join the thoracic duct. The thoracic duct empties into a large vein carrying blood to the heart to complete the circulation process for T and B cells. A typical circulation cycle for these lymphocytes takes about 12–24 hours.

blood to the site of infection (see Figure 25–5), whereas others stay in the lymphoid organ and help activate B cells or other T cells there. Some effector B cells (plasma cells) remain in the peripheral lymphoid organ and secrete antibodies into the blood for days until they die; others migrate to the bone marrow, where they secrete antibodies into the blood for months or years. The memory T and B cells produced join the recirculating pool of lymphocytes.

Lymphocyte recirculation depends on specific interactions between the lymphocyte cell surface and the surface of the endothelial cells lining the blood vessels in the peripheral lymphoid organs. Many cell types in the blood come into contact with the specialized endothelial cells lining the postcapillary venules in lymph nodes, but only lymphocytes adhere and then migrate out of the bloodstream into the nodes. The lymphocytes initially adhere to the endothelial cells via *homing receptors* that bind to specific ligands (often called *counterreceptors*) on the endothelial cell surface. Lymphocyte migration into lymph nodes depends on a homing receptor protein called *L-selectin*, a member of the selectin family of cell-surface lectins. This protein binds to specific sugar groups on a counterreceptor that is expressed exclusively on the surface of the specialized endothelial cells lining the postcapillary venules in lymph nodes, causing the lymphocytes to adhere weakly to the endothelial cells and to roll slowly along their surface. The rolling continues until another, much stronger adhesion system is called into play by the chemoattractant proteins (called *chemokines;* see below) secreted by endothelial cells. This strong adhesion is mediated by members of the *integrin* family of cell adhesion molecules, which become activated on the lymphocyte surface. Now the lymphocytes stop rolling and crawl out of the blood vessel into the lymph node (**Figure 25–15**). Both selectins and integrins are discussed in Chapter 19.

Chemokines are a type of cytokine. They are small, secreted, positively charged proteins that have a crucial role in guiding the migrations of various types of cells, including white blood cells. <ACCG> They are all structurally related and bind to the surface of endothelial cells, as well as to negatively charged proteoglycans of the extracellular matrix in organs. By binding to G-protein-coupled chemokine receptors (discussed in Chapter 15) on the surface of specific blood cells, chemokines attract these cells from the bloodstream into an organ, guide them to specific locations within the organ, and then help stop migration. (Unfortunately, the AIDS virus, HIV, also binds to certain chemokine receptors, as well as to the CD4 co-receptor that we discuss later, and thus allows the virus to infect white blood cells.) The T and B cells initially enter the same region of a lymph node, but then different chemokines guide them to separate regions of the node—T cells to the *paracortex* and B cells to *lymphoid follicles* (**Figure 25–16**).

Unless they encounter their antigen, both T and B cells soon leave the lymph node via efferent lymphatic vessels. If they encounter their antigen, however, they are stimulated to display adhesion receptors that trap the cells in the node; the cells accumulate at the junction between the T cell and B cell areas, where the rare specific T and B cells can interact, leading to their proliferation and differentiation

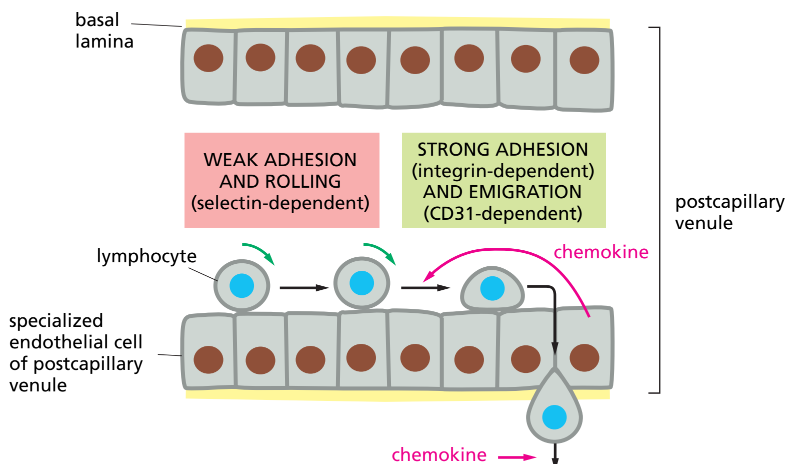


Figure 25–15 Migration of a lymphocyte out of the bloodstream into a lymph node. <CCCC> A circulating lymphocyte adheres weakly to the surface of the specialized endothelial cells lining a postcapillary venule in a lymph node. This initial adhesion is mediated by L-selectin on the lymphocyte surface. The adhesion is sufficiently weak to enable the lymphocyte to roll along the surface of the endothelial cells, pushed along by the flow of blood. Stimulated by chemokines secreted by the endothelial cells (curved *red* arrow), the lymphocyte rapidly activates a stronger adhesion system, mediated by an integrin. This strong adhesion enables the cell to stop rolling. The lymphocyte then uses an Ig-like cell adhesion protein (CD31) to bind to the junctions between adjacent endothelial cells and migrate out of the venule. CD31 is located both on the surface of the lymphocyte and at the junctions between the endothelial cells. The subsequent migration of the lymphocytes in the lymph node depends on chemokines produced within the node (straight *red* arrow).

The migration of other white blood cells out of the bloodstream into sites of infection occurs in a similar way.

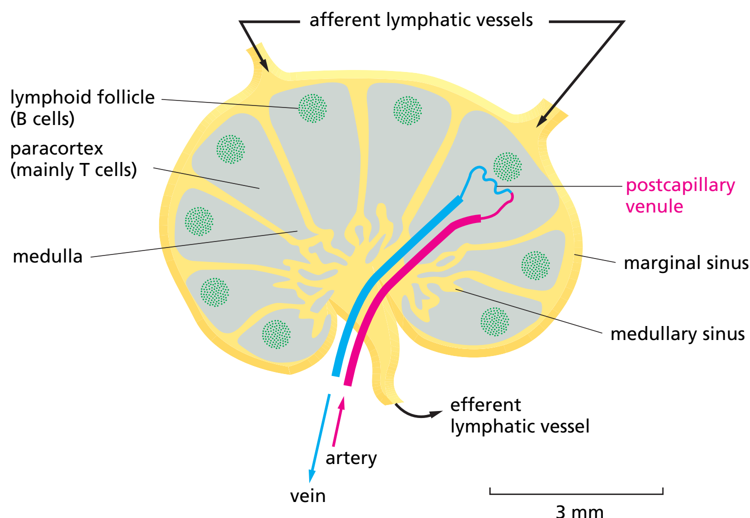


Figure 25–16 A simplified drawing of a human lymph node. B cells are primarily clustered in structures called lymphoid follicles, whereas T cells are found mainly in the paracortex. Chemokines attract both types of lymphocytes into the lymph node from the blood via postcapillary venules (see Figure 25–15). T and B cells then migrate to their respective areas, attracted by different chemokines. If they do not encounter their specific antigen, both T cells and B cells then enter the medullary sinuses and leave the node via the efferent lymphatic vessel. This vessel ultimately empties into the bloodstream, allowing the lymphocytes to begin another cycle of circulation through a peripheral lymphoid organ (see Figure 25–14).

If they encounter their specific antigen, T and B cells are retained in the node and are activated to become effector cells or memory cells; T cells and B cells responding to the same pathogen can interact in and around lymphoid follicles.

into either effector cells or memory cells. Many of the effector cells leave the node, expressing different chemokine receptors that help guide them to their new destinations—T cells to sites of infection and B cells to the bone marrow.

Summary

Innate immune responses are triggered at sites of infection by pathogen-associated molecular patterns (PAMPs), which are recognized by pattern recognition receptors made by cells of the innate immune system. In addition to fighting infection directly, these innate immune responses help activate adaptive immune responses in peripheral lymphoid organs. Unlike innate immune responses, adaptive responses display immunological memory and thereby provide specific and long-lasting protection against the particular pathogen that induced them.

The adaptive immune system is composed of many millions of lymphocyte clones, with the cells in each clone sharing a unique cell-surface receptor that enables them to bind a particular antigen. The binding of antigen to these receptors, however, is usually not sufficient to stimulate a lymphocyte to proliferate and differentiate into an effector cell that can help eliminate the pathogen. Membrane-bound co-stimulatory signals and a variety of secreted signals (cytokines) provided by another specialized cell in a peripheral lymphoid organ are also required. Helper T cells provide such signals for B cells, while dendritic cells usually provide them for T cells. Effector B cells secrete antibodies, which can act over long distances to help eliminate extracellular pathogens and their toxins. Effector T cells, by contrast, act locally to either kill infected host cells or help other cells to eliminate the pathogen. As part of the adaptive immune response, some lymphocytes proliferate and differentiate into memory cells, which are able to respond faster and more efficiently the next time the same pathogen invades. Both B and T cells circulate continuously between one peripheral lymphoid organ and another via the blood and lymph. Only if they encounter their specific foreign antigen in a peripheral lymphoid organ do they stop migrating, proliferate, and differentiate into effector cells or memory cells. Lymphocytes that would react against self molecules either alter their receptors or are eliminated, inactivated, or suppressed by regulatory T cells, so that the adaptive immune system normally avoids attacking the molecules and cells of the host.

B CELLS AND ANTIBODIES

Vertebrates inevitably die of infection if they are unable to make antibodies. Antibodies defend us against infection by binding to viruses and microbial toxins, thereby inactivating them (see Figure 25–2). When antibodies bind to invading pathogens, they also recruit some of the components of the innate immune system, including various types of white blood cells and components of the

complement system (discussed in Chapter 24). The white blood cells and a ctivated complement components work together to attack the invaders.

Synthesized exclusively by B cells, antibodies are produced in billions of forms, each with a different amino acid sequence. Collectively called **immunoglobulins** (abbreviated as **Ig**), they are among the most abundant protein components in the blood, constituting about 20% of the total protein in plasma by weight. Mammals make five classes of antibodies, each of which mediates a characteristic biological response following antigen binding. In this section, we discuss the structure and function of antibodies and how they interact with antigen.

B Cells Make Antibodies as Both Cell-Surface Antigen Receptors and Secreted Proteins

All antibody molecules made by an individual B cell have the same antigen-binding site. The first antibodies made by a newly formed B cell are not secreted but are instead inserted into the plasma membrane, where they serve as receptors for antigen. Each B cell has approximately 10^5 such receptors in its plasma membrane. As we discuss later, each of these receptors is stably associated with a complex of transmembrane proteins that activate intracellular signaling pathways when antigen on the outside of the cell binds to the receptor.

Each B cell clone produces a single species of antibody, with a unique antigen-binding site. When an antigen (with the aid of a helper T cell) activates a naïve or a memory B cell, that B cell proliferates and differentiates into an antibody-secreting effector cell. Such effector cells make and secrete large amounts of soluble (rather than membrane-bound) antibody, which has the same unique antigen-binding site as the cell-surface antibody that served earlier as the antigen receptor (**Figure 25–17**). Effector B cells can begin secreting antibody while they are still small lymphocytes, but the end stage of their maturation pathway is a large plasma cell (see Figure 25–7B), which continuously secretes antibodies at the astonishing rate of about 5000 molecules per second. Although most plasma cells die after several days, some survive in the bone marrow for months or years and continue to secrete antibodies into the blood, helping to provide long-term protection against the pathogen that stimulated their production.

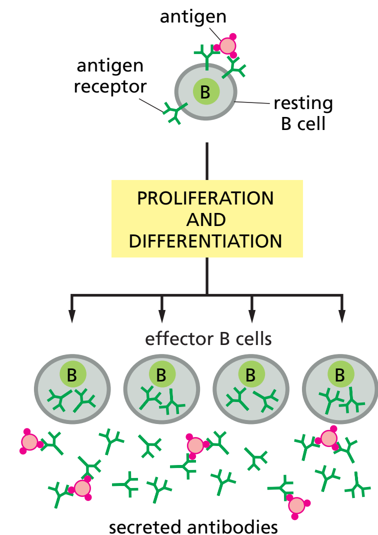


Figure 25–17 The membrane-bound and secreted antibodies made by a B cell clone. When an antigen (aided by a helper T cell—not shown) binds to and thereby activates either a naïve or a memory B cell, the cell proliferates and differentiates into effector cells. The effector cells produce and secrete antibodies with a unique antigen-binding site, which is the same as that of their original membrane-bound antibody that served as their antigen receptors.

A Typical Antibody Has Two Identical Antigen-Binding Sites

The simplest antibodies are Y-shaped molecules with two identical antigen-binding sites, one at the tip of each arm of the Y (**Figure 25–18**). Because of their two antigen-binding sites, they are described as *bivalent*. As long as an antigen has three or more antigenic determinants, bivalent antibody molecules can cross-link it into a large lattice (**Figure 25–19**) that macrophages can readily phagocytose and degrade. The efficiency of antigen binding and cross-linking is greatly increased by the flexible *hinge region* in most antibodies, which allows the distance between the two antigen-binding sites to vary (**Figure 25–20**).

The protective effect of antibodies is not due simply to their ability to bind and cross-link antigen. The tail of the Y-shaped molecule mediates many other activities of antibodies. As we discuss later, antibodies with the same antigen-binding sites can have any one of several different tail regions. Each type of tail region gives the antibody different functional properties, such as the ability to activate the complement system, to bind to phagocytic cells, or to cross the placenta from mother to fetus.

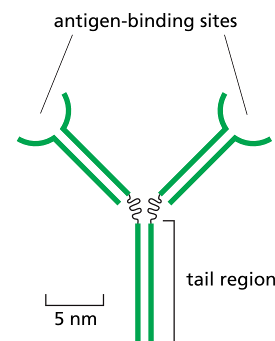


Figure 25–18 A simple representation of an antibody molecule. Note that its two antigen-binding sites are identical.

An Antibody Molecule Is Composed of Heavy and Light Chains

The basic structural unit of an antibody molecule consists of four polypeptide chains, two identical **light (L) chains** (each containing about 220 amino acids) and two identical **heavy (H) chains** (each usually containing about 440 amino

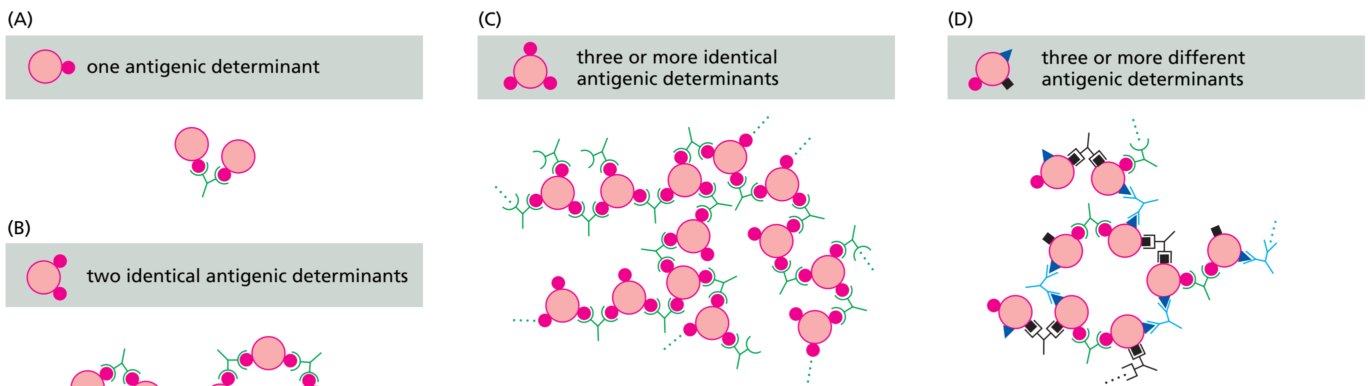


Figure 25–19 Antibody–antigen interactions. Because antibodies have two identical antigen-binding sites, they can cross-link antigens. The types of antibody–antigen complexes that form depend on the number of antigenic determinants on the antigen. (A–C) A single species of antibody (a monoclonal antibody) is shown binding to antigens containing one, two, or three copies of a single type of antigenic determinant. Antigens with two identical antigenic determinants can form small cyclic complexes or linear chains with the antibodies, while antigens with three or more identical antigenic determinants can form large three-dimensional lattices that readily precipitate out of solution. (D) Most antigens have many different antigenic determinants (see Figure 25–29A), and different antibodies that recognize different determinants can cooperate in cross-linking the antigen into large three-dimensional lattices.

acids). A combination of noncovalent and covalent (disulfide) bonds holds the four chains together. The molecule is composed of two identical halves, each with the same antigen-binding site. Both light and heavy chains usually cooperate to form the antigen-binding surface (**Figure 25–21**).

There Are Five Classes of Antibody Heavy Chains, Each with Different Biological Properties

In mammals, there are five *classes* of antibodies, IgA, IgD, IgE, IgG, and IgM, each with its own class of heavy chain—α, δ, ε, γ, and μ, respectively. IgA molecules have α chains, IgG molecules have γ chains, and so on. In addition, there are a number of subclasses of IgG and IgA immunoglobulins; for example, there are four human IgG subclasses (IgG1, IgG2, IgG3, and IgG4), having γ_1, γ_2, γ_3, and γ_4 heavy chains, respectively. The various heavy chains give a distinctive conformation to the hinge and tail regions of antibodies, so that each class (and subclass) has characteristic properties of its own.

IgM, which has μ heavy chains, is always the first class of antibody that a developing B cell makes, although many B cells eventually switch to making other classes of antibody when an antigen stimulates them (discussed below). The first cells in the B cell lineage that make Ig are *pro-B cells*, which make only μ chains. They give rise to *pre-B cells*, in which the μ chains associate with so-called *surrogate light chains* (substituting for genuine light chains) and insert into the plasma membrane. Signaling from this pre-B cell receptor is required for the cell to progress to the next stage of development, where it makes bona fide light chains. The light chains combine with the μ chains, replacing the surrogate light chains, to form four-chain IgM molecules (each with two μ chains and two light chains). These molecules then insert into the plasma membrane, where they function as receptors for antigen. At this point, the cell is called an *immature naïve B cell*. After leaving the bone marrow, the cell starts to produce cell-surface **IgD** molecules as well, with the same antigen-binding site as the IgM molecules. It is now called a *mature naïve B cell*. It is this cell that can respond to foreign antigen in peripheral lymphoid organs (**Figure 25–22**).

IgM is not only the first class of antibody to appear on the surface of a developing B cell. It is also the major class secreted into the blood in the early stages of a primary antibody response, on first exposure to an antigen. (Unlike IgM, IgD

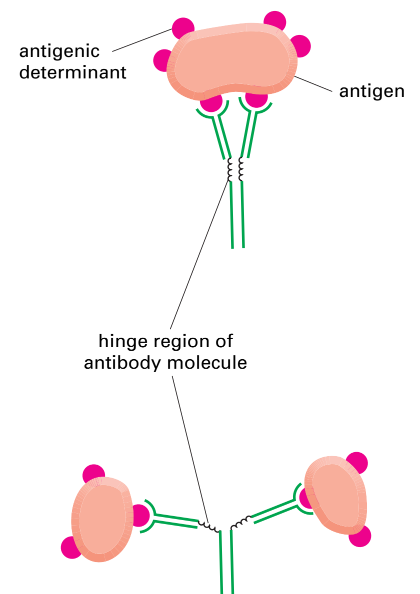


Figure 25–20 The hinge region of an antibody molecule. Because of its flexibility, the hinge region improves the efficiency of antigen binding and cross-linking.

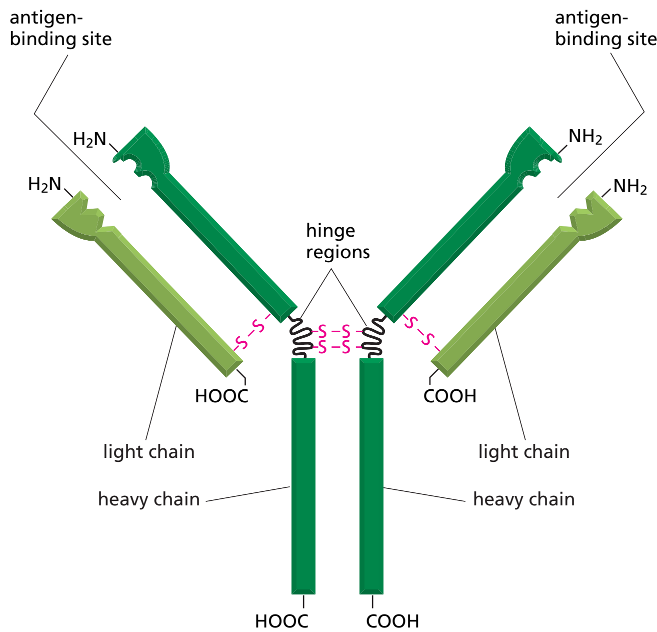


Figure 25–21 A schematic drawing of a bivalent antibody molecule. It is composed of four polypeptide chains—two identical heavy chains and two identical light chains. The two antigen-binding sites are identical, each formed by the N-terminal region of a light chain and the N-terminal region of a heavy chain. The two heavy chains also form both the tail and hinge region of the antibody.

molecules are secreted in only small amounts and seem to function mainly as cell-surface receptors for antigen.) In its secreted form, IgM is a pentamer composed of five four-chain units, giving it a total of 10 antigen-binding sites. Each pentamer contains one copy of another polypeptide chain, called a *J (joining) chain*. The J chain is produced by IgM-secreting cells and is covalently inserted between two adjacent tail regions (**Figure 25–23**).

When an antigen with multiple identical antigenic determinants (see Figure 25–19) binds to a single secreted pentameric IgM molecule, it alters the structure of the pentamer, allowing it to activate the complement system. As discussed in Chapter 24, when the antigen is on the surface of an invading pathogen, this activation of complement can either mark the pathogen for phagocytosis or kill it directly. As we discuss later, complement activation can also greatly increase the immune response to an antigen: the binding of an activated complement component to an antibody–antigen complex, for example, can increase the ability of the antigen to stimulate a B cell response more than a thousand fold (see Figure 25–71A).

The major class of immunoglobulin in the blood is **IgG**, which is a four-chain monomer (see Figure 25–21) produced in large quantities during secondary antibody responses. Besides activating complement, the tail region of an IgG molecule binds to specific receptors on macrophages and neutrophils.

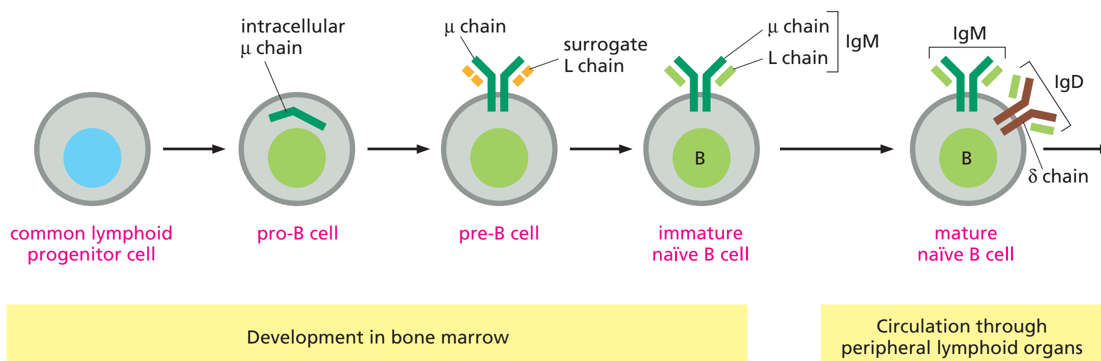


Figure 25–22 The main stages in B cell development. All of the stages shown occur independently of antigen. The pro-B cell makes μ chains, but they remain in the endoplasmic reticulum until surrogate light chains are made. Although not shown, all of the cell-surface Ig molecules are associated with transmembrane proteins that help convey signals to the cell interior (see Figure 25–70). When they are activated by their specific foreign antigen and helper T cells in peripheral lymphoid organs, mature naïve B cells proliferate and differentiate into either antibody-secreting cells or memory cells (not shown).

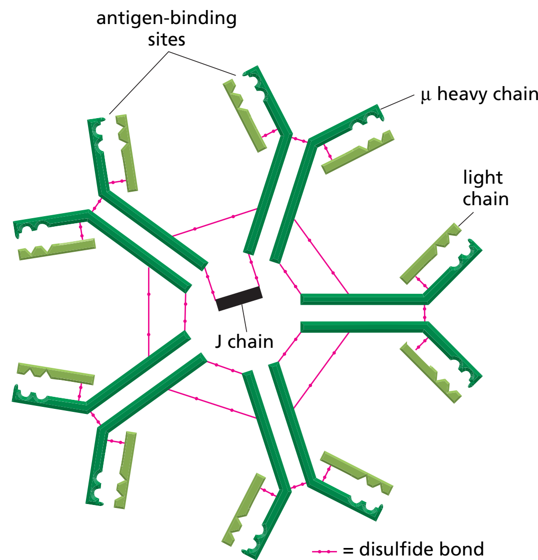


Figure 25–23 A pentameric IgM molecule. Disulfide bonds *(red)* hold the five four-chain units together. A single J chain, which has a structure similar to that of a single Ig domain (discussed later), is covalently attached by disulfide bonds to the tails of two μ heavy chains. The J chain is required for pentamer formation. The addition of each successive four-chain IgM unit requires a J chain, which is then discarded, except for the last one, which is retained. Note that IgM molecules do not have hinge regions.

Largely by means of such **Fc receptors** (so-named because antibody tails are called *Fc* regions), these phagocytic cells bind, ingest, and destroy infecting microorganisms that have become coated with the IgG antibodies produced in response to the infection (**Figure 25–24**).

Some IgG subclasses are the only antibodies that can pass from mother to fetus via the placenta. Cells of the placenta that are in contact with maternal blood have Fc receptors that bind these blood-borne IgG molecules and direct their passage to the fetus. The antibody molecules bound to the receptors are first taken into the placental cells by receptor-mediated endocytosis. They are then transported across the cell in vesicles and released by exocytosis into the fetal blood (a process called *transcytosis*—see Figure 25–26). Because other classes of antibodies do not bind to these particular Fc receptors, they cannot pass across the placenta. Later, IgG is secreted into the mother's milk and is then

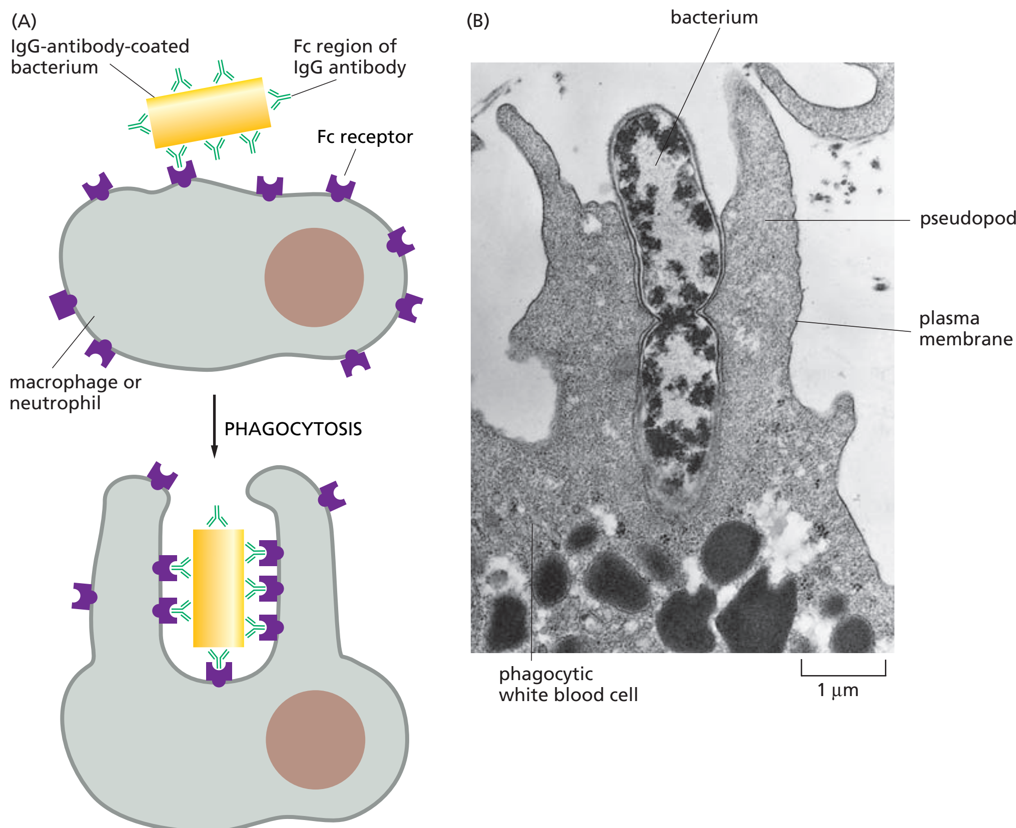


Figure 25–24 Antibody-activated phagocytosis. (A) An IgG-antibody-coated bacterium is efficiently phagocytosed by a macrophage or neutrophil, which has cell-surface receptors that bind the tail (Fc) region of IgG molecules. The binding of the antibody-coated bacterium to these Fc receptors activates the phagocytic process. The tail of an antibody molecule is called an Fc region because, when antibodies are cleaved with the proteolytic enzyme papain, the fragments containing the tail region readily crystallize. (B) Electron micrograph of a neutrophil phagocytosing an IgG-coated bacterium, which is in the process of dividing. The process in which antibody (or complement) coating of a pathogen increases the efficiency with which the pathogen is phagocytosed is called *opsonization*. (B, courtesy of Dorothy F. Bainton, from R.C. Williams, Jr. and H.H. Fudenberg, Phagocytic Mechanisms in Health and Disease. New York: Intercontinental Medical Book Corporation, 1971.)

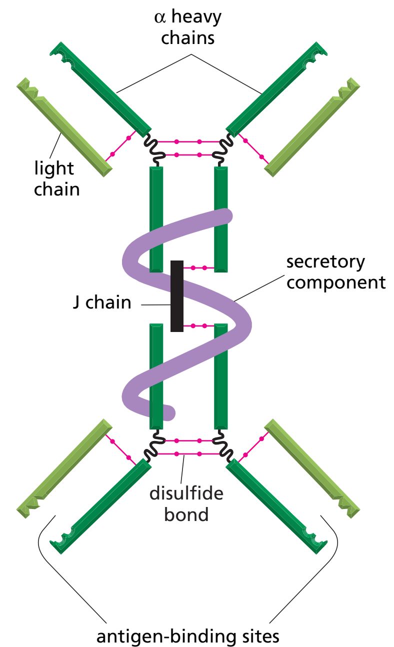


Figure 25–25 A highly schematized diagram of a dimeric IgA molecule found in secretions. In addition to the two IgA monomers, there is a single J chain and an additional polypeptide chain called the *secretory component*, which is derived from the Fc receptor (see Figure 25–26) and is thought to protect the IgA molecules from digestion by proteolytic enzymes in secretions.

taken up from the gut of the neonate into the blood by transcytosis, providing protection for the baby against infection.

IgA is the principal class of antibody in secretions, including saliva, tears, milk, and respiratory and intestinal secretions. IgA is a four-chain monomer in the blood which is assembled into a dimer by the addition of two other polypeptide chains before it is released into secretions (**Figure 25–25**). It is transported through secretory epithelial cells from the extracellular fluid into the secreted fluid by transcytosis mediated by another type of Fc receptor that is unique to secretory epithelia (**Figure 25–26**). This Fc receptor can also transport IgM into secretions (but less efficiently), which is probably why individuals with a selective IgA deficiency, the most common form of antibody deficiency, are only mildly affected by the defect.

The tail region of **IgE** molecules, which are four-chain monomers, binds with unusually high affinity (K_a ~ 10^{10} liters/mole) to yet another class of Fc receptors. These receptors are located on the surface of *mast cells* in tissues and of *basophils* in the blood. The IgE molecules bound to them function as passively acquired receptors for antigen. Antigen binding triggers the mast cell or basophil to secrete a variety of cytokines and biologically active amines, especially *histamine* (**Figure 25–27**). The histamine causes blood vessels to dilate and become leaky, which in turn helps white blood cells, antibodies, and complement components to enter sites where mast cells have been activated. The release of amines from mast cells and basophils is largely responsible for the symptoms of such *allergic* reactions as hay fever, asthma, and hives. In addition, mast cells secrete factors that attract and activate white blood cells called *eosinophils*. Eosinophils also have Fc receptors that bind IgE molecules, and they can kill extracellular parasitic worms, especially if the worms are coated with IgE antibodies.

In addition to the five classes of heavy chains found in antibody molecules, higher vertebrates have two types of light chains, **κ** and **λ**, which seem to be functionally indistinguishable. Either type of light chain may be associated with any of the heavy chains. An individual antibody molecule, however, always contains identical light chains and identical heavy chains: an IgG molecule, for instance, may have either κ or λ light chains, but not one of each. As a result, an antibody's antigen-binding sites are always identical. Such symmetry is crucial for the cross-linking function of secreted antibodies (see Figure 25–19).

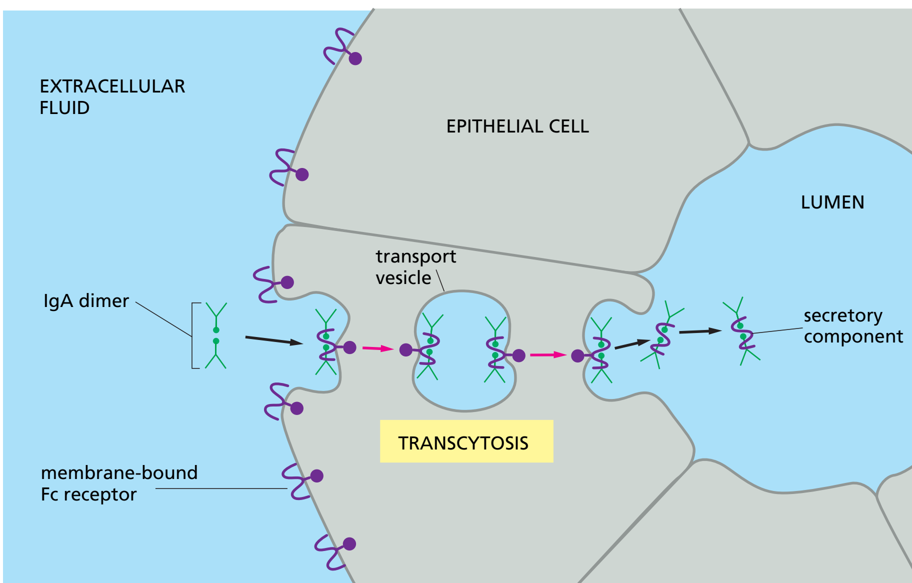


Figure 25–26 The mechanism of transport of a dimeric IgA molecule across an epithelial cell. The IgA molecule, as a J-chain-containing dimer, binds to a transmembrane receptor protein on the nonluminal surface of a secretory epithelial cell. (The J chain has been omitted in this diagram for clarity.) The receptor–IgA complexes are ingested by receptor-mediated endocytosis, transferred across the epithelial cell cytoplasm in vesicles, and secreted into the lumen on the opposite side of the cell by exocytosis. When exposed to the lumen, the part of the Fc receptor protein that is bound to the IgA dimer (the *secretory component*) is cleaved from its transmembrane tail, thereby releasing the antibody in the form shown in Figure 25–25.

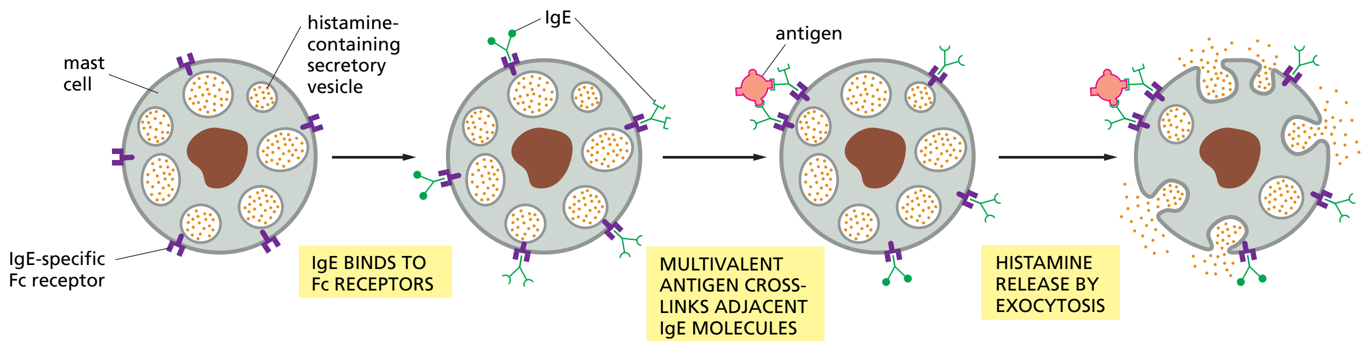


Figure 25–27 The role of IgE in histamine secretion by mast cells. A mast cell (or a basophil) binds IgE molecules after they are secreted by effector B cells. The soluble IgE antibodies bind to Fc receptor proteins on the mast cell surface that specifically recognize the Fc region of these antibodies. The bound IgE molecules serve as cell-surface receptors for antigen. Thus, unlike B cells, each mast cell (and basophil) has a set of cell-surface antibodies with a wide variety of antigen-binding sites. When an antigen molecule binds to these membrane-bound IgE antibodies so as to cross-link them to their neighbors, it signals the mast cell to release its histamine and other local mediators by exocytosis.

All classes of antibody can be made in a membrane-bound form, as well as in a soluble, secreted form. The two forms differ only in the C-terminus of their heavy chain. The heavy chains of membrane-bound antibody molecules have a transmembrane hydrophobic C-terminus, which anchors them in the lipid bilayer of the B cell's plasma membrane. The heavy chains of secreted antibody molecules, by contrast, have instead a hydrophilic C-terminus, which allows them to escape from the cell. The switch in the character of the antibody molecules made occurs because the activation of B cells by antigen (and helper T cells) induces a change in the way in which the H-chain RNA transcripts are made and processed in the nucleus (see Figure 7–99).

The properties of the various classes of antibodies in humans are summarized in **Table 25–1**.

The Strength of an Antibody–Antigen Interaction Depends on Both the Number and the Affinity of the Antigen-Binding Sites

The binding of an antigen to an antibody, like the binding of a substrate to an enzyme, is reversible. The sum of many relatively weak non-covalent forces, including hydrogen bonds, hydrophobic and van der Waals forces, and ionic interactions determine the strength of the interaction. These weak forces are effective only when the antigen molecule is close enough to allow some of its atoms to fit into complementary recesses on the surface of the antibody. The complementary regions of a four-chain antibody unit are its two identical antigen-binding sites; the corresponding region on the antigen is an antigenic determinant (**Figure 25–28**). Most antigenic macromolecules have many different antigenic determinants and are said to be *multivalent;* if two or more of the determinants are identical (as in a polymer with a repeating structure), the antigen is said to be *polyvalent* (**Figure 25–29**).

Table 25–1 Properties of the Major Classes of Antibodies in Humans

PROPERTIES	CLASS OF ANTIBODY				
	IgM	IgD	IgG	IgA	IgE
Heavy chains	μ	δ	γ	α	ε
Light chains	κ or λ	κ or λ	κ or λ	κ or λ	κ or λ
Number of four-chain units	5	1	1	1 or 2	1
Percentage of total Ig in blood	10	<1	75	15	<1
Activates complement	++++	–	++	–	–
Crosses placenta	–	–	+	–	–
Binds to macrophages and neutrophils	–	–	+	–	–
Binds to mast cells and basophils	–	–	–	–	+

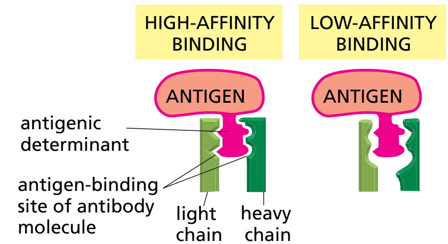
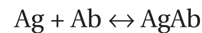


Figure 25–28 Antigen binding to antibody. In this highly schematized diagram, an antigenic determinant on a macromolecule is shown interacting with one of the antigen-binding sites of two different antibody molecules, one of high affinity and one of low affinity. Various weak noncovalent forces hold the antigenic determinant in the binding site, and the site with the better fit to the antigen has a greater affinity. Note that both the light and heavy chains of the antibody molecule usually contribute to the antigen-binding site.

The reversible binding reaction between an antigen with a single antigenic determinant (denoted Ag) and a single antigen-binding site (denoted Ab) can be expressed as



$$Ag + Ab \leftrightarrow AgAb$$

The equilibrium point depends both on the concentrations of Ab and Ag and on the strength of their interaction. Clearly, a larger fraction of Ab will become associated with Ag as the concentration of Ag increases. The strength of the interaction is generally expressed as the **affinity constant** (**K_a**) (see Figure 3–43), where

$$K_a = [AgAb]/[Ag][Ab]$$

(the square brackets indicate the concentration of each component at equilibrium).

One can determine the affinity constant, also known as the association constant, by measuring the concentration of free Ag required to fill half of the antigen-binding sites on the antibody. When half the sites are filled, [AgAb] = [Ab] and $K_a = 1/[Ag]$. Thus, the reciprocal of the antigen concentration that produces half the maximum binding is equal to the affinity constant of the antibody for the antigen. Common values range from as low as 5×10^4 to as high as 10^{11} liters/mole.

The **affinity** of an antibody for an antigenic determinant describes the strength of binding of a single copy of the antigenic determinant to a single antigen-binding site, and it is independent of the number of antigen-binding sites. When, however, a polyvalent antigen, carrying multiple copies of the same antigenic determinant, combines with a polyvalent IgM antibody (see Figure 25–23), the binding strength is greatly increased because all of the antigen–antibody bonds must be broken simultaneously before the antigen and antibody can dissociate. Even a bivalent IgG molecule can bind at least 100 times more strongly to a polyvalent antigen if both antigen-binding sites are engaged than if only one site is engaged. The total binding strength of a bivalent or polyvalent antibody with a polyvalent antigen is referred to as the **avidity** of the interaction.

If the affinity of the antigen-binding sites in an IgG and an IgM molecule is the same, the IgM molecule (with 10 binding sites) will have a much greater avidity for a polyvalent antigen than an IgG molecule (which has two binding sites). This difference in avidity, often 10^4-fold or more, is important because antibodies produced early in an immune response usually have much lower affinities than those produced later. Because of its high total avidity, IgM—the major Ig class produced early in primary immune responses—can function effectively even when each of its binding sites has only a low affinity.

So far, we have considered the general structure and function of antibodies. Next, we look at the details of their structure, as revealed by studies of their amino acid sequence and three-dimensional structure.

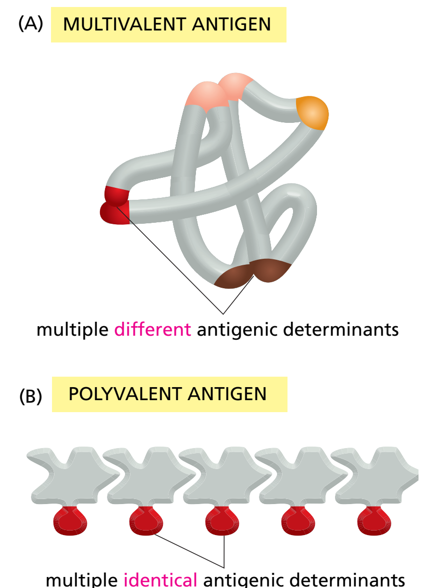


Figure 25–29 Molecules with multiple antigenic determinants. (A) A globular protein is shown with a number of *different* antigenic determinants. Different regions of a polypeptide chain usually come together in the folded structure to form each antigenic determinant on the surface of the protein, as shown for three of the four determinants. (B) A polymeric structure is shown with many *identical* antigenic determinants.

Antibody Light and Heavy Chains Consist of Constant and Variable Regions

Comparison of the amino acid sequences of different antibody molecules reveals a striking feature with important genetic implications. Both light and heavy chains have a variable sequence at their N-terminal ends but a constant sequence at their C-terminal ends. Consequently, when we compare the amino acid sequences of many different κ chains, the C-terminal halves are the same or show only minor differences, whereas the N-terminal halves all differ. Light chains have a **constant region** about 110 amino acids long and a **variable region**

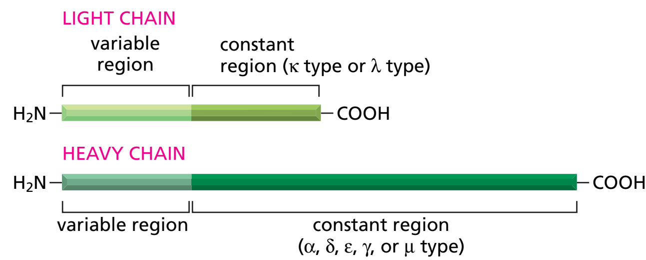


Figure 25–30 Constant and variable regions of immunoglobulin chains. The variable regions of the light and heavy chains form the antigen-binding sites, while the constant regions of the heavy chains determine the other biological properties of an antibody.

of the same size. The variable region of the heavy chains is also about 110 amino acids long, but the constant region is about three or four times longer (330 or 440 amino acids), depending on the class (**Figure 25–30**).

It is the N-terminal ends of the light and heavy chains that come together to form the antigen-binding site, and the variability of their amino acid sequences provides the structural basis for the diversity of antigen-binding sites. The greatest diversity occurs in three small **hypervariable regions** in the variable regions of both light and heavy chains; the remaining parts of the variable region, known as *framework regions*, are relatively constant.

Only about 5–10 amino acids in each hypervariable region form the actual antigen-binding site (**Figure 25–31**). As a result, the size of the antigenic determinant that an antibody recognizes is generally comparably small. It can consist of fewer than 10 amino acids on the surface of a globular protein, for example.

The Light and Heavy Chains Are Composed of Repeating Ig Domains

Both light and heavy chains are made up of repeating segments—each about 110 amino acids long and each containing one intrachain disulfide bond. Each repeating segment folds independently to form a compact functional unit called an **immunoglobulin (Ig) domain**. As shown in **Figure 25–32**, a light chain consists of one variable (V_L) and one constant (C_L) domain (equivalent to the variable and constant regions shown in the top half of Figure 25–30). V_L pairs with the variable (V_H) domain of the heavy chain to form the antigen-binding region. C_L pairs with the first constant domain of the heavy chain (C_H1), and the remaining constant domains of the heavy chains form the Fc region, which determines the other biological properties of the antibody. Most heavy chains have three constant domains (C_H1, C_H2, and C_H3), but those of IgM and IgE antibodies have four.

The similarity in their domains suggests that antibody chains arose during evolution by a series of gene duplications, beginning with a primordial gene coding for a single 110 amino acid domain of unknown function. Each domain of the constant region of a heavy chain is encoded by a separate coding sequence (exon), which supports this hypothesis (**Figure 25–33**).

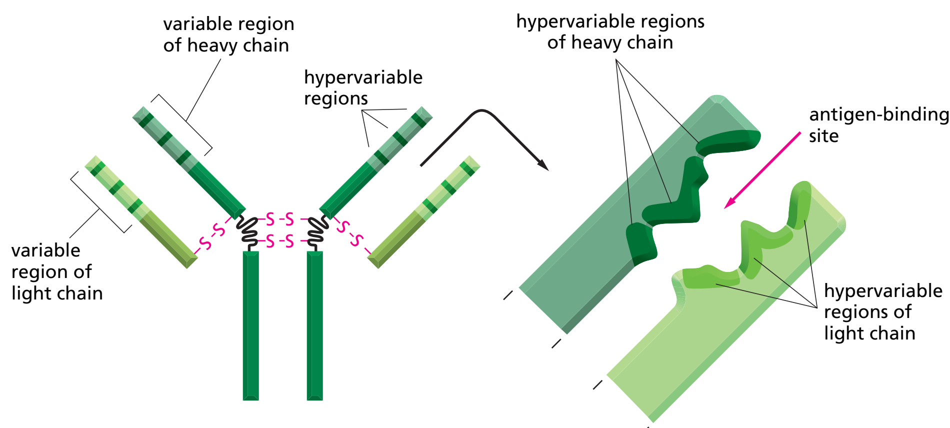


Figure 25–31 Antibody hypervariable regions. Highly schematized drawing of how the three hypervariable regions in each light and heavy chain together form the antigen-binding site of an antibody molecule.

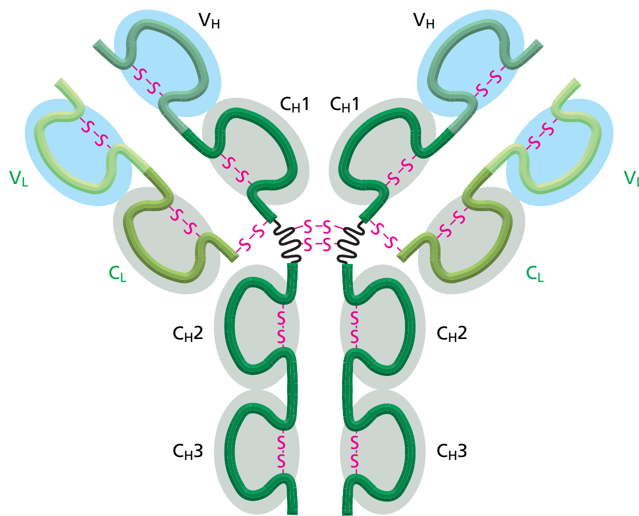


Figure 25–32 Immunoglobulin domains. The light and heavy chains in an antibody molecule are each folded into similar repeating domains. The variable domains (shaded in *blue*) of the light and heavy chains (V_L and V_H) make up the antigen-binding sites, while the constant domains of the heavy chains (mainly C_H2 and C_H3) determine the other biological properties of the molecule. The heavy chains of IgM and IgE antibodies do not have a hinge region and have an extra constant domain (C_H4). Hydrophobic interactions between domains on adjacent chains help hold the chains together in the antibody molecule: V_L binds to V_H, C_L binds to C_H1, and so on (see Figure 25–34). All antibodies are glycosylated on their C_H2 domains (not shown); the attached oligosaccharide chains vary from antibody to antibody and influence the biological properties of the antibody.

An Antigen-Binding Site Is Constructed from Hypervariable Loops

Many antibody fragments, as well as some intact antibody molecules, have been studied by x-ray crystallography. From these examples, we can understand the way in which billions of different antigen-binding sites are constructed on a common structural theme.

As illustrated in **Figure 25–34**, each Ig domain has a very similar three-dimensional structure consisting of a sandwich of two β sheets held together by a disulfide bond. As we discuss later, many other proteins on the surface of lymphocytes and other cells, many of which function as cell–cell adhesion molecules (discussed in Chapter 19), contain similar domains and hence are members of a very large *immunoglobulin (Ig) superfamily* of proteins.

The variable domains of antibody molecules are unique in that each has its particular set of three hypervariable regions, which are arranged in three *hypervariable loops* (see Figure 25–34). The hypervariable loops of both the light and heavy variable domains cluster together to form the antigen-binding site. Because the variable domain of an antibody molecule consists of a highly conserved rigid framework, with hypervariable loops attached at one end, changes in only the lengths and amino acid sequences of the hypervariable loops can generate an enormous diversity of antigen-binding sites. The overall three-dimensional structure necessary for antibody function remains constant.

X-ray analyses of crystals of antibody fragments bound to an antigenic determinant reveal exactly how the hypervariable loops of the light and heavy variable domains cooperate to form an antigen-binding surface in particular

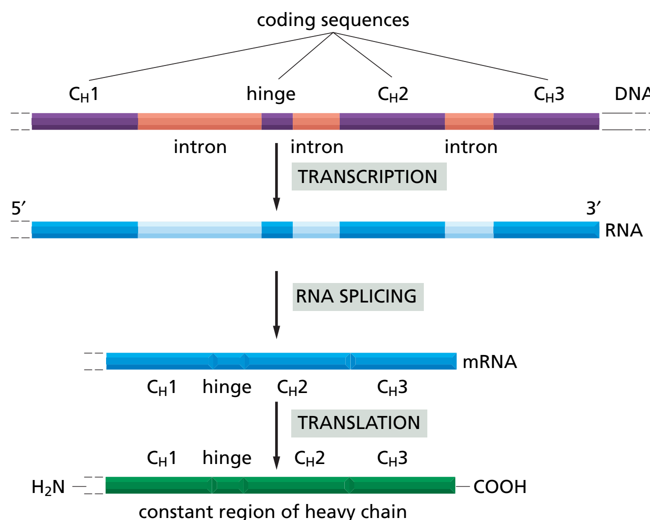


Figure 25–33 The organization of the DNA sequences that encode the constant region of an antibody heavy chain, such as that found in IgG. The coding sequences (exons) for each domain and for the hinge region are separated by noncoding sequences (introns). The intron sequences are removed by splicing the primary RNA transcripts to form mRNA. The presence of introns in the DNA is thought to have facilitated accidental duplications of DNA segments that gave rise to the antibody genes during evolution (discussed in Chapter 4). The DNA and RNA sequences that encode the variable region of the heavy chain are not shown.

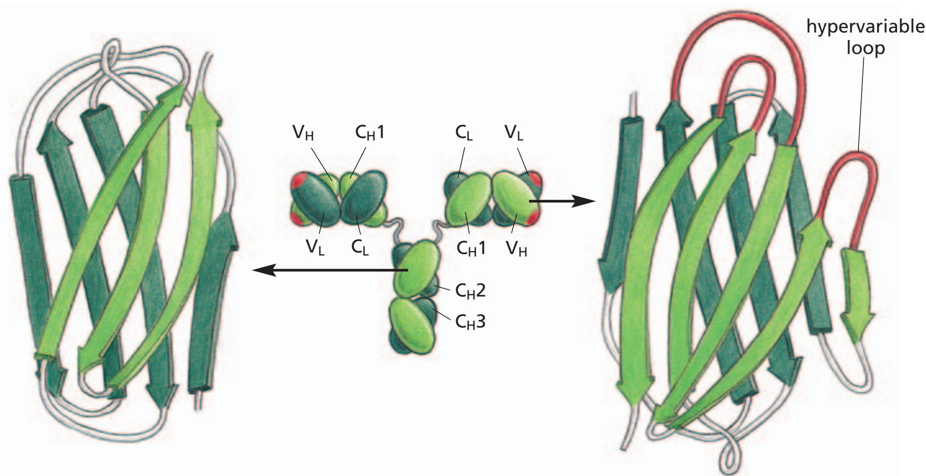


Figure 25–34 The folded structure of an IgG antibody molecule, based on x-ray crystallography studies. <GCCG> The structure of the whole protein is shown in the middle, while the structure of a constant domain is shown on the left and that of a variable domain on the right. Both domains consist of two β sheets, which are joined by a disulfide bond (not shown). Note that all the hypervariable regions *(red)* form loops at the far end of the variable domain, where they come together to form part of the antigen-binding site (see also Figure 3–41).

cases. The dimensions and shape of each different site vary depending on the conformations of the polypeptide chain in the hypervariable loops, which in turn are determined by the sequences of the amino acid side chains in the loops. The shapes of binding sites vary greatly—from pockets, to grooves, to undulating flatter surfaces, and even to protrusions—depending on the antibody (**Figure 25–35**). Smaller ligands tend to bind to deeper pockets, whereas larger ones tend to bind to flatter surfaces. In addition, the binding site can alter its shape after antigen binding to fit the ligand better.

Now that we have discussed the structure and functions of antibodies, we are ready to consider the crucial question that puzzled immunologists for many years—what are the genetic mechanisms that enable each of us to make many billions of different antibody molecules?

Summary

Antibodies defend vertebrates against infection by inactivating viruses and microbial toxins and by recruiting the complement system and various types of white blood cells to kill invading pathogens. A typical antibody molecule is Y-shaped, with two identical antigen-binding sites at the tips of the Y, plus binding sites for complement components and various cell-surface receptors on the tail of the Y.

Each B cell clone makes antibody molecules with a unique antigen-binding site. Initially, during B cell development in the bone marrow, the antibody molecules are inserted into the plasma membrane, where they serve as receptors for antigen. In peripheral lymphoid organs, antigen binding to these receptors, together with co-stimulatory signals provided by helper T cells, activates the B cells to proliferate and differentiate into either memory cells or antibody-secreting effector cells. The effector cells secrete large amounts of antibodies with the same unique antigen-binding site as the membrane-bound antibodies.

A typical antibody molecule is composed of four polypeptide chains, two identical heavy chains and two identical light chains. Parts of both the heavy and light chains usually combine to form the antigen-binding sites. There are five classes of antibodies (IgA, IgD, IgE, IgG, and IgM), each with a distinctive heavy chain (α, δ, ε, γ, and μ, respectively). The heavy chains also form the tail (Fc region) of the antibody, which determines what other proteins will bind to the antibody and therefore what biological properties the antibody class has. Either type of light chain (κ or λ) can be associated with any class of heavy chain; this choice has no effect on the properties of the antibody, except for its specificity for antigen.

Each light and heavy chain is composed of a number of Ig domains—β sheet structures constructed from about 110 amino acids. A light chain has one variable (V_L) and one constant (C_L) domain, while a heavy chain has one variable (V_H) and either three or four constant (C_H) domains. The amino acid sequence variation in the variable domains of both light and heavy chains is concentrated in several small hypervariable regions, which protrude as loops at one end of these domains to form the antigen-binding site.

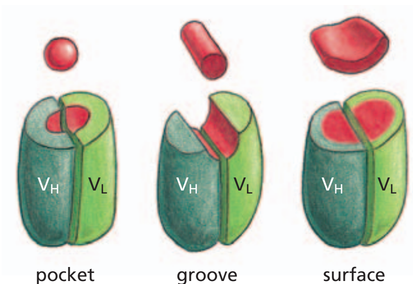


Figure 25–35 The variety of antigen-binding surfaces in antibodies. The hypervariable loops of different V_L and V_H domains can combine to form a large variety of binding surfaces. The antigenic determinants and the antigen-binding site of the antibodies are shown in *red*. Only one antigen-binding site is shown for each antibody.

THE GENERATION OF ANTIBODY DIVERSITY

Even in the absence of antigen stimulation, a human can probably make more than 10^{12} different antibody molecules—its preimmune, **primary antibody repertoire**. The primary repertoire consists of IgM and IgD antibodies and is apparently large enough to ensure that there will be an antigen-binding site to fit almost any potential antigenic determinant, albeit with low affinity. (The antigen-binding sites of many antibodies can cross-react with a variety of related but different antigenic determinants, making this primary antibody defense force even more formidable.)

After stimulation by antigen (and helper T cells), B cells can switch from making IgM and IgD to making other classes of antibodies—a process called *class switching*. In addition, the affinity of these antibodies for their antigen progressively increases over time—a process called *affinity maturation*. Thus, antigen stimulation generates a **secondary antibody repertoire**, with a greatly increased diversity of both Ig classes and antigen-binding sites.

Antibodies are proteins, and proteins are encoded by genes. Antibody diversity therefore poses a special genetic problem: how can an animal make more antibodies than there are genes in its genome? (The human genome, for example, contains only about 25,000 genes.) This problem is not quite as formidable as it might first appear. Recall that the variable regions of the light and heavy chains of antibodies usually combine to form the antigen-binding site. Thus, an animal with 1000 genes encoding light chains and 1000 genes encoding heavy chains could, in principle, combine their products in 1000×1000 different ways to make 10^6 different antigen-binding sites (although, in reality, not every light chain can combine with every heavy chain to make an antigen-binding site). Nonetheless, unique genetic mechanisms have evolved to enable adaptive immune systems to generate an almost unlimited number of different light and heavy chains in a remarkably economical way.

Not all vertebrates use the same genetic mechanisms to diversify antibodies, and there are even substantial differences in the mechanisms used by different mammals. We discuss the mechanisms used by mice and humans, in which antibody diversity is generated in two steps. First, before antigen stimulation, developing B cells join together separate *gene segments* in DNA in order to create the genes that encode the primary repertoire of low-affinity IgM and IgD antibodies. Second, after antigen stimulation, the assembled antibody-coding genes can undergo two further changes—mutations that can increase the affinity of the antigen-binding site and DNA rearrangements that switch the class of antibody made. Together, these changes produce the secondary repertoire of high-affinity IgG, IgA, and IgE antibodies.

We begin this section by discussing the mechanisms that B cells use to produce the primary antibody repertoire and then discuss the mechanisms that they use to produce the secondary repertoire.

Antibody Genes Are Assembled From Separate Gene Segments During B Cell Development

Mice and humans produce their primary antibody repertoire by joining separate antibody **gene segments** together during B cell development. Each type of antibody chain—κ light chains, λ light chains, and heavy chains—is encoded by a separate locus on a separate chromosome. Each locus contains a large number of gene segments encoding the V region of an antibody chain, and one or more gene segments encoding the C region. During the development of a B cell in the bone marrow (or fetal liver), a complete coding sequence for each of the two antibody chains to be synthesized is assembled by site-specific genetic recombination (discussed in Chapter 5). In addition to bringing together the separate gene segments of the antibody gene, these rearrangements also activate transcription from the gene promoter through changes in the relative positions of the enhancers and silencers acting on the gene. Thus, a complete antibody chain can be synthesized only after the DNA has been rearranged.

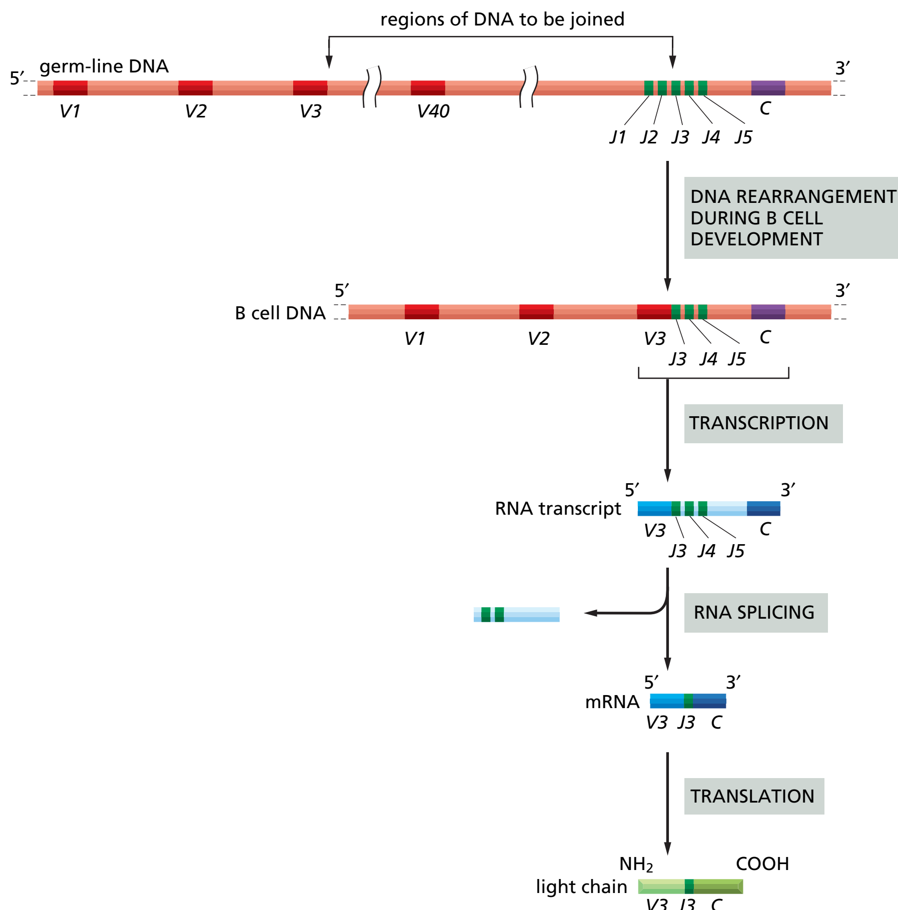


Figure 25–36 The *V–J* joining process involved in making a human κ light chain. In the "germ-line" DNA (where the antibody genes are not rearranged and are therefore not being expressed), the cluster of five *J* gene segments is separated from the *C*-region coding sequence by a short intron and from the 40 *V* gene segments by thousands of nucleotide pairs. During the development of a B cell, a randomly chosen *V* gene segment *(V3* in this case) is moved to lie precisely next to one of the *J* gene segments *(J3* in this case). The "extra" *J* gene segments *(J4* and *J5)* and the intron sequence are transcribed (along with the joined *V3* and *J3* gene segments and the C-region coding sequence) and then removed by RNA splicing to generate mRNA molecules with contiguous *V3, J3,* and *C* sequences, as shown. These mRNAs are then translated into κ light chains. A *J* gene segment encodes the C-terminal 15 or so amino acids of the V region, and a short sequence containing the *V–J* segment junction encodes the third hypervariable region of the light chain, which is the most variable part of the V region.

Each light-chain V region is encoded by a DNA sequence assembled from two gene segments—a long ***V* gene segment** and a short *joining*, or ***J* gene segment** (not to be confused with the protein *J chain* (see Figure 25–23), which is encoded elsewhere in the genome). **Figure 25–36** illustrates the sequence of events involved in the production of a human κ light-chain polypeptide from its separate gene segments. Each heavy-chain V region is similarly constructed by combining gene segments, but here an additional *diversity segment*, or ***D* gene segment**, is also required (**Figure 25–37**).

The large number of inherited *V, J,* and *D* gene segments available for encoding antibody chains contributes substantially to antibody diversity, and the combinatorial joining of these segments (called *combinatorial diversification*) greatly increases this contribution. Any of the 40 *V* segments in the human κ light-chain locus, for example, can be joined to any of the 5 *J* segments (see Figure 25–36), so that this locus can encode at least 200 (40 × 5) different κ-chain V regions. Similarly, any of the 40 *V* segments in the human heavy-chain locus can be joined to any of the 25 *D* segments and to any of the 6 *J* segments to encode at least 6000 (40 × 25 × 6) different heavy-chain V regions.

The combinatorial diversification resulting from the assembly of different combinations of inherited *V, J,* and *D* gene segments is an important mechanism for diversifying the antigen-binding sites of antibodies. By this mechanism alone, called *V(D)J recombination,* a human can produce 320 different V_L regions (200 κ and 120 λ) and 6000 different V_H regions. In principle, these could then be combined to make about 1.9×10^6 (320 × 6000) different antigen-binding sites. In addition, as we discuss next, the joining mechanism itself greatly increases this number of possibilities (probably more than 10^8-fold), making the primary antibody repertoire much larger than the total number of B cells (about 10^{12}) in a human.

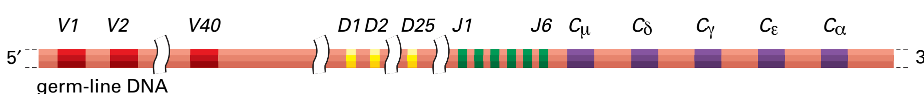


Figure 25–37 The human heavy-chain locus. There are 40 *V* segments, 25 *D* segments, 6 *J* segments, and an ordered cluster of *C*-region coding sequences, each cluster encoding a different class of heavy chain. The *D* segment (and part of the *J* segment) encodes amino acids in the third hypervariable region, which is the most variable part of the heavy-chain V region. The genetic mechanisms involved in producing a heavy chain are the same as those shown in Figure 25–36 for light chains, except that two DNA rearrangement steps are required instead of one. First a *D* segment joins to a *J* segment, and then a *V* segment joins to the rearranged *DJ* segment. The figure is not drawn to scale and omits detail: for example, the total length of the heavy chain locus is over 2 megabases.

Imprecise Joining of Gene Segments Greatly Increases the Diversity of V Regions

In the process of **V(D)J recombination**, site-specific recombination joins separate antibody gene segments together to form a functional V_L- or V_H-region coding sequence. Conserved *recombination signal sequences* flank each gene segment and serve as recognition sites for the joining process, ensuring that only appropriate gene segments recombine. Thus, for example, a light-chain *V* segment will always join to a *J* segment but not to another *V* segment. An enzyme complex called the *V(D)J recombinase* mediates joining. This complex contains two proteins that are specific to developing lymphocytes, as well as enzymes that help repair damaged DNA in all our cells.

Two closely linked genes called *Rag1* and *Rag2* (*Rag* = *r*ecombination *a*ctivating *g*enes) encode the lymphocyte-specific proteins of the V(D)J recombinase, RAG1 and RAG2. To mediate V(D)J joining, the two proteins come together to form a complex (called **RAG**), which functions as an endonuclease, introducing double-strand breaks precisely between the gene segments to be joined and their flanking recombination signal sequences. RAG then initiates the rejoining process by recruiting enzymes involved in DNA double-strand repair in all cells (**Figure 25–38**). Mice or humans deficient in either of the two *Rag* genes or in nonhomologous end joining are highly susceptible to infection because they are unable to carry out V(D)J recombination and consequently do not have functional B or T cells, a condition called *severe combined immunodeficiency (SCID)*. (As we discuss later, T cells use the same V(D)J recombinase to assemble the gene segments that encode their antigen-specific receptors.)

During the joining of antibody (and T cell receptor) gene segments, as in nonhomologous end-joining (see Figure 5–51A), a variable number of nucleotides are often lost from the ends of the recombining gene segments, and one or more randomly chosen nucleotides may also be inserted. This random loss and gain of nucleotides at joining sites is called **junctional diversification**, and it enormously increases the diversity of V-region coding sequences created by V(D)J recombination, specifically in the third hypervariable region. This

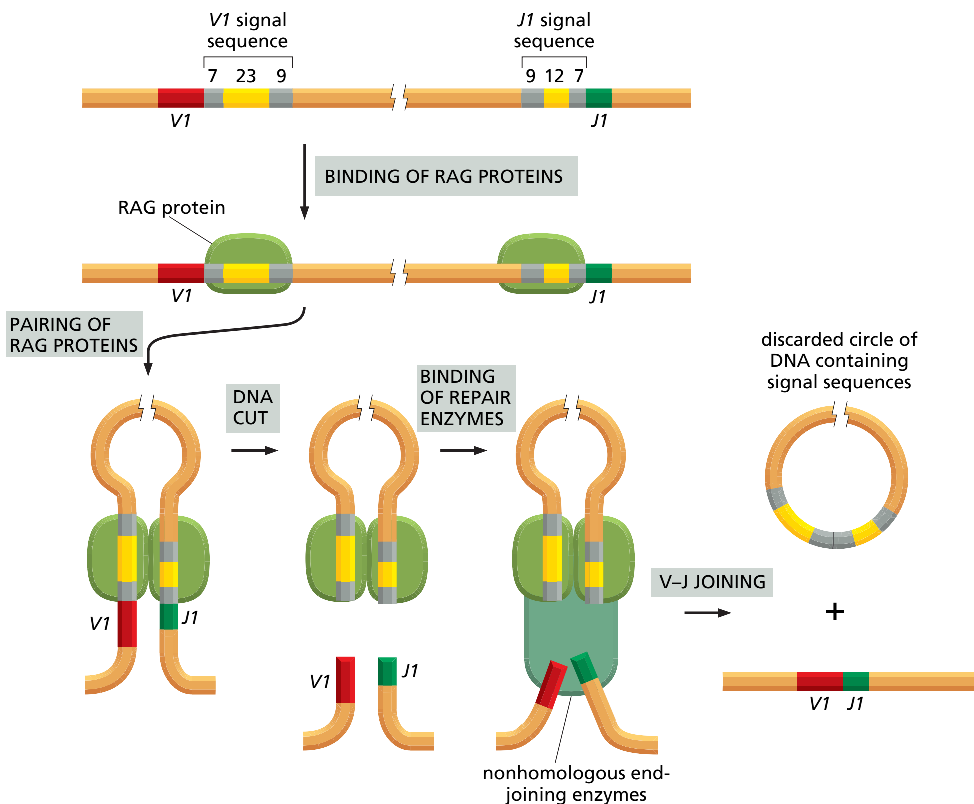


Figure 25–38 The role of recombination signal sequences in RAG-mediated gene segment joining. In the case shown, V1 is joined to J1 in a light-chain locus. Two types of DNA signal sequences are involved in V(D)J recombination, and recombination can only occur between different types: both have the same 7-base-pair (bp) sequence at one end and the same 9-bp sequence at the other end, but in one type the ends are separated by a 12-bp spacer and in the other they are separated by a 23-bp spacer, as shown. When one RAG protein binds to a 12-bp spacer and another to a 23-bp spacer, and the two RAG proteins bind to each other, the two different signal sequences are juxtaposed. The RAG complex then cuts the two signal sequences at their 7-bp ends, and DNA repair enzymes join the cut V1 and J1 segments together. The signal sequences are also joined together and discarded as a small circle of DNA that contains all the DNA originally located between V1 and J1.

The same process and signal sequences are used to join V, D, and J gene segments in a heavy-chain locus. The arrangement of signal sequences and the "12/23 rule" just described ensure that only appropriate gene segments recombine.

increased diversification comes at a price, however. In many cases, it will shift the reading frame to produce a nonfunctional gene. Because roughly two in every three rearrangements are "nonproductive" in this way, many developing B cells never make a functional antibody molecule and consequently die in the bone marrow.

B cells making functional antibody molecules that bind strongly to self antigens in the bone marrow would be dangerous. Such B cells maintain expression of the RAG proteins and can undergo a second round of V(D)J recombination in a light-chain locus (usually a κ locus), thereby changing the specificity of the cell-surface antibody they make—a process referred to as **receptor editing**. To provide a further layer of protection, clonal deletion eliminates those self-reactive B cells that fail to change their specificity (see Figure 25–13).

The Control of V(D)J Recombination Ensures That B Cells Are Monospecific

B cells are *monospecific*. That is, all the antibodies that any one B cell produces have identical antigen-binding sites. This property enables antibodies to cross-link antigens into large aggregates, thereby promoting antigen elimination (see Figure 25–19). It also means that an activated B cell secretes antibodies with the same specificity as that of its membrane-bound antibody receptor, guaranteeing the specificity of antibody responses (see Figure 25–17).

To achieve monospecificity, each B cell must make only one type of V_L region and one type of V_H region. Since B cells, like other somatic cells, are diploid, each cell has six loci encoding antibody chains: two heavy-chain loci (one from each parent) and four light-chain loci (one κ and one λ from each parent). If DNA rearrangements occurred independently in each heavy-chain locus and each light-chain locus, a single B cell could make up to eight different antibodies, each with a different antigen-binding site.

In fact, however, each B cell uses only two of the six antibody loci: one of the two heavy-chain loci and one of the four light-chain loci. Thus, each B cell must choose not only between its κ and λ light-chain loci, but also between its maternal and paternal light-chain and heavy-chain loci. This second choice is called **allelic exclusion**. Allelic exclusion also occurs in the expression of some genes that encode T cell receptors and genes that encode olfactory receptors in the nose (discussed in Chapter 15). However, for most proteins that are encoded by autosomal genes, both the maternal and paternal gene copies in a cell are expressed about equally.

Allelic exclusion and κ versus λ light-chain choice during B cell development depend on negative feedback regulation of the V(D)J recombination process. A functional rearrangement in one antibody locus suppresses rearrangements in all remaining loci that encode the same type of antibody chain (**Figure 25–39**). In B cell clones isolated from transgenic mice expressing a rearranged μ-chain gene, for example, the rearrangement of all of the endogenous heavy-chain genes is usually suppressed. Comparable results have been obtained for light chains. The suppression does not occur if the product of the rearranged gene fails to assemble into a receptor that inserts into the plasma membrane. It has therefore been proposed that either the receptor assembly process itself or extracellular signals that act on the receptor suppress further gene rearrangements.

Although no biological differences between the constant regions of κ and λ light chains have been discovered, there is an advantage in having two separate loci encoding light-chain variable regions. Having two separate loci increases the chance that a pre-B cell that has successfully assembled a V_H-region coding sequence will then successfully assemble a V_L-region coding sequence to become a B cell. This chance is further increased because, before a developing pre-B cell produces ordinary light chains, it makes surrogate light chains (see Figure 25–22), which assemble with μ heavy chains. The resulting receptors are displayed on the cell surface and allow the cell to proliferate, producing large numbers of progeny cells, some of which are likely to succeed in producing bona fide light chains.

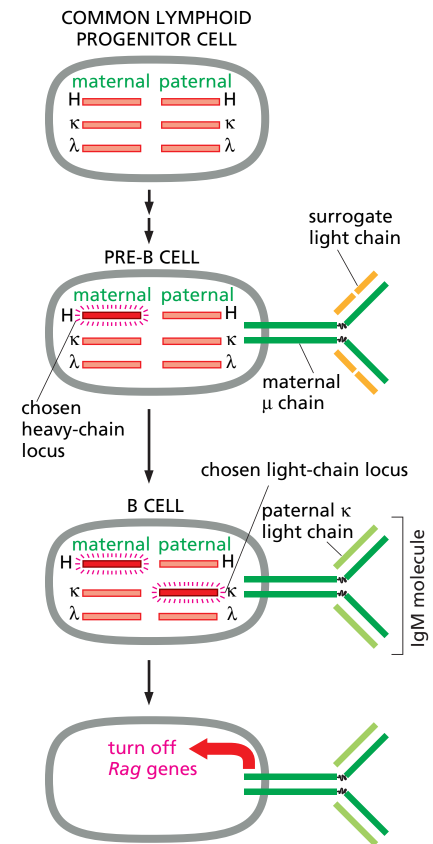


Figure 25–39 Selection of antibody loci during B cell development in the bone marrow. To produce antibodies with only one type of antigen-binding site, a developing B cell must use only one L-chain locus and one H-chain locus. Although the choice between maternal and paternal loci is thought to be random, the assembly of V-region coding sequences in a developing B cell proceeds in an orderly sequence, one segment at a time, usually beginning with the H-chain locus. In this locus, *D* segments first join to J_H segments on both parental chromosomes; then V_H to DJ_H joining occurs on one of these chromosomes (not shown). If this rearrangement produces a functional gene, the resulting production of complete μ chains (always the first H chains made) leads to their expression on the cell surface in association with surrogate light chains (see Figure 25–22). The cell now shuts down all further rearrangements of V_H-region-encoding gene segments and initiates V_L rearrangement. V_L rearrangement usually occurs first in a κ locus, and only if that fails does it occur at the other κ locus or at a λ locus. If, at any point, "in-phase" V_L-to-J_L joining leads to the production of light chains, these combine with preexisting μ chains to form IgM antibody molecules, which insert into the plasma membrane. The IgM cell-surface receptors are thought to enable the newly formed B cell to receive extracellular signals that shut down all further V(D)J recombination, by turning off the expression of the *Rag1* and *Rag2* genes.
If a developing B cell makes a receptor with high affinity for a self antigen, *Rag* gene expression is maintained and the cell undergoes another round of V(D)J recombination in a light-chain locus (called receptor editing—see Figure 25–13), thereby changing the specificity of its receptor (not shown). If a cell fails to assemble both a functional V_H-region and a functional V_L-region coding sequence, it is unable to make antibody molecules and dies by apoptosis (not shown).

The production of a functional B cell is a complex and highly selective process: in the end, all B cells that fail to produce intact antibody molecules die by apoptosis.

We turn now from the mechanisms responsible for generating the primary antibody repertoire before antigen stimulation to those responsible for generating the secondary antibody repertoire after antigen stimulation. We begin with the remarkable Darwinian-like mechanism responsible for increasing the affinity of the antigen-binding sites of antibodies for their specific antigen.

Antigen-Driven Somatic Hypermutation Fine-Tunes Antibody Responses

As mentioned earlier, with the passage of time after immunization, there is usually a progressive increase in the affinity of the antibodies produced against the immunizing antigen. This phenomenon, known as **affinity maturation**, is due to the accumulation of point mutations in both heavy-chain and light-chain V-region coding sequences. The mutations occur long after the coding regions have been assembled. After B cells have been stimulated by antigen and helper T cells in a peripheral lymphoid organ, some of the activated B cells proliferate rapidly in the lymphoid follicles (see Figure 25–16) and form structures called *germinal centers.* Here, the B cells mutate at the rate of about one mutation per V-region coding sequence per cell generation. Because this is about a million times greater than the spontaneous mutation rate in other genes and occurs in somatic cells rather than germ cells (discussed in Chapter 21), the process is called **somatic hypermutation**.

Very few of the altered antibodies generated by hypermutation will have an increased affinity for the antigen. Because the same antibody genes produce the antigen receptors on the B cell surface, the antigen will stimulate preferentially those few B cells that do make such antibodies with increased affinity for the antigen. Clones of these altered B cells will preferentially survive and proliferate, especially as the amount of antigen decreases to very low levels late in the response. Most other B cells in the germinal center will die by apoptosis. Thus, as a result of repeated cycles of somatic hypermutation, followed by antigen-driven proliferation of selected clones of effector and memory B cells, antibodies of increasingly higher affinity become abundant during an immune response, providing progressively better protection against the pathogen. (In

some mammals, including sheep and cows, a similar somatic hypermutation also plays a major part in diversifying the primary antibody repertoire before B cells encounter their antigen.)

A breakthrough in understanding the molecular mechanism of somatic hypermutation came with the identification of an enzyme that is required for the process. It is called **activation-induced deaminase** (**AID**) because it is expressed specifically in activated B cells and deaminates cytosine (C) to uracil (U) in transcribed V-region coding DNA. The deamination produces U:G mismatches in the DNA double helix, and the repair of these mismatches produces various types of mutations, depending on the repair pathway used (**Figure 25–40**). Somatic hypermutation affects only actively transcribed V-region coding sequences, possibly because the AID enzyme is specifically loaded onto RNA transcripts (discussed in Chapter 7). AID is also required when activated B cells switch from IgM production to the production of other classes of antibody, as we now discuss.

B Cells Can Switch the Class of Antibody They Make

As discussed earlier, all B cells begin their antibody-synthesizing lives by making IgM molecules and inserting them into the plasma membrane as receptors for antigen. After the B cells leave the bone marrow, but before they interact with antigen, they begin making both IgM and IgD molecules as membrane-bound antigen receptors, both with the same antigen-binding sites (see Figure 25–22). Stimulation by antigen and helper T cells activates many of these cells to become IgM-secreting effector cells, so that IgM antibodies dominate the primary antibody response. Later in the immune response, however, when activated B cells are undergoing somatic hypermutation, the combination of antigen and helper-T-cell-derived cytokines stimulates many of the B cells to switch from making membrane-bound IgM and IgD to making IgG, IgA, or IgE antibodies—a process called **class switching**. Some of these cells become memory cells that express the corresponding class of antibody molecules on their surface, while others become effector cells that secrete the antibodies. The IgG, IgA, and IgE molecules are collectively referred to as *secondary classes* of antibodies, because they are produced only after antigen stimulation, dominate secondary antibody responses, and make up the secondary antibody repertoire. As we saw earlier, each different class of antibody is specialized to attack pathogens in different ways and in different sites.

The constant region of an antibody heavy chain determines the class of the antibody. Thus, the ability of B cells to switch the class of antibody they make without changing the antigen-binding site implies that the same assembled V_H-region coding sequence (which specifies the antigen-binding part of the heavy chain) can sequentially associate with different C_H-coding sequences. This has important functional implications. It means that, in an individual animal, a

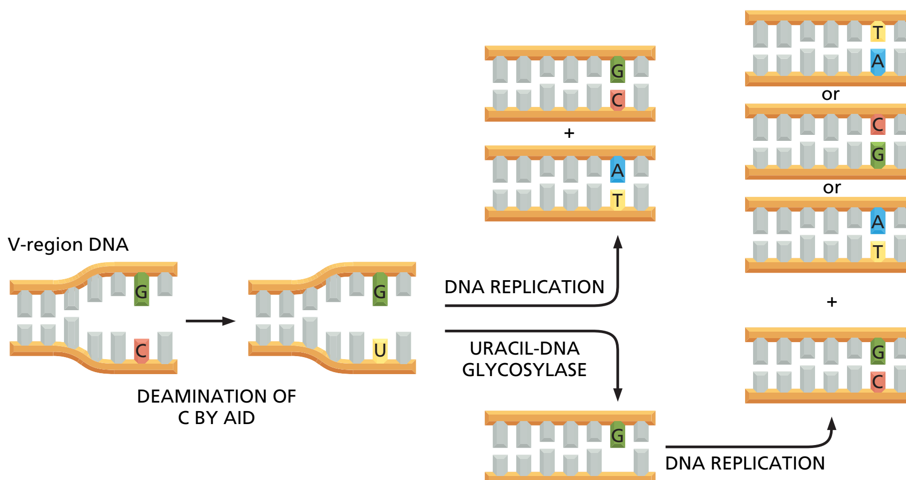


Figure 25–40 Some ways in which AID can cause mutations during somatic hypermutation. AID deaminates some cytosines to uracil in transcribed V-region coding DNA, causing U:G mismatches, which lead to mutations in various ways. Some mutations occur when DNA containing unprocessed U:G mismatches are replicated (see Figure 5–49A). Others occur when the uracil is removed by uracil-DNA glycosylase before the DNA is replicated, as this generates a position on one of the DNA template strands that lacks a base for DNA polymerase to copy. Still others (not shown) occur when the area around the U:G mismatch is excised by the mismatch repair system (discussed in Chapter 5), producing a gap that can be repaired by error-prone DNA polymerases, thereby generating mutations at A:T as well as C:G pairs.

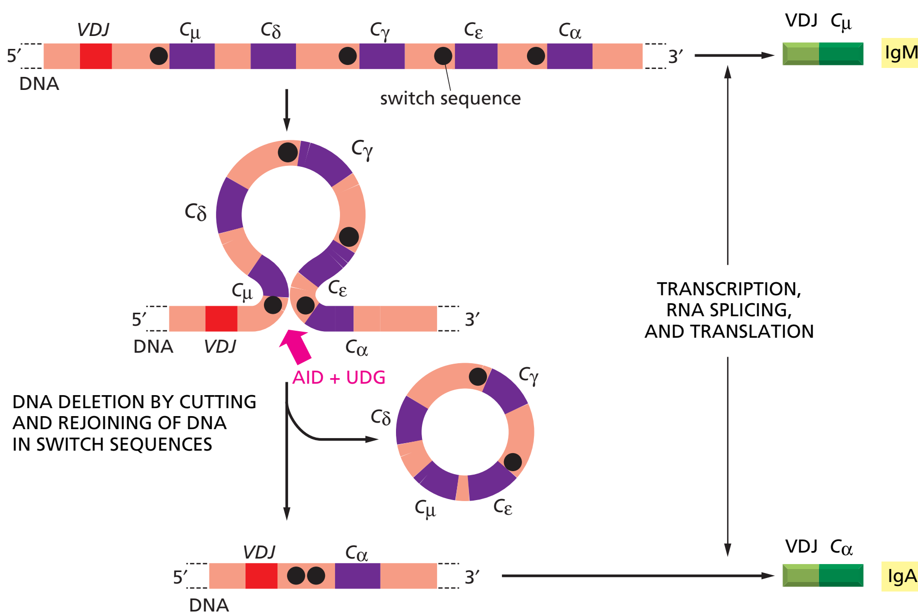


Figure 25–41 An example of the DNA rearrangement that occurs in class switch recombination. A B cell making an IgM antibody from an assembled *VDJ* DNA sequence is stimulated to switch to making an IgA antibody. In the process, it deletes the DNA between the *VDJ* sequence and the C_{α}-coding sequence. Specific DNA sequences *(switch sequences)* located upstream of each C_H-coding sequence (except C_{δ}) recombine with each other, with the deletion of the intervening DNA. As discussed in the text, the recombination process depends on AID and uracil-DNA glycosylase (UDG), the same enzymes that are involved in somatic hypermutation (see Figure 25–40).

particular antigen-binding site that has been selected by environmental antigens can be distributed among the various classes of antibodies, thereby acquiring the different biological properties of each class.

When a B cell switches from making IgM and IgD to one of the secondary classes of antibody, an irreversible change at the DNA level occurs—a process called **class-switch recombination**. It entails the deletion of all the C_H-coding sequences between the assembled VDJ-coding sequence and the particular C_H-coding sequence that the cell is destined to express. Class-switch recombination differs from V(D)J recombination in several ways. (1) It happens after antigen stimulation, mainly in germinal centers, and depends on helper T cells. (2) It uses different recombination signal sequences, called *switch sequences*, which are composed of short motifs tandemly repeated over several kilobases. (3) It involves cutting and joining the switch sequences, which are noncoding sequences, and so the coding sequence is unaffected (**Figure 25–41**). (4) Most importantly, the molecular mechanism is different. It depends on AID, which is also involved in somatic hypermutation, rather than on RAG, which is responsible for V(D)J recombination.

The cytokines that activate class switching induce the production of transcription factors that activate transcription from the relevant switch sequences, allowing AID to bind to these sequences. Once bound, AID initiates switch recombination by deaminating some cytosines to uracil in the vicinity of these switch sequences. Excision of these uracils by uracil-DNA glycosylase (see Figure 25–40) is thought to lead somehow to double-strand breaks in the participating switch regions, which are then joined by a form of nonhomologous end-joining (discussed in Chapter 5).

Thus, whereas the primary antibody repertoire in mice and humans is generated by V(D)J joining mediated by RAG, the secondary antibody repertoire is generated by somatic hypermutation and class-switch recombination, both of which are mediated by AID. **Figure 25–42** summarizes the main mechanisms involved in diversifying antibodies that we have discussed in this chapter.

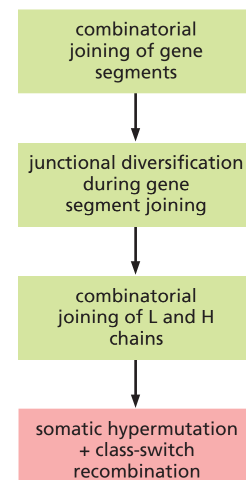


Figure 25–42 The main mechanisms of antibody diversification in mice and humans. Those shaded in *green* occur during B cell development in the bone marrow (or fetal liver), whereas the mechanisms shaded in *red* occur when B cells are stimulated by foreign antigen and helper T cells in peripheral lymphoid organs, either late in a primary response or in a secondary response.

Summary

Antibodies are encoded by three loci on separate chromosomes, each of which produces a different polypeptide chain. One encodes κ light chains, one encodes λ light chains, and one encodes heavy chains. Each antibody locus contains separate gene segments that code for different parts of the variable region of the particular antibody chain. Each light-chain locus contains one or more constant- (C-) region coding sequences and sets of variable (V) and joining (J) gene segments. The heavy-chain locus contains sets of C-region coding sequences and sets of V, diversity (D), and J gene segments.

During B cell development in the bone marrow (or fetal liver), before antigen stimulation, separate gene segments are brought together by site-specific recombination that depends on the RAG complex. A V_L gene segment recombines with a J_L gene segment to produce a DNA sequence coding for the V region of a light chain, and a V_H gene segment recombines with a D and a J_H gene segment to produce a DNA sequence coding for the V region of a heavy chain. Each of the assembled V-region coding sequences is then co-transcribed with the appropriate C-region sequence to produce an RNA molecule that codes for the complete polypeptide chain. Once a B cell makes functional heavy and light chains that form antigen-binding sites (that do not bind a self antigen with high affinity), it turns off the V(D)J recombination process, thereby ensuring that the cell makes only one species of antigen-binding site.

By randomly combining inherited gene segments that code for V_L and V_H regions during B cell development, humans can make hundreds of different light chains and thousands of different heavy chains. Because the antigen-binding site is formed where the hypervariable loops of the V_L and V_H come together in the final antibody, the heavy and light chains can potentially pair to form antibodies with millions of different antigen-binding sites. The loss and gain of nucleotides at the site of gene-segment joining increases this number enormously. These antibodies, made by RAG-dependent V(D)J recombination before antigen stimulation, are low-affinity IgM and IgD antibodies, and they constitute the primary antibody repertoire.

Antibodies are further diversified following antigen stimulation in peripheral lymphoid organs by the AID- and helper-T-cell-dependent processes of somatic hypermutation and class-switch recombination, which produce the high-affinity IgG, IgA, and IgE antibodies that constitute the secondary antibody repertoire. Class switching allows the same antigen-binding site to be incorporated into antibodies that have different biological properties.

T CELLS AND MHC PROTEINS

Like antibody responses, T-cell-mediated immune responses are exquisitely antigen-specific, and they are at least as important as antibodies in defending vertebrates against infection. Indeed, most adaptive immune responses, including most antibody responses, require helper T cells for their initiation. Most importantly, unlike B cells, T cells can help eliminate pathogens that would otherwise be invisible inside host cells. Much of the rest of this chapter is concerned with how T cells accomplish this feat.

T cell responses differ from B cell responses in at least two crucial ways. First, T cells are activated by foreign antigen to proliferate and differentiate into effector cells only when the antigen is displayed on the surface of *antigen-presenting cells*, usually dendritic cells in peripheral lymphoid organs. T cells require antigen-presenting cells for activation because the form of antigen they recognize is different from that recognized by B cells. Whereas B cells recognize intact protein antigens, for example, T cells recognize fragments of protein antigens that have been partly degraded inside the antigen-presenting cell. Special proteins, called *MHC proteins* (introduced in Chapter 24), bind to the peptide fragments and carry them to the surface of the antigen-presenting cell, where T cells can recognize them.

The second difference is that, once activated, effector T cells act only at short range, either within a secondary lymphoid organ or after they have migrated into a site of infection. Effector B cells, by contrast, secrete antibodies that can act far away. Effector T cells interact directly with another host cell in the body, which they either kill (as in the case of an infected host cell, for example) or signal in some way (as in the case of a B cell or macrophage, for example). We shall refer to such host cells as *target cells*. However, because these traget cells must display an antigen bound to an MHC protein on their surface for a T cell to recognize them, they are also antigen-presenting cells.

There are three main classes of T cells—cytotoxic T cells, helper T cells, and regulatory (suppressor) T cells. Effector *cytotoxic T cells* directly kill cells that are infected with a virus or some other intracellular pathogen. Effector *helper T cells*

help stimulate the responses of other cells—mainly macrophages, dendritic cells, B cells, and cytotoxic T cells. Effector *regulatory T cells* suppress the activity of other cells, especially of self-reactive effector T cells.

In this section, we describe these three main classes of T cells and their respective functions. We discuss how they recognize foreign antigens on the surface of antigen-presenting cells and target cells and consider the crucial part played by MHC proteins in the recognition process. Finally, we describe how T cells are selected during their development in the thymus to ensure that only cells with potentially useful receptors survive and mature. We begin by considering the nature of the cell-surface receptors that T cells use to recognize antigen.

T Cell Receptors (TCRs) Are Antibodylike Heterodimers

Because T cell responses depend on direct contact with an antigen-presenting cell or a target cell, **T cell receptors** (**TCRs**), unlike antibodies made by B cells, exist only in membrane-bound form and are not secreted. For this reason, TCRs were difficult to isolate, and it was not until the 1980s that researchers identified their molecular structure. TCRs resemble antibodies. They are composed of two disulfide-linked polypeptide chains, each of which contains two Ig-like domains, one variable and one constant (**Figure 25–43**A). Moreover, the three-dimensional structure of the extracellular part of a TCR has been determined by x-ray diffraction, and it looks very much like one arm of a Y-shaped antibody molecule (Figure 25–43B).

On most T cells, the TCRs have one α chain and one β chain. The genetic loci that encode the α and β chains are located on different chromosomes. Like an antibody heavy-chain locus, the TCR loci contain separate *V, D,* and *J* gene segments (or just *V* and *J* gene segments in the case of the α chain locus), which are brought together by site-specific recombination during T cell development in the thymus. With one exception, T cells use the same mechanisms to generate TCR diversity as B cells use to generate antibody diversity. Indeed, they use the same V(D)J recombinase, including the RAG proteins discussed earlier. The mechanism that does not operate in TCR diversification is antigen-driven somatic hypermutation. Thus, the affinity of the receptors tends to be low (K_a ~ 10^5–10^7 liters/mole), although T cells with the highest affinities are preferentially selected by antigen to persist as memory cells. T cells can partly compensate for their low affinity by increased avidity, which results when multiple TCRs bind simultaneously to multiple membrane-bound ligands (the

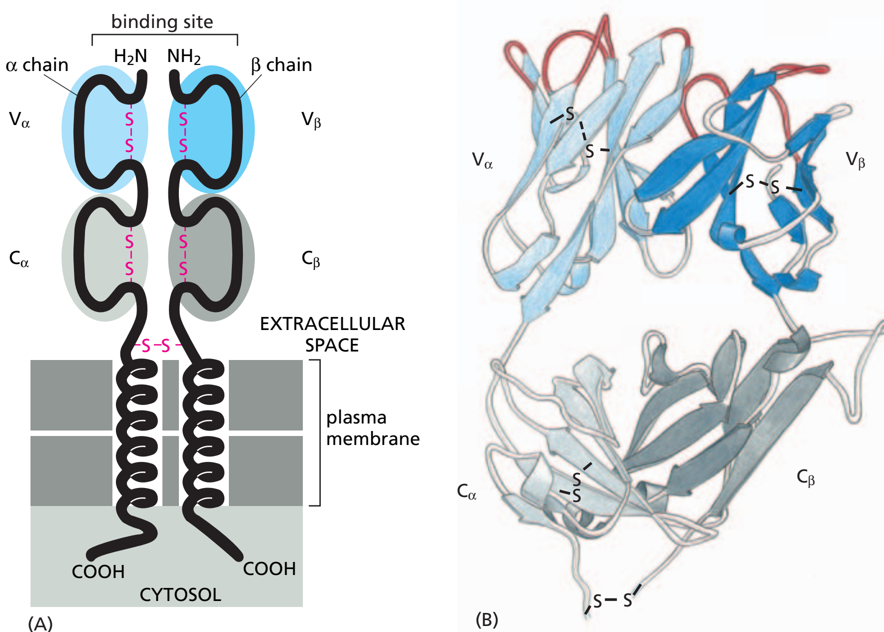


Figure 25–43 A T cell receptor (TCR) heterodimer. (A) Schematic drawing showing that the receptor is composed of an α and a β polypeptide chain. Each chain is about 280 amino acids long and has a large extracellular part that is folded into two Ig-like domains—one variable (V) and one constant (C). The antigen-binding site is formed by a V_α and a V_β domain (shaded in *blue*). Unlike antibodies, which have two binding sites for antigen, TCRs have only one. The αβ heterodimer is noncovalently associated with a large set of invariant membrane-bound proteins (not shown), which help activate the T cell when the TCRs bind to antigen. A typical T cell has about 30,000 such receptor complexes on its surface. (B) The three-dimensional structure of the extracellular part of a TCR. The antigen-binding site is formed by the hypervariable loops of both the V_α and V_β domains *(red),* and it is similar in its overall dimensions and geometry to the antigen-binding site of an antibody molecule. (B, based on K.C. Garcia et al., *Science* 274:209–219, 1996. With permission from AAAS.)

peptide–MHC complexes that we describe later). Moreover, various co-receptors and cell–cell adhesion proteins greatly strengthen the binding of a T cell to an antigen-presenting cell or a target cell.

A minority of T cells, instead of making α and β chains, make a different but related type of receptor heterodimer, composed of γ chains and δ chains. Although these cells normally make up 5–10% of the T cells in human blood, they can be the dominant T cell population in epithelia (in the skin and gut, for example). The functions of these cells are less well understood than those of T cells expressing α/β TCRs, and we will not discuss them further.

As with antigen receptors on B cells, the TCRs are tightly associated in the plasma membrane with a number of invariant membrane-bound proteins that are involved in passing the signal from an antigen-activated receptor to the cell interior (see Figure 25–66). We will discuss these proteins in more detail later. First, we must consider the special ways in which T cells recognize foreign antigen on the surface of an antigen-presenting cell.

Antigen Presentation by Dendritic Cells Can Either Activate or Tolerize T Cells

Naïve cytotoxic or helper T cells must be activated to proliferate and differentiate into effector cells before they can kill or help their target cells, respectively. This activation occurs in peripheral lymphoid organs on the surface of activated **dendritic cells** (**Figure 25–44**) that display foreign antigen complexed with MHC proteins on their surface, along with co-stimulatory proteins. By contrast, memory T cells can be activated by other types of antigen-presenting cells, including macrophages and B cells, as well as by dendritic cells.

Various kinds of dendritic cells interact with T cells, but they all have a single known function, which is to present antigens that either activate or suppress the T cells. Dendritic cells are located in tissues throughout the body, including the central and peripheral lymphoid organs. Wherever they encounter invading microbes, they endocytose the pathogens or their products. If the encounter is not in a lymphoid organ, the dendritic cells carry the foreign antigens via the lymph to local lymph nodes or gut-associated lymphoid organs. The encounter with a pathogen activates pattern recognition receptors of the dendritic cell, which is thereby induced to mature from an antigen-capturing cell to an activated antigen-presenting cell that can activate T cells (see Figure 25–5). Dendritic cells have to be activated in order to activate naïve T cells, and they can also be activated by tissue injury or by effector helper T cells. Tissue injury is thought to activate dendritic cells by the release of heat shock proteins and uric acid crystals when cells die by necrosis rather than by apoptosis (discussed in Chapter 18).

Activated dendritic cells display three types of protein molecules on their surface that have a role in activating a T cell to become an effector cell or a memory cell (**Figure 25–45**): (1) *MHC proteins*, which present foreign antigen to the TCR, (2) *co-stimulatory proteins,* which bind to complementary receptors on the T cell surface, and (3) *cell–cell adhesion molecules,* which enable a T cell to bind to the antigen-presenting cell for long enough to become activated, which is usually hours. In addition, activated dendritic cells secrete a variety of cytokines that can influence the type of effector helper T cell that develops (discussed later), as well as where the T cell migrates after it has been stimulated. T cells activated by dendritic cells isolated from gut-associated Peyer's patches (see Figure 25–3), for example, but not by those isolated from lymph nodes, migrate to the small intestine where their antigens are likely to be located.

Nonactivated dendritic cells also have important roles. They help induce self-reactive T cells to become tolerant, both in the thymus and in other organs; such dendritic cells present self antigens in the absence of the co-stimulatory molecules required to activate naïve T cells. They induce tolerance in at least two ways: they can stimulate abortive responses in the T cell that lead to either inactivation or apoptosis, and they can activate regulatory T cells to suppress the activity of another T cell.

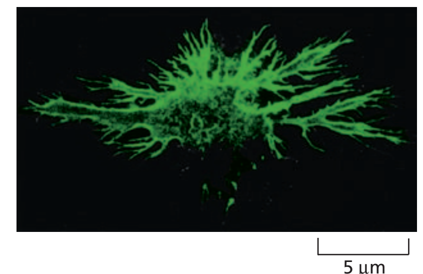


Figure 25–44 Immunofluorescence micrograph of a dendritic cell in culture. These antigen-presenting cells derive their name from their long processes, or "dendrites." The cell has been labeled with a monoclonal antibody that recognizes a surface antigen on these cells. (Courtesy of David Katz.)

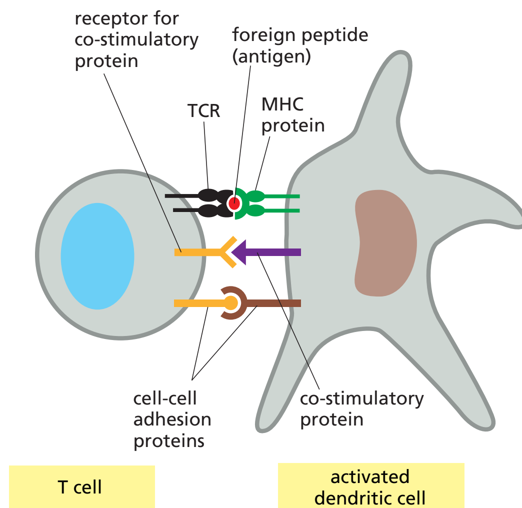


Figure 25–45 Three types of proteins on the surface of an activated dendritic cell involved in activating a T cell. The invariant polypeptide chains that are always stably associated with the T cell receptor (TCR) are not shown.

Before discussing the role of MHC proteins in presenting antigen to T cells, we consider the functions of the three major classes of T cells.

Effector Cytotoxic T Cells Induce Infected Target Cells to Kill Themselves

Cytotoxic T cells protect vertebrates against intracellular pathogens such as viruses and some bacteria and parasites that multiply in the host-cell cytoplasm, where they are sheltered from antibody-mediated attack. Cytotoxic T cells do this by killing the infected cell before the microbes can proliferate and escape from the infected cell to infect neighboring cells. As we discuss later, the intracellular microbes can be recognized by T cells because vertebrate cells have mechanisms for displaying fragments of their intracellular proteins on the cell surface, where they are bound to MHC proteins.

Once a cytotoxic T cell has been activated by an infected antigen-presenting cell to become an effector cell, it can kill any target cell harboring the same pathogen. Using its TCR, the effector cytotoxic T cell first recognizes a microbial antigen bound to an MHC protein on the surface of an infected target cell. This causes the T cell to reorganize its cytoskeleton and focus its killing apparatus on the target (**Figure 25–46**). Focus is achieved when the TCRs actively aggregate—

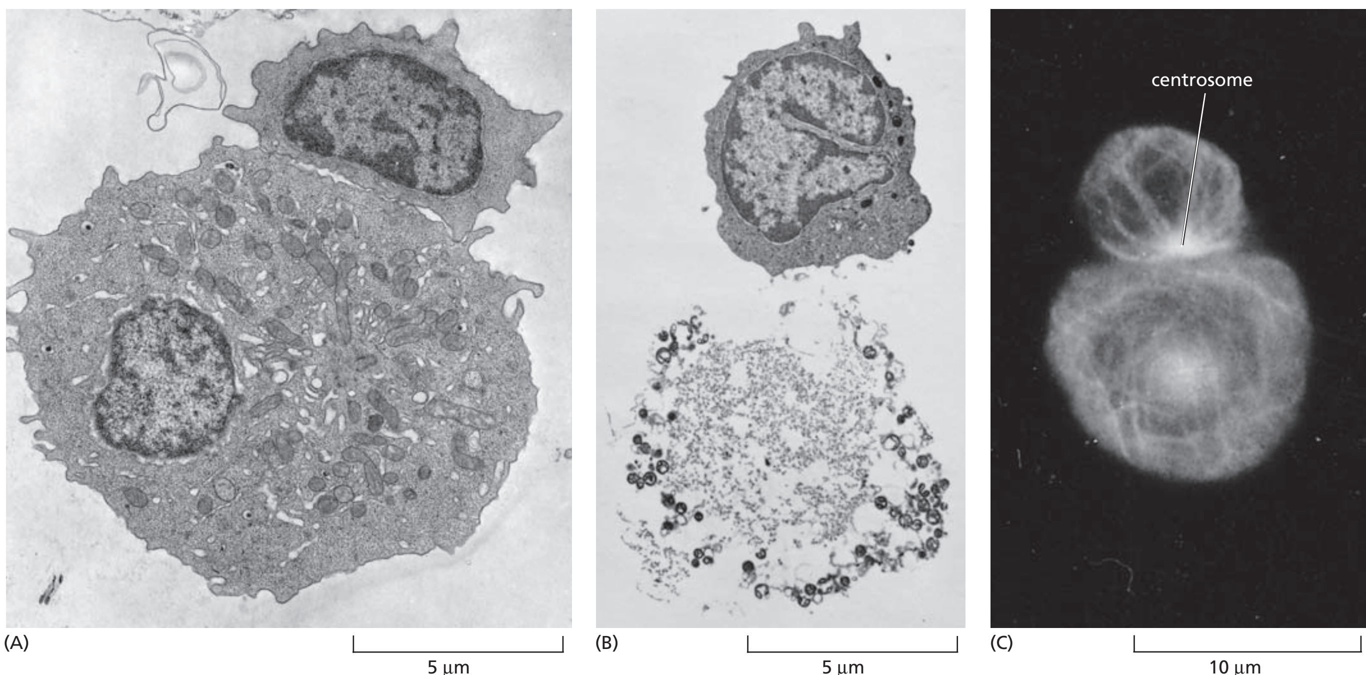


Figure 25–46 Effector cytotoxic T cells killing target cells in culture. <GTCA> (A) Electron micrograph showing an effector cytotoxic T cell binding to a target cell. The cytotoxic T cells were obtained from mice immunized with the target cells, which are foreign tumor cells. (B) Electron micrograph showing a cytotoxic T cell and a tumor cell that the T cell has killed. In an animal, as opposed to in a culture dish, the killed target cell would be phagocytosed by neighboring cells long before it disintegrated in the way that it has here. (C) Immuno-fluorescence micrograph of a T cell and tumor cell after staining with anti-tubulin antibodies. Note that the centrosome in the T cell is located at the point of cell–cell contact with the target cell—an immunological synapse. The secretory granules (not visible) in the T cell are initially transported along microtubules to the centrosome, which then moves to the synapse, delivering the granules to where they can release their contents. See also Figure 16–103. (A and B, from D. Zagury et al., *Eur. J. Immunol.* 5:818–822, 1975. With permission from John Wiley & Sons, Inc. C, reproduced from B. Geiger, D. Rosen and G. Berke, *J. Cell Biol*. 95:137–143, 1982. With permission from The Rockefeller University Press.)

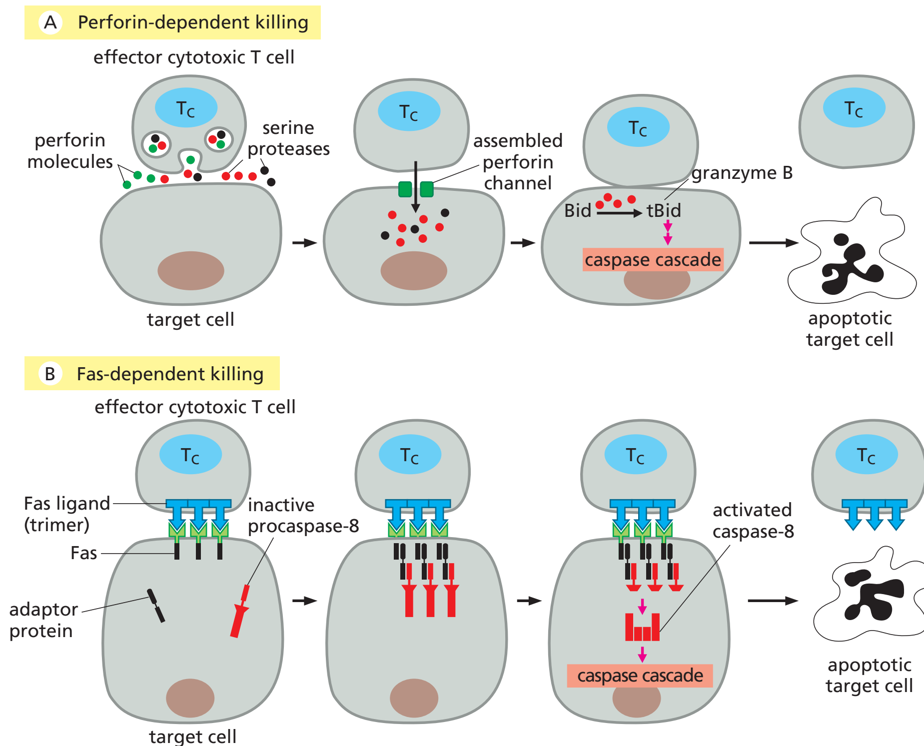


Figure 25–47 Two strategies by which effector cytotoxic T cells kill their target cells. In both cases, the T cell has to contact the target cell to kill it, and a single cytotoxic T cell can kill multiple target cells in sequence. (A) The cytotoxic T cell (T_C) releases perforin and proteolytic enzymes onto the surface of an infected target cell by localized exocytosis. The high concentration of Ca^{2+} in the extracellular fluid causes the perforin to assemble into transmembrane channels in the target cell plasma membrane. The channels are thought to allow the proteolytic enzymes to enter the target cell cytosol. One of the enzymes, granzyme B, cleaves the Bid protein to produce the truncated form tBid, which releases cytochrome *c* from mitochondria to initiate a caspase cascade leading to apoptosis. (B) The homotrimeric Fas ligand on the surface of the cytotoxic T cell binds to and activates the Fas protein on the surface of a target cell. The cytosolic tail of Fas contains a *death domain,* which, when activated, binds to an adaptor protein, which in turn recruits a specific procaspase *(procaspase-8)*. Clustered procaspase-8 molecules become activated and initiate a proteolytic caspase cascade leading to apoptosis (see Figure 18–6).

along with various co-receptors, adhesion molecules, and signaling proteins—at the T cell/target cell interface, forming an *immunological synapse*. A similar synapse forms when an effector helper T cell interacts with its target cell. In this way, effector T cells avoid delivering their signals to neighboring cells.

Once bound to its target cell, an effector cytotoxic T cell can employ one of two strategies to kill the target, both of which operate by inducing the target cell to kill itself by undergoing apoptosis (discussed in Chapter 18). In killing an infected target cell, the cytotoxic T cell usually releases a pore-forming protein called **perforin**, which is homologous to the complement component C9 (see Figure 24–49). The cytotoxic T cell stores perforin in secretory vesicles and releases it by local exocytosis at the point of contact with the target cell. Perforin then polymerizes in the target cell plasma membrane to form transmembrane channels. The secretory vesicles also contain serine proteases, which are thought to enter the target cell cytosol through the perforin channels. One of the proteases, called *granzyme B,* activates a pro-apoptotic Bcl2 protein called *Bid* by producing a truncated form of the protein called *tBid;* tBid then releases cytochrome *c* from mitochondria, triggering a proteolytic caspase cascade that kills the cell by apoptosis (discussed in Chapter 18) (**Figure 25–47**A). Mice with an inactivated perforin gene cannot generate pathogen-specific cytotoxic T cells, and they show an increased susceptibility to certain viral and intracellular bacterial infections.

In the second killing strategy, the cytotoxic T cell activates a death-inducing caspase cascade in the target cell less directly. A homotrimeric protein on the cytotoxic T cell surface called **Fas ligand** binds to transmembrane receptor proteins on the target cell called **Fas**. The binding alters the Fas proteins so that their clustered cytosolic tails recruit procaspase-8 into a complex via an adaptor protein. The recruited procaspase-8 molecules thereby become activated and initiate a caspase cascade that leads to apoptosis (Figure 25–47B).

Effector Helper T Cells Help Activate Other Cells of the Innate and Adaptive Immune Systems

In contrast to cytotoxic T cells, **helper T cells** are crucial for defense against both extracellular and intracellular pathogens. They help stimulate B cells to make

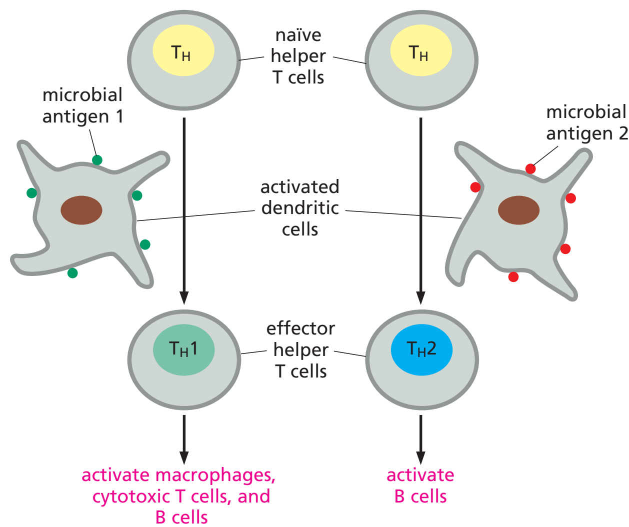


Figure 25–48 Differentiation of naïve helper T cells into either T_H1 or T_H2 effector helper cells in a peripheral lymphoid organ. The nature of the activated dendritic cell and the characteristics of the pathogen that activated it mainly determine which type of effector helper cell develops.

antibodies that help inactivate or eliminate extracellular pathogens and their toxic products. They also activate macrophages to destroy any intracellular pathogens multiplying within the macrophage's phagosomes, and they help activate cytotoxic T cells to kill infected target cells. They can also stimulate a dendritic cell to maintain it in an activated state.

Once an antigen-presenting cell activates a helper T cell to become an effector cell, the helper cell can then help activate other cells. It does this both by secreting a variety of co-stimulatory cytokines and by displaying co-stimulatory proteins on its surface. When activated by its binding to an antigen on a dendritic cell, a naïve helper T cell usually differentiates into either of two distinct types of effector helper cell, called T_H1 and T_H2. *T_H1 cells* are mainly involved in immunity to intracellular microbes and help activate macrophages, cytotoxic T cells, and B cells. *T_H2 cells* are mainly involved in immunity to extracellular pathogens, especially multicellular parasites, and they help activate B cells to make antibodies against the pathogen (**Figure 25–48**). As we discuss later, the nature of the invading pathogen and the types of innate immune responses it elicits largely determine which type of helper T cell develops. This, in turn, determines the nature of the adaptive immune responses mobilized to fight the invaders.

In some cases, a naïve helper T cell that encounters its antigen in a peripheral lymphoid organ develops into an effector cell that suppresses rather than helps an immune response. Such *regulatory T cells,* however, mostly develop in the thymus, as a distinct class of T cell, as we now discuss.

Regulatory T Cells Suppress the Activity of Other T Cells

Regulatory T cells have been difficult to study and characterize, largely because, until recently, there were no good markers to identify them. Indeed, for many years immunologists questioned whether such cells existed or not. They were originally identified by their ability to suppress the activity of other lymphocytes and were therefore called *suppressor T cells.* When markers became available, they were renamed *regulatory T cells* and shown to suppress the activity of effector helper and cytotoxic T cells and of dendritic cells. Although they make up less than 10% of the T cells in the blood and peripheral lymphoid organs, regulatory T cells play a crucial part in immunological self tolerance by suppressing the activity of self-reactive effector helper and cytotoxic T cells. They also help prevent excessive T cell responses to microbial antigens in chronic infections. In both these ways, they help prevent adaptive immune responses from damaging host tissues.

A breakthrough in understanding regulatory T cells was the discovery that they alone express the transcription factor *Foxp3*, which serves as both an unambiguous marker of these cells and a master controller of their development. When the gene encoding this protein is inactivated in mice or humans, for example, the individuals specifically fail to produce regulatory T cells and

develop an early and fatal autoimmune disease involving multiple organs. It is still uncertain how regulatory T cells suppress the action of effector T cells or dendritic cells, but one pathway is thought to involve the secretion of the inhibitory cytokines *TGFβ* and *interleukin 10 (IL10)*.

We now turn to the crucial role of MHC proteins in presenting antigen to T cells.

T Cells Recognize Foreign Peptides Bound to MHC Proteins

As discussed earlier, both cytotoxic T cells and helper T cells are initially activated in peripheral lymphoid organs by recognizing foreign antigen on the surface of an antigen-presenting cell, which is a dendritic cell in the case of a naïve T cell. The antigen is usually in the form of peptide fragments that are generated by the degradation of foreign protein antigens inside the antigen-presenting cell. The recognition process depends on the presence of **MHC proteins** in the antigen-presenting cell. These bind fragments, carry them to the cell surface, and present them there—along with co-stimulatory signals—to the T cells. Once activated, effector T cells then recognize the same peptide–MHC complex on the surface of the target cell they influence, which may be a B cell, a cytotoxic T cell, or an infected macrophage in the case of a helper T cell, or any infected host cell in the case of a cytotoxic T cell; for helper T cells, it may also be the dendritic cell itself.

A large complex of genes called the **major histocompatibility complex** (**MHC**) encodes MHC proteins. There are two main classes of MHC proteins, and they are both structurally and functionally distinct. *Class I MHC proteins* mainly present foreign peptides to cytotoxic T cells, and *class II MHC proteins* mainly present foreign peptides to helper and regulatory T cells (**Figure 25–49**).

Before examining the mechanisms by which protein antigens are processed for presentation to T cells, we must look more closely at the MHC proteins themselves, which have such a critical role in T cell function.

MHC Proteins Were Identified in Transplantation Reactions Before Their Functions Were Known

MHC proteins were initially identified as the main antigens recognized in **transplantation reactions**. When organ grafts are exchanged between adult individuals, either of the same species *(allografts)* or of different species *(xenografts),* they are usually rejected. In the 1950s, skin grafting experiments between different strains of mice demonstrated that *graft rejection* is an adaptive immune response to the foreign antigens on the surface of the grafted cells. Rejection is mediated mainly by T cells, which react against genetically "foreign" versions of cell-surface proteins called *histocompatibility molecules* (from the Greek word *histos,* meaning "tissue"). The MHC proteins encoded by the clustered genes of the major histocompatibility complex (MHC) are by far the most important of these. MHC proteins are expressed on the cells of all higher vertebrates. They were first demonstrated in mice, where they are called *H-2 antigens (h*istocompatibility-*2*

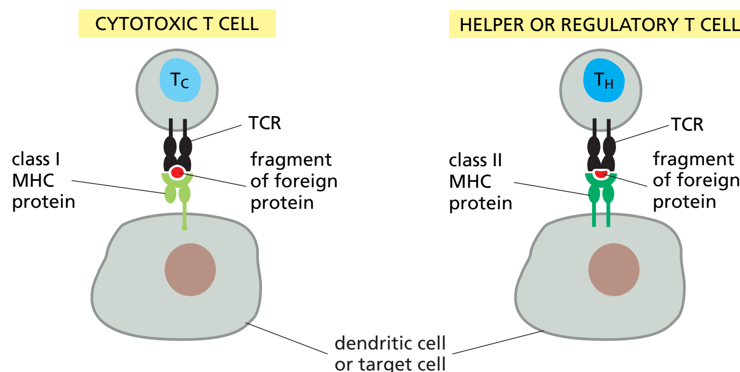


Figure 25–49 Recognition by T cells of foreign peptides bound to MHC proteins. Cytotoxic T cells recognize foreign peptides in association with class I MHC proteins, whereas helper T cells and regulatory T cells recognize foreign peptides in association with class II MHC proteins. In both cases, the T cell recognizes the peptide–MHC complexes on the surface of a dendritic cell or a target cell.

antigens). In humans they are called *HLA antigens (h*uman-*l*eucocyte-*a*ssociated antigens) because they were first demonstrated on leucocytes (white blood cells).

Three remarkable properties of MHC proteins baffled immunologists for many years. First, MHC proteins are overwhelmingly the antigens that are recognized in T-cell-mediated transplantation reactions. Second, an unusually large fraction of T cells are able to recognize foreign MHC proteins: whereas fewer than 0.001% of an individual's naïve T cells respond to a typical viral antigen, up to 10% of them respond to the foreign MHC proteins of another individual. Third, some of the genes that code for MHC proteins are the most *polymorphic* known in higher vertebrates. That is, within a species, there are an extraordinarily large number of *alleles* (alternative forms of the same gene) present (in some cases more than 400), without any one allele predominating. As each individual has at least 12 genes encoding MHC proteins (as discussed later), it is very rare for two unrelated individuals to have an identical set of MHC proteins. These differences make it very difficult to match donor and recipient for organ transplantation unless they are closely related.

Of course, a vertebrate does not need to protect itself against invasion by foreign vertebrate cells. So the apparent obsession of its T cells with foreign MHC proteins and the extreme polymorphism of these molecules were a great puzzle. The puzzle was at least partly solved when researchers discovered that (1) MHC proteins bind fragments of foreign proteins and display them on the surface of host cells for T cells to recognize, and (2) T cells respond to foreign MHC proteins in the same way they respond to self MHC proteins that have foreign antigen bound to them.

Class I and Class II MHC Proteins Are Structurally Similar Heterodimers

Class I and class II MHC proteins have very similar overall structures. They are both transmembrane heterodimers with extracellular N-terminal domains that bind antigen for presentation to T cells.

Class I MHC proteins consist of a transmembrane α chain, which is encoded by a class I MHC gene, and a small extracellular protein called *β_2-microglobulin* (**Figure 25–50**A). The β_2-microglobulin does not span the membrane and is

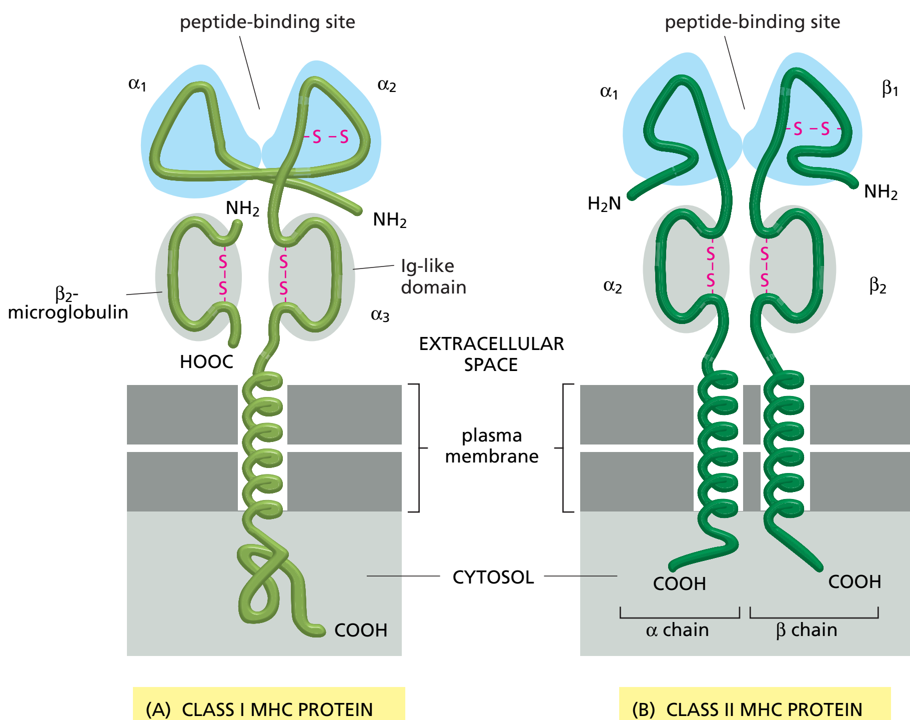


Figure 25–50 Class I and class II MHC proteins. (A) The α chain of the class I molecule has three extracellular domains, α_1, α_2, and α_3, encoded by separate exons. It is noncovalently associated with a smaller polypeptide chain, β_2-microglobulin, which is not encoded within the MHC. The α_3 domain and β_2-microglobulin are Ig-like. While β_2-microglobulin is invariant, the α chain is extremely polymorphic, mainly in the α_1 and α_2 domains. (B) In class II MHC proteins, both chains are polymorphic, mainly in the α_1 and β_1 domains; the α_2 and β_2 domains are Ig-like. Thus, there are striking similarities between class I and class II MHC proteins. In both, the two outermost domains (shaded in *blue*) are polymorphic and interact to form a groove that binds peptide fragments of foreign proteins and presents them to T cells.

encoded by a gene that does not lie in the MHC gene cluster. The α chain is folded into three extracellular globular domains (α_1, α_2, and α_3), and the α_3 domain and the β_2-microglobulin, which are closest to the membrane, are both similar to an Ig domain. The two N-terminal domains of the α chain, which are farthest from the membrane, contain the polymorphic (variable) amino acids that T cells recognize in transplantation reactions. These domains bind a peptide and present it to cytotoxic T cells.

Like class I MHC proteins, **class II MHC proteins** are heterodimers with two conserved Ig-like domains close to the membrane, and two polymorphic (variable) N-terminal domains farthest from the membrane. In these proteins, however, both chains (α and β) are encoded by genes within the MHC, and both span the membrane (Figure 25–50B). The two polymorphic domains bind a peptide and present it to helper or regulatory T cells.

The presence of Ig-like domains in class I and class II proteins suggests that MHC proteins and antibodies have a common evolutionary history. The locations of the genes that encode class I and class II MHC proteins in humans are shown in **Figure 25–51**, where we illustrate how an individual can make six types of class I MHC proteins and more than six types of class II proteins.

In addition to the classic class I MHC proteins, there are many *class-I-MHC-like proteins*, which form dimers with β_2-microglobulin. These proteins are encoded by genes outside the MHC and are much less polymorphic than MHC proteins, but some of them present specific microbial antigens, including some lipids and glycolipids, to T cells. Although the functions of most of them are unknown, some have a role in brain development.

An MHC Protein Binds a Peptide and Interacts with a T Cell Receptor

Any individual can make only a small number of different classic MHC proteins, which together must be able to present peptide fragments from almost any foreign protein to T cells. Thus, unlike an antibody molecule, each MHC protein has to be able to bind a very large number of different peptides. X-ray crystallographic analyses of MHC proteins have revealed the structural basis for this versatility.

As shown in **Figure 25–52**A, a class I MHC protein has a single *peptide-binding site* located at one end of the molecule, facing away from the plasma membrane. This site consists of a deep groove between two long α helices; the groove narrows at both ends so that it is only large enough to accommodate an extended peptide about 8–10 amino acids long. In fact, when a class I MHC protein was first analyzed by x-ray crystallography, this groove contained bound peptides that had co-crystallized with the MHC protein (Figure 25–52B), suggesting that once a peptide binds to this site it does not normally dissociate.

A typical peptide binds in the groove of a class I MHC protein in an extended conformation, with its terminal amino group bound to invariant amino acids of the MHC protein at one end of the groove and its terminal carboxyl group bound to invariant amino acids at the other end of the groove (**Figure 25–53**). Some amino acid side chains of the peptide bind to variable (polymorphic) amino acids of the MHC protein distributed along the groove, while other side chains point outward, in a position to be recognized by TCRs on cytotoxic T cells. Because the invariant amino acids of the MHC protein at the ends of the groove recognize features of the peptide backbone that are common to all peptides, each allelic form of a class I MHC protein can bind a large variety of peptides of

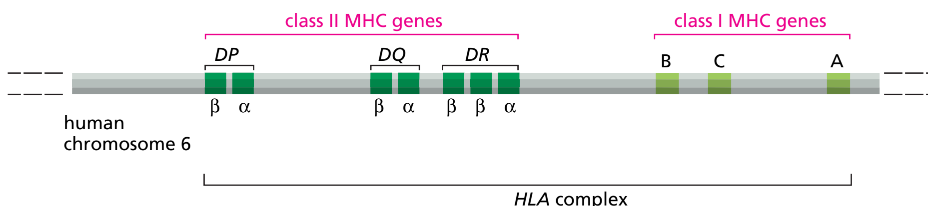


Figure 25–51 Human MHC genes. This simplified schematic drawing shows the location of the genes that encode the transmembrane subunits of class I *(light green)* and class II *(dark green)* MHC proteins. The genes shown encode three types of class I proteins (HLA-A, HLA-B, and HLA-C) and three types of class II MHC proteins (HLA-DP, HLA-DQ, and HLA-DR). An individual can therefore make six types of class I MHC proteins (three encoded by maternal genes and three by paternal genes) and more than six types of class II MHC proteins. The number of class II MHC proteins that can be made is greater than six because there are two DR β genes and because maternally encoded and paternally encoded polypeptide chains can sometimes pair.

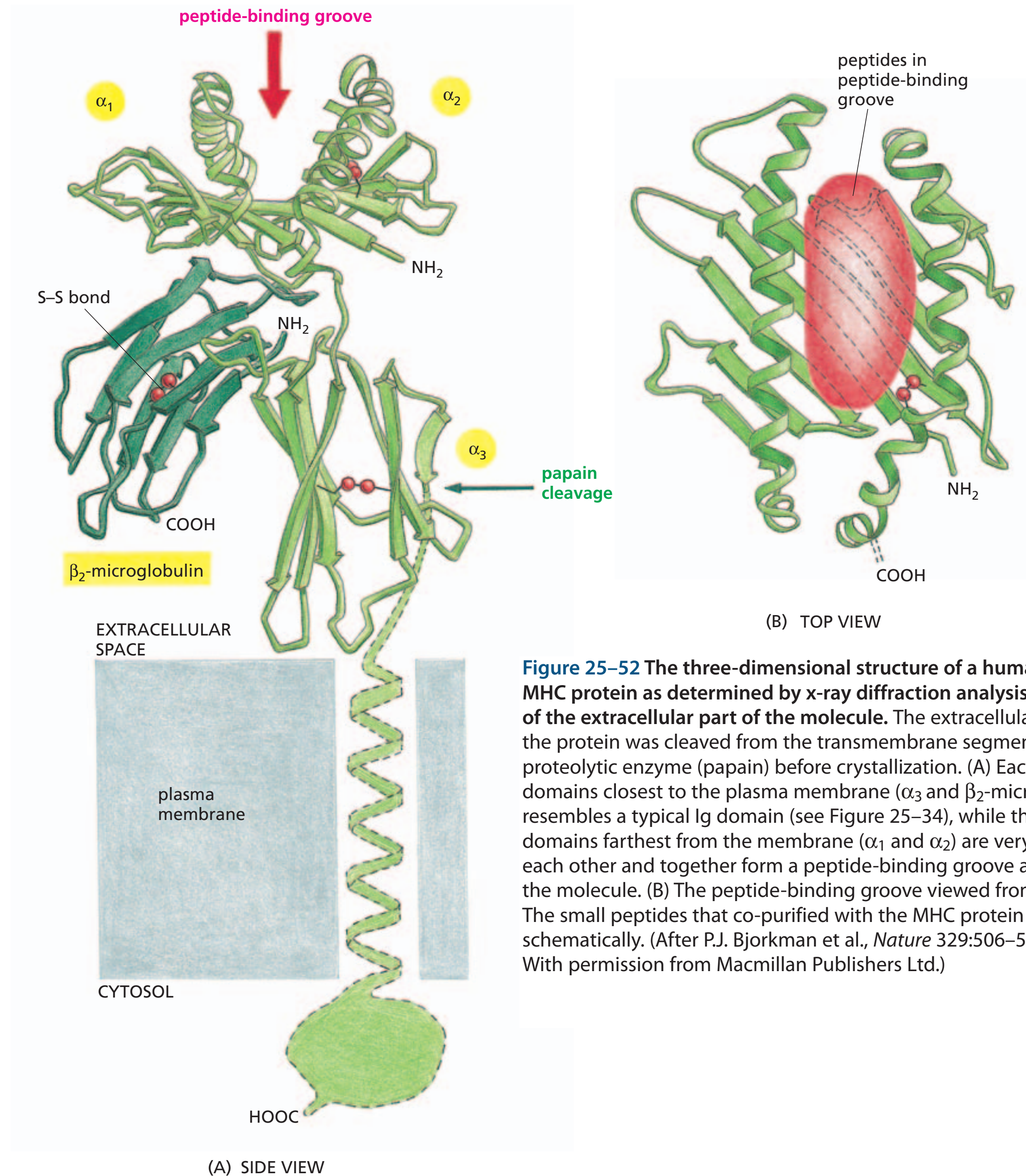


Figure 25–52 The three-dimensional structure of a human class I MHC protein as determined by x-ray diffraction analysis of crystals of the extracellular part of the molecule. The extracellular part of the protein was cleaved from the transmembrane segment by a proteolytic enzyme (papain) before crystallization. (A) Each of the two domains closest to the plasma membrane (α_3 and β_2-microglobulin) resembles a typical Ig domain (see Figure 25–34), while the two domains farthest from the membrane (α_1 and α_2) are very similar to each other and together form a peptide-binding groove at the top of the molecule. (B) The peptide-binding groove viewed from above. The small peptides that co-purified with the MHC protein are shown schematically. (After P.J. Bjorkman et al., *Nature* 329:506–512, 1987. With permission from Macmillan Publishers Ltd.)

diverse sequence. At the same time, the polymorphic MHC amino acids along the groove, which bind specific side chains of the peptide, ensure that each allelic form binds and presents a distinct characteristic set of peptides. Thus, the six types of class I MHC proteins in an individual can present a broad range of foreign peptides to the cytotoxic T cells, but in each individual they do so in slightly different ways.

The three-dimensional structure of class II MHC proteins is very similar to that of class I proteins, but the antigen-binding groove does not narrow at the ends, so it can accommodate longer peptides, which are usually 12–20 amino acids long. Moreover, the peptide is not bound at its ends but is instead held by interactions with invariant amino acids of the MHC protein distributed along the length of the groove (**Figure 25–54**). As in the case of class I MHC proteins, side chains of other amino acids in the peptide either bind to polymorphic MHC amino acids along the groove or point upward to be recognized by TCRs on helper or regulatory T cells. A class II MHC groove can accommodate a more heterogeneous set of peptides than can a class I MHC groove. Thus, although an individual makes only a small number of types of class II proteins, each with its

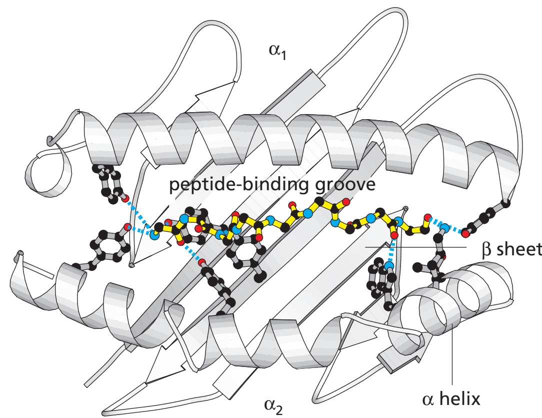


Figure 25–53 A peptide bound in the groove of a class I MHC protein. <AAGT> Schematic drawing of a top view of the groove. A ribbon drawing of the MHC groove is shown in *gray;* it is formed by the α_1 and α_2 domains of the protein (see Figures 25–50A and 25–52A). The backbone of the bound peptide is shown in *yellow,* with the carbon atoms in *black,* oxygen atoms in *red,* and nitrogen atoms in *blue;* the amino terminus of the peptide is to the left. Note that the terminal amino and carboxyl groups of the peptide backbone bind via hydrogen and ionic bonds (shown as *dotted blue lines*) to the side chains of invariant MHC amino acids of the MHC protein towards the ends of the groove (shown in full). Although not illustrated in the drawing, the side chains of some amino acids of the peptide bind to variable (polymorphic) amino acids of the groove, while others point upward and can be recognized by TCRs on a cytotoxic T cell. (Courtesy of Paul Travers.)

own unique peptide-binding groove, together these proteins can bind and present an enormous variety of foreign peptides to helper T cells, which have a crucial role in almost all adaptive immune responses.

X-ray crystallographic analyses of complexes formed between a soluble TCR and a soluble MHC protein with peptide in its binding groove revealed the way in which the TCR recognizes a peptide–MHC complex. Recombinant DNA technology produced the soluble proteins for these experiments. In each case studied, the TCR fits diagonally across the peptide-binding groove and binds through its V_α and V_β hypervariable loops to both the walls of the groove and the peptide (**Figure 25–55**). Soluble peptide–MHC complexes are now widely used to detect T cells with a particular specificity; they are usually cross-linked into tetramers so that they can bind to four TCRs on the T cell surface with strong avidity.

MHC Proteins Help Direct T Cells to Their Appropriate Targets

Virtually all nucleated vertebrate cells express class I MHC proteins. This is presumably because effector cytotoxic T cells must be able to focus on and kill any cell in the body that becomes infected with an intracellular microbe such as a virus. Class II proteins, by contrast, are normally confined largely to cells that take up foreign antigens from the extracellular fluid and interact with helper T cells. These cells also express class I MHC proteins. They include dendritic cells, which initially activate naïve helper T cells, as well as the targets of effector helper T cells, such as macrophages and B cells.

It is important that effector cytotoxic T cells focus their attack mainly on cells that *make* the foreign antigens (such as viral proteins), while helper T cells focus their help mainly on cells that have taken up foreign antigens from the extracellular fluid. Since the former type of target cell is always a menace, while

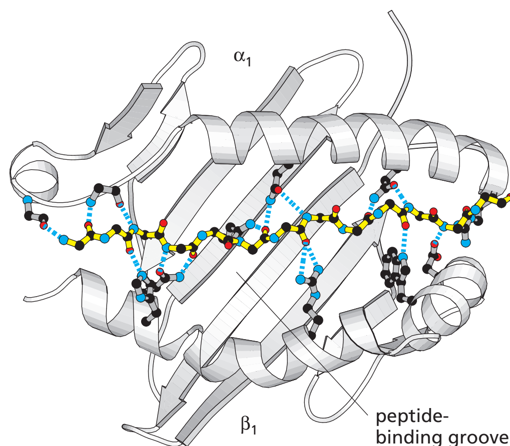


Figure 25–54 A peptide bound in the groove of a class II MHC protein. <GAAA> Schematic drawing similar to that shown in Figure 25–53. The groove is formed by the amino terminal domains of the α and β chains (α_1 and β_1—see Figure 25–50B). Note that the peptide extends beyond the ends of the groove and that its backbone binds through hydrogen bonds distributed along the length of the peptide to invariant amino acid side chains in the groove. (Courtesy of Paul Travers.)

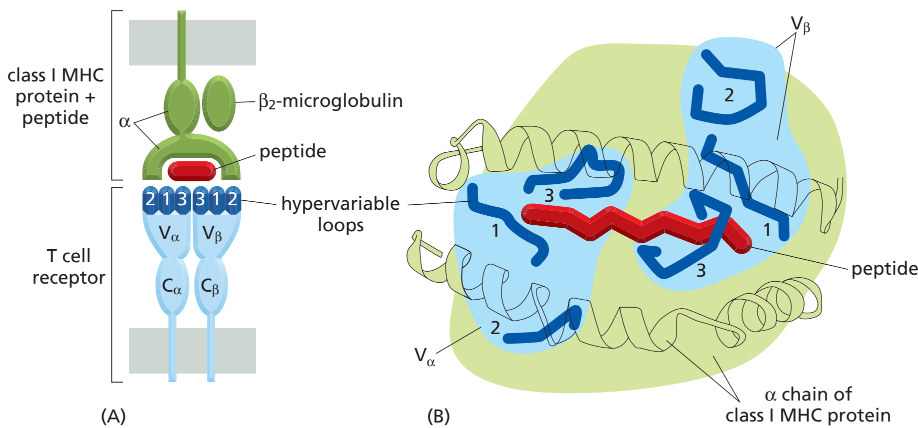


Figure 25–55 The interaction of a T cell receptor with a viral peptide bound to a class I MHC protein. (A) Schematic view of the hypervariable loops of the V_α and V_β domains of the T cell receptor interacting with the peptide and the walls of the peptide-binding groove of the MHC protein. Note that the third hypervariable loops, which are the most variable, primarily interact with the peptide, whereas the other hypervariable loops primarily bind to the walls of the peptide-binding groove. (B) Drawing of the "footprint" of the V domains *(light blue)* and hypervariable loops *(dark blue)* of the receptor over the peptide-binding groove, as determined by x-ray diffraction. The V_α domain covers the amino half of the peptide, while the V_β domain covers the carboxyl half. Note that the receptor is oriented diagonally across the peptide-binding groove. (B, adapted from D.N. Garboczi et al., *Nature* 384:134–141, 1996. With permission from Macmillan Publishers Ltd.)

the latter type is essential for the body's adaptive immune defenses, it is vitally important that T cells do not confuse the two target cells and misdirect their cytotoxic and helper functions. Therefore, in addition to the antigen receptor that recognizes a peptide–MHC complex, each of the three major classes of T cells also expresses a *co-receptor* that recognizes a separate, invariant part of the appropriate class of MHC protein. These two co-receptors, called CD4 and CD8, help direct helper (and regulatory) T cells and cytotoxic T cells, respectively, to their appropriate targets. **Table 25–2** reviews the properties of class I and class II MHC proteins.

CD4 and CD8 Co-Receptors Bind to Invariant Parts of MHC Proteins

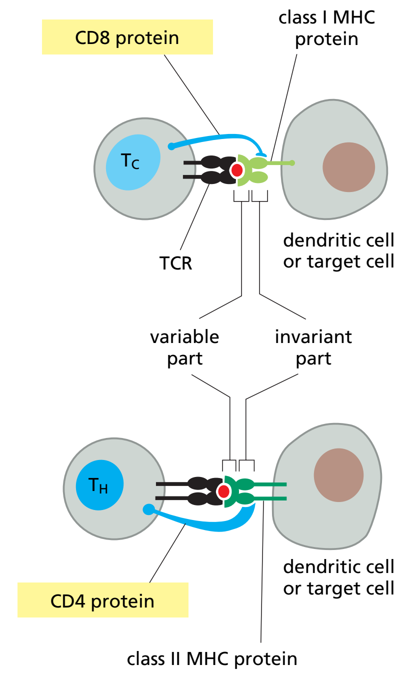
The affinity of TCRs for peptide–MHC complexes on an antigen-presenting cell or target cell is usually too low by itself to mediate a functional interaction between the two cells. T cells normally require *accessory receptors* to help stabilize the interaction by increasing the overall strength of the cell–cell adhesion. Unlike TCRs or MHC proteins, the accessory receptors do not bind foreign antigens and are invariant.

When accessory receptors have a direct role in activating the T cell by generating their own intracellular signals, they are called **co-receptors**. The most important and best understood of the co-receptors on T cells are the CD4 and CD8 proteins, both of which are single-pass transmembrane proteins with extracellular Ig-like domains. Like TCRs, they recognize MHC proteins, but, unlike TCRs, they bind to invariant parts of the protein, far away from the peptide-binding groove. **CD4** is expressed on both helper T cells and regulatory T cells and binds to class II MHC proteins, whereas **CD8** is expressed on cytotoxic T cells and binds to class I MHC proteins (**Figure 25–56**). Thus, CD4 and CD8 contribute to T cell recognition by helping the T cell to focus on particular MHC proteins, and thereby on particular types of target cells: the recognition of class I

Table 25–2 Properties of Human Class I and Class II MHC Proteins

	CLASS I	CLASS II
Genetic loci	*HLA-A, HLA-B, HLA-C*	*DP, DQ, DR*
Chain structure	α chain + β_2-microglobulin	α chain + β chain
Cell distribution	most nucleated cells	dendritic cells, B cells, macrophages, thymus epithelial cells, some others
Presents antigen to	cytotoxic T cells	helper T cells, regulatory T cells
Source of peptide fragments	mainly proteins made in cytoplasm	mainly endocytosed plasma membrane and extracellular proteins
Polymorphic domains	$\alpha_1 + \alpha_2$	$\alpha_1 + \beta_1$
Recognition by co-receptor	CD8	CD4

Figure 25–56 CD4 and CD8 co-receptors on the surface of T cells. Cytotoxic T cells (T_C) express CD8, which recognizes class I MHC proteins, whereas helper T cells (T_H) and regulatory T cells (not shown) express CD4, which recognizes class II MHC proteins. Note that the co-receptors bind to the same MHC protein that the TCR has engaged, so that they are brought together with TCRs during the antigen recognition process. Whereas the TCR binds to the variable (polymorphic) parts of the MHC protein that form the peptide-binding groove, the co-receptor binds to the invariant part, well away from the groove.



MHC proteins allows cytotoxic T cells to focus on any host cell, while the recognition of class II MHC proteins allows helper T cells to focus on a small subset of cells—most notably dendritic cells, macrophages, and B cells. The cytoplasmic tail of the CD4 and CD8 proteins is associated with a member of the Src family of cytoplasmic tyrosine kinases called *Lck*, which phosphorylates various intracellular proteins on tyrosines and thereby participates in the activation of the T cell (discussed in Chapter 15). Antibodies to CD4 and CD8 are widely used as tools to help distinguish between the main classes of T cells, in both humans and experimental animals: whereas only cytotoxic T cells express CD8, both helper and regulatory T cells express CD4.

Ironically, the AIDS virus (HIV) uses CD4 molecules (as well as chemokine receptors) to enter helper T cells. AIDS patients are susceptible to infection by microbes that are not normally dangerous because HIV depletes helper T cells. As a result, most AIDS patients die of infection within several years of the onset of symptoms, unless they are treated with a combination of powerful anti-HIV drugs. HIV also uses CD4 and chemokine receptors to enter macrophages, which also have both of these receptors on their surface.

Before a T cell can recognize a foreign protein, the protein has to be processed inside an antigen-presenting cell or target cell so that it can be displayed as peptide–MHC complexes on the cell surface. We now consider how a virus-infected antigen-presenting cell or target cell processes viral proteins for presentation to a cytotoxic T cell. We then discuss how ingested foreign proteins are processed for presentation to a helper T cell.

Cytotoxic T Cells Respond to Fragments of Foreign Cytosolic Proteins in Association with Class I MHC Proteins

An experiment performed in the 1970s provided dramatic evidence that class I MHC proteins are involved in the recognition of viral antigens by cytotoxic T cells. Researchers found that effector cytotoxic T cells from a virus-infected mouse could kill cultured cells infected with the same virus only if these target cells expressed some of the same class I MHC proteins as the infected mouse. This experiment demonstrated that the cytotoxic T cells of any individual can recognize a specific foreign antigen on a target cell only when the target cell expresses at least some of the allelic forms of class I MHC proteins expressed by that individual, a phenomenon known as *MHC restriction* (**Figure 25–57**).

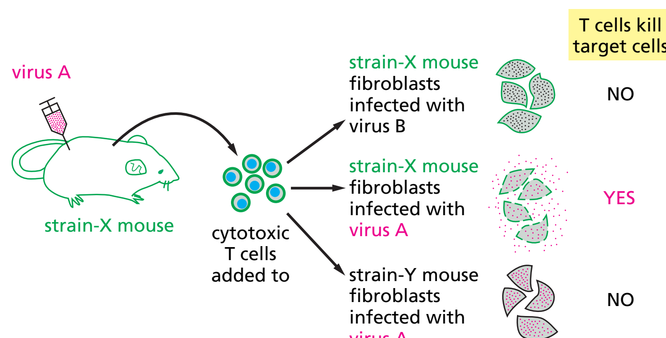


Figure 25–57 The classic experiment showing that an effector cytotoxic T cell recognizes some aspect of the surface of the host target cell in addition to a viral antigen. Mice of strain X are infected with virus A. Seven days later, the spleens of these mice contain effector cytotoxic T cells able to kill virus-infected, strain-X fibroblasts in cell culture. As expected, they kill only fibroblasts infected with virus A and not those infected with virus B. Thus, the cytotoxic T cells are virus-specific. The same T cells, however, are also unable to kill fibroblasts from strain-Y mice infected with the same virus A, indicating that the cytotoxic T cells recognize a genetic difference between the two kinds of fibroblasts and not just the virus. Pinning down the difference required the use of special strains of mice (known as *congenic strains*) that either were genetically identical except for the alleles at their class I MHC loci or were genetically different except for these alleles. In this way, it was found that the killing of infected target cells required that they express at least one of the same class I MHC alleles as expressed by the original infected mouse. This result suggested that class I MHC proteins are somehow necessary to present cell-surface-bound viral antigens to effector cytotoxic T cells and that they do so in a highly specific manner.

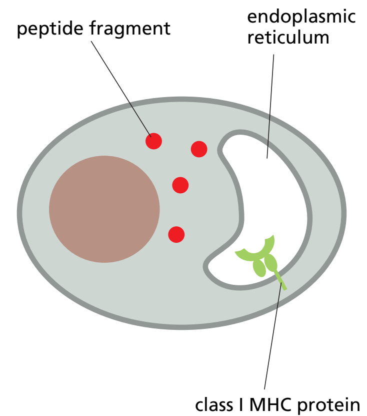


Figure 25–58 The peptide-transport problem. How do peptide fragments get from the cytosol, where they are produced, into the ER lumen, where the peptide-binding grooves of class I MHC proteins are located? A special transport process is required.

Subsequent evidence indicated that, in killing a virus-infected cell, a cytotoxic T cell recognizes degraded fragments of internal proteins of the virus that are bound to class I MHC proteins on the infected cell surface. Because a T cell can recognize tiny amounts of foreign antigen (as few as 1–10 peptide–MHC complexes for T cells with the highest affinity receptors), only a tiny fraction of the fragments generated from viral proteins have to bind to class I MHC proteins and get to the cell surface to trigger an effector cytotoxic T cell to attack.

The internal viral proteins are synthesized in the cytosol of the infected cell. As discussed in Chapter 3, proteolytic degradation in the cytosol is mainly mediated by an ATP- and ubiquitin-dependent mechanism that operates in *proteasomes*—large proteolytic enzyme complexes constructed from many different protein subunits. All proteasomes are probably able to generate peptide fragments of appropriate length to fit in the groove of class I MHC proteins, as even bacterial proteasomes can do so. This suggests that the MHC groove evolved to fit peptides of around this length. Nonetheless, some proteasomes are apparently specialized for producing peptides for class I MHC proteins, as they contain two subunits that are encoded by genes located within the MHC chromosomal region.

How do peptides generated in the cytosol make contact with the peptide-binding groove of class I MHC proteins in the lumen of the endoplasmic reticulum (**Figure 25–58**)? The answer initially came through observations of mutant cells in which class I MHC proteins are not expressed at the cell surface but are instead degraded within the cell. The mutant genes in these cells proved to encode subunits of a protein belonging to the family of *ABC transporters,* which we discuss in Chapter 11. This transporter is located in the ER membrane and uses the energy of ATP hydrolysis to pump peptides from the cytosol into the ER lumen. The genes encoding its two subunits are located in the MHC chromosomal region, and if either gene is inactivated by mutation, cells are unable to supply peptides to class I MHC proteins. It is striking that the class I MHC proteins in such mutant cells are degraded inside the cell without reaching the cell surface. This occurs because peptide binding is normally required for the proper folding of these proteins: until it binds a peptide, a class I MHC protein remains in the ER, tethered to an ABC transporter by a chaperone protein; without a peptide bound, the trapped MHC proteins in the mutant cells eventually undergo proteolysis (**Figure 25–59**).

In all cells, peptide fragments come from the cells' own cytosolic and nuclear proteins that are degraded in the processes of normal protein turnover and quality control mechanisms. (Surprisingly, more than 30% of the proteins made by mammalian cells are apparently faulty and are degraded in proteasomes soon after they are synthesized.) These peptides are constantly being pumped into the ER and are carried to the cell surface by class I MHC proteins. They are not antigenic, however, because the cytotoxic T cells that could recognize them have either been eliminated, inactivated, or suppressed by regulatory T cells in the process of self-tolerance (see Figure 25–13).

When an antigen activates cytotoxic T cells or T_H1 helper T cells to become effector cells, the effector cells secrete the cytokine **interferon-γ** (**IFNγ**), which greatly enhances anti-viral responses. The IFNγ acts on virus-infected host cells in two ways. It blocks viral replication, and it increases the expression of many genes within the MHC chromosomal region. These genes include those that encode class I MHC proteins, the two specialized proteasome subunits, and the two subunits of the peptide transporter located in the ER (**Figure 25–60**). Thus, the machinery in a host cell that is required for presenting viral antigens to cytotoxic T cells is coordinately called into action by IFNγ, creating a positive feedback loop that amplifies the immune response and culminates in the death of the infected cell.

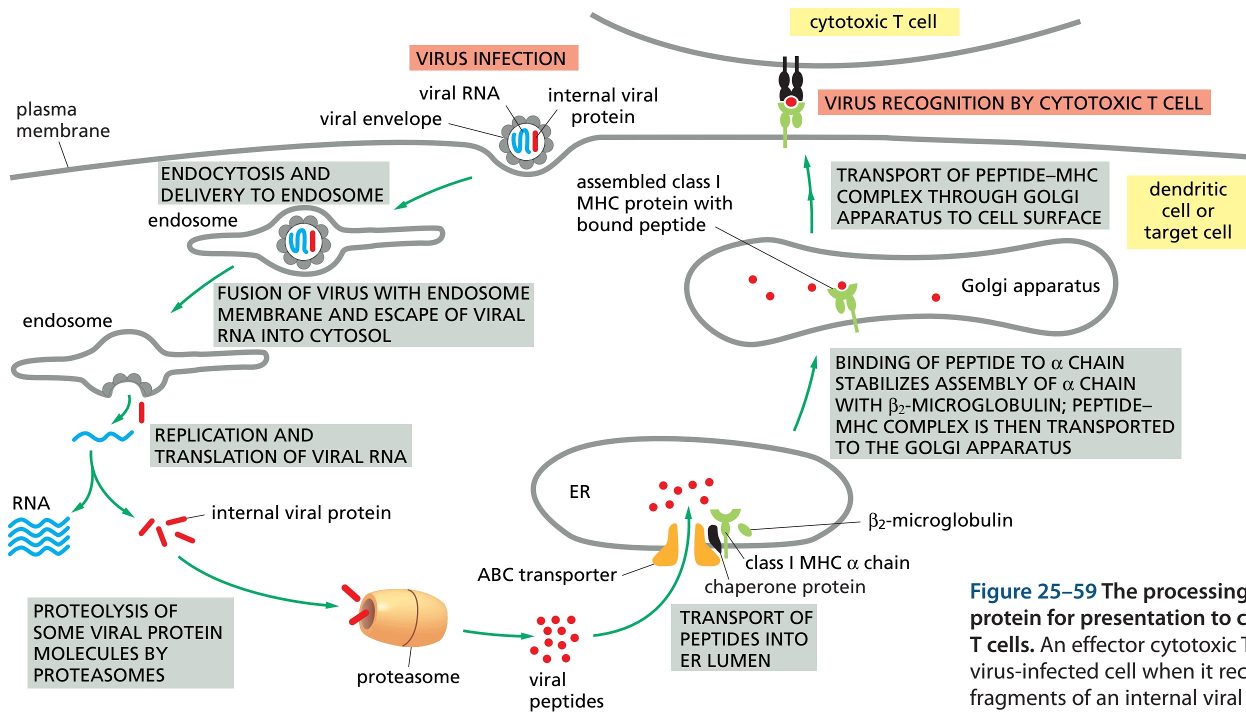


Figure 25–59 The processing of a viral protein for presentation to cytotoxic T cells. An effector cytotoxic T cell kills a virus-infected cell when it recognizes fragments of an internal viral protein bound to class I MHC proteins on the surface of the infected cell. Not all viruses enter the cell in the way that this enveloped RNA virus does, but fragments of internal viral proteins always follow the pathway shown. Only a small proportion of the viral proteins synthesized in the cytosol are degraded and transported to the cell surface, but this is sufficient to attract an attack by a cytotoxic T cell. Several chaperone proteins (only one of which is shown) in the ER lumen aid the folding and assembly of class I MHC proteins. The chaperones bind to the class I MHC α chain and act sequentially. The last one binds the MHC protein to the ABC transporter, as shown. The assembly of class I MHC proteins and their transport to the cell surface requires the binding of peptide.

Helper T Cells Respond to Fragments of Endocytosed Foreign Protein Associated with Class II MHC Proteins

Unlike cytotoxic T cells, helper T cells do not act directly to kill infected cells. Instead, they stimulate macrophages to be more effective in destroying intracellular microorganisms, and they help B cells and cytotoxic T cells to respond to microbial antigens.

Like the viral proteins presented to cytotoxic T cells, the proteins presented to helper T cells on dendritic cells or target cells are degraded fragments of foreign proteins. The fragments are bound to class II MHC proteins in much the same way that virus-derived peptides are bound to class I MHC proteins. But both the source of the peptide fragments presented and the route they take to find the MHC proteins generally differ.

Rather than being derived from foreign protein synthesized in the cytosol of a cell, the foreign peptides presented to helper T cells are derived from endosomes. Some come from extracellular microbes or their products that the antigen-presenting cell has endocytosed and degraded in the acidic environment of its endosomes. Others come from microbes growing within the endocytic compartment of the antigen-presenting cell. In either case, the peptides do not have to be pumped across a membrane because they are generated in a compartment that is topologically equivalent to the extracellular space. Instead of entering the lumen of the ER, where the class II MHC proteins are synthesized and assembled, they bind to preassembled class II heterodimers in an endosomal compartment. When the peptide binds, the class II MHC protein alters its conformation, trapping the

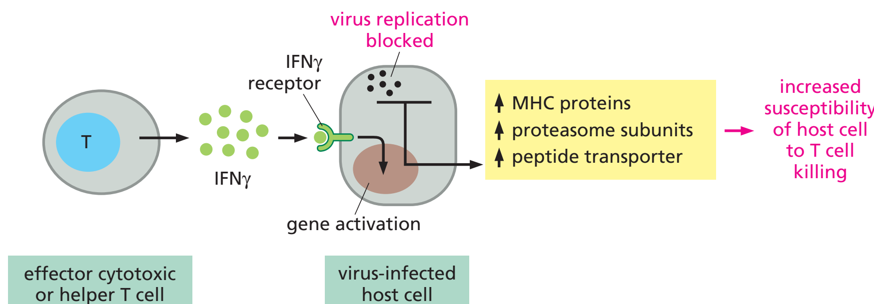


Figure 25–60 Some effects of interferon-γ (IFNγ) on virus-infected cells. The activated IFNγ receptors signal to the nucleus, altering gene transcription, which leads to the effects indicated. The effects shaded in *yellow* tend to make the infected cell a better target for killing by an effector cytotoxic T cell.

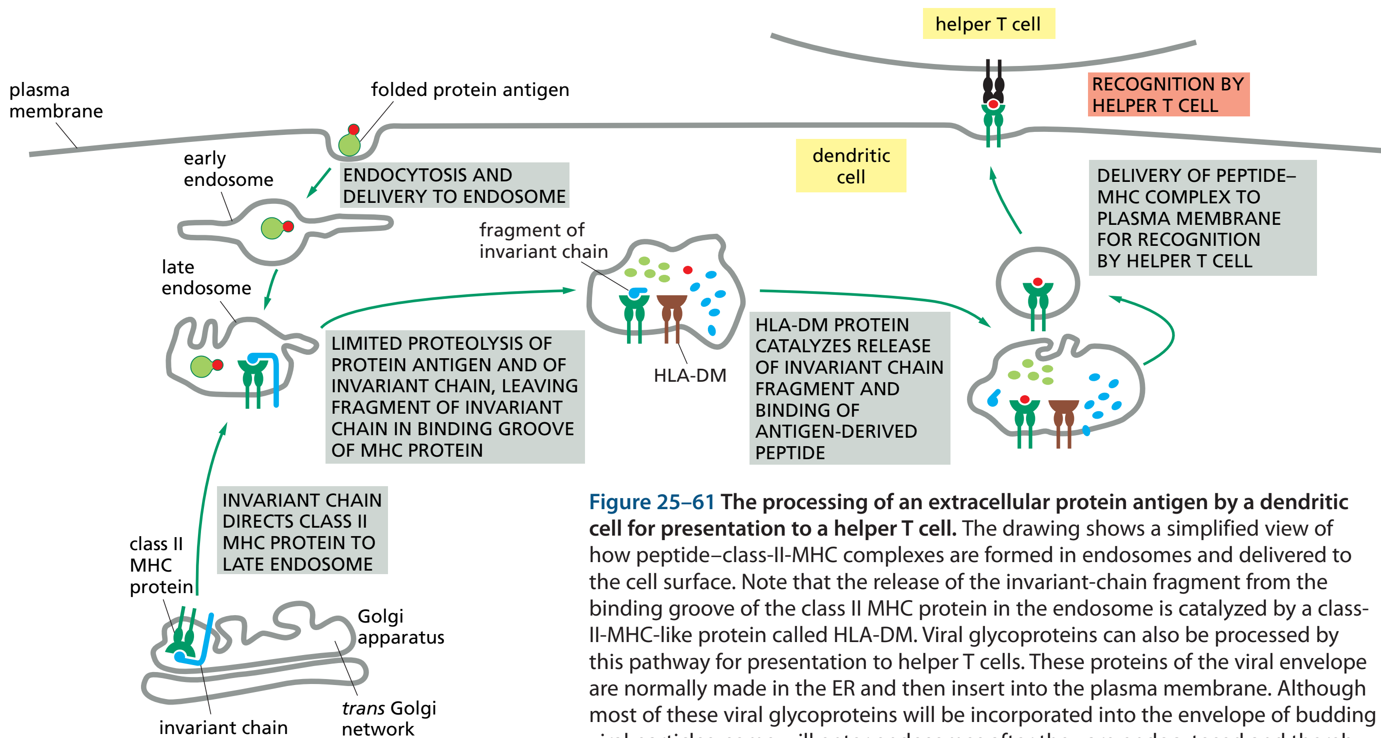


Figure 25–61 The processing of an extracellular protein antigen by a dendritic cell for presentation to a helper T cell. The drawing shows a simplified view of how peptide–class-II-MHC complexes are formed in endosomes and delivered to the cell surface. Note that the release of the invariant-chain fragment from the binding groove of the class II MHC protein in the endosome is catalyzed by a class-II-MHC-like protein called HLA-DM. Viral glycoproteins can also be processed by this pathway for presentation to helper T cells. These proteins of the viral envelope are normally made in the ER and then insert into the plasma membrane. Although most of these viral glycoproteins will be incorporated into the envelope of budding viral particles, some will enter endosomes after they are endocytosed and thereby enter the class II MHC pathway.

peptide in the binding groove and transferring it to the cell surface for presentation to helper T cells.

A newly synthesized class II MHC protein must avoid clogging its binding groove prematurely in the ER lumen with peptides pumped in from the cytosol. A special polypeptide, called the **invariant chain**, ensures this by associating with newly synthesized class II MHC heterodimers in the ER. Part of its polypeptide chain lies within the peptide-binding groove of the MHC protein, thereby blocking the groove from binding peptides in the lumen of the ER. The invariant chain also directs class II MHC proteins from the *trans* Golgi network to a late endosomal compartment. Here, proteases cleave the invariant chain, leaving only a short fragment bound in the peptide-binding groove of the MHC protein. This fragment is then released, freeing the MHC protein to bind peptides derived from endocytosed proteins (**Figure 25–61**). In this way, the functional differences between class I and class II MHC proteins are ensured—the former mainly presenting molecules that come from the cytosol, the latter mainly presenting molecules that come from the endocytic compartment.

This distinction between antigen presentation to cytotoxic T cells and to helper T cells, however, is not absolute. Dendritic cells, for example, need to be able to activate cytotoxic T cells to kill virus-infected cells even when the virus does not infect dendritic cells themselves. To do so, dendritic cells use a process called **cross-presentation**, which begins when they phagocytose fragments of virus-infected cells. They then actively transport viral proteins out of the phagosome into the cytosol, where they are degraded in proteasomes; resulting fragments of the viral proteins are then transported into the ER lumen, where they load onto assembling class I MHC proteins. Cross-presentation in dendritic cells also operates to activate cytotoxic T cells against tumor antigens of cancer cells and against the MHC proteins of foreign organ grafts.

Most of the class I and class II MHC proteins on the surface of a target cell have peptides derived from self proteins in their binding groove. For class I proteins, the fragments mainly derive from degraded cytosolic and nuclear proteins. For class II proteins, they mainly derive from degraded proteins that originate in the plasma membrane or extracellular fluid and are endocytosed. Only a small fraction of the 10^5 or so class II MHC proteins on the surface of an antigen-presenting cell will have foreign peptides bound to them. This is sufficient, however:

even a single copy of such a peptide–MHC complex on a dendritic cell can activate a helper T cell that has a TCR that binds the complex with a high enough affinity.

Potentially Useful T Cells Are Positively Selected in the Thymus

We have seen that T cells recognize antigen in association with self MHC proteins but not in association with foreign MHC proteins (see Figure 25–57): that is, T cells show *MHC restriction*. This restriction results from a process of **positive selection** during T cell development in the thymus. In this process, those immature T cells *(thymocytes)* that will be capable of recognizing foreign peptides presented by self MHC proteins are selected to survive and mature, while most of the remainder, which would be of no use to the animal, undergo apoptosis. Thus, MHC restriction is an acquired property of the immune system that emerges as T cells develop in the thymus.

The most direct way to study the selection process is to follow the fate of a set of developing T cells of known specificity. This can be done by using transgenic mice that express a specific pair of rearranged α and β TCR genes derived from a T cell clone of known antigen and MHC specificity. Such experiments show that the transgenic T cells mature in the thymus and populate the peripheral lymphoid organs only if the transgenic mouse also expresses the same allelic form of MHC protein as is recognized by the transgenic TCR. If the mouse does not express the appropriate MHC protein, the transgenic T cells die in the thymus. Thus, the survival and maturation of a developing T cell depend on a match between its TCR and the MHC proteins expressed in the thymus (which have self peptides derived from the body's own proteins bound to them). Similar experiments using transgenic mice in which MHC expression is confined to specific cell types in the thymus indicate that it is MHC proteins on epithelial cells in the cortex of the thymus that are responsible for this positive selection (**Figure 25–62**).

After positively selected T cells leave the thymus, their continued survival as naïve T cells depends on their continual stimulation by self-peptide–MHC complexes (and the cytokine *IL7*); this stimulation is enough to promote cell survival but not enough to activate the T cells to proliferate or become effector or memory cells.

As part of the positive selection process in the thymus, developing T cells that express TCRs recognizing class I MHC proteins are selected to become

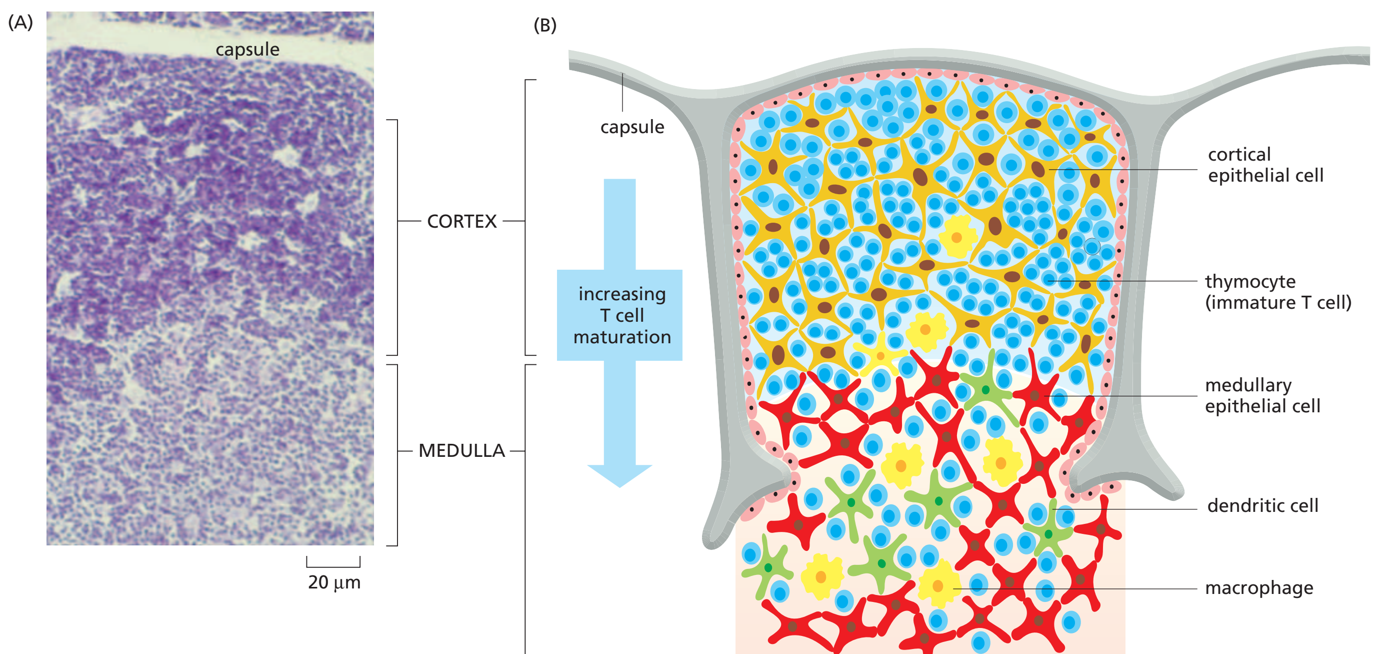


Figure 25–62 The cellular organization of the human thymus. (A) A light micrograph of a stained section of one thymus lobule showing the outer cortex and inner medulla. (B) A schematic drawing of the lobule showing the cellular composition. The cortex contains immature thymocytes, and the medulla contains mature thymocytes. The thymocytes, macrophages, and dendritic cells develop from cells that migrate in from the bone marrow. The functions of these different regions and cell types will be discussed later, when we consider how developing thymocytes are selected for survival. Because of these selection processes, more than 95% of the thymocytes produced in the thymus die by apoptosis. The dead cells are rapidly phagocytosed and digested by the macrophages. (Adapted from K. Murphy et al., Janeway's Immunobiology, 7th ed. New York: Garland Science, 2008.)

cytotoxic cells, while T cells that express TCRs recognizing class II MHC proteins are selected to become either helper cells or regulatory cells. Thus, genetically engineered mice that lack cell-surface class I MHC proteins lack cytotoxic T cells specifically, whereas mice that lack class II MHC proteins lack both helper and regulatory T cells specifically. The development of regulatory T cells depends on special groups of epithelial cells in the thymus medulla called *Hassall's corpuscles*.

The cells that are undergoing positive selection initially express both CD4 and CD8 co-receptors, and these are required for the selection process: without CD4, helper and regulatory T cells fail to develop, and without CD8, cytotoxic T cells fail to develop. Once they develop, cytotoxic T cells lose CD4, and helper and regulatory T cells lose CD8.

Positive selection still leaves a large problem to be solved. If developing cytotoxic and helper T cells with receptors that recognize self peptides associated with self MHC proteins were to mature in the thymus and migrate to peripheral lymphoid tissues, they might wreak havoc. A second, *negative selection* process in the thymus is required to help avoid this potential disaster.

Most Developing Cytotoxic and Helper T Cells That Could Be Activated by Self-Peptide–MHC Complexes Are Eliminated in the Thymus

As discussed previously, a fundamental feature of the adaptive immune system is that it can learn to distinguish self from nonself and normally does not react against self molecules. An important mechanism in achieving this state of immunological self tolerance is the deletion in the thymus of developing self-reactive cytotoxic and helper T cells—that is, T cells whose TCRs bind strongly enough to the complex of a self peptide and a self MHC protein to become activated. Because, as we discuss later, most B cells require helper T cells to respond to antigen, the elimination of self-reactive helper T cells also helps ensure that any self-reactive B cells that escape from the mechanisms responsible for B cell tolerance induction are harmless (see Figure 25–13).

Before discussing the negative selection process that removes self-reactive T cells in the thymus, it is useful to speculate on the logic behind a two-step system that ends up selecting for the small fraction of developing T cells that express a TCR that binds weakly, but not strongly, to a self MHC protein carrying a self-peptide. As illustrated in **Figure 25–63**, it is thought that the production of a large repertoire of such T cells guarantees that at least a few of the T cells will be able to bind strongly to the complex of a foreign peptide with the same MHC protein, thus triggering an adaptive immune response. However, it is of course not enough for the thymus to select *for* T cells that recognize self MHC proteins; it must also select *against* cytotoxic and helper T cells that could become activated by self MHC proteins complexed with self peptides in peripheral lymphoid

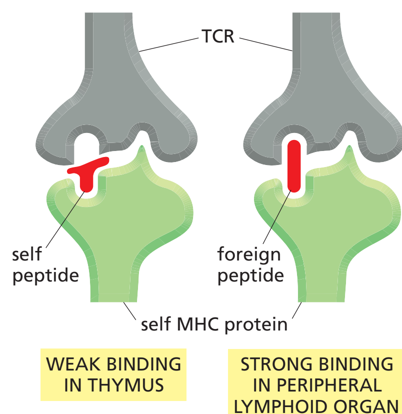


Figure 25–63 Schematic drawing showing how a TCR selected in the thymus because it binds weakly to a self MHC protein complexed with a self peptide can bind strongly to the same MHC protein complexed with a foreign peptide. Because the self peptide complex gives weak binding and the foreign peptide is not present in the thymus, a T cell expressing this TCR in the thymus would be positively selected and also avoid negative selection.

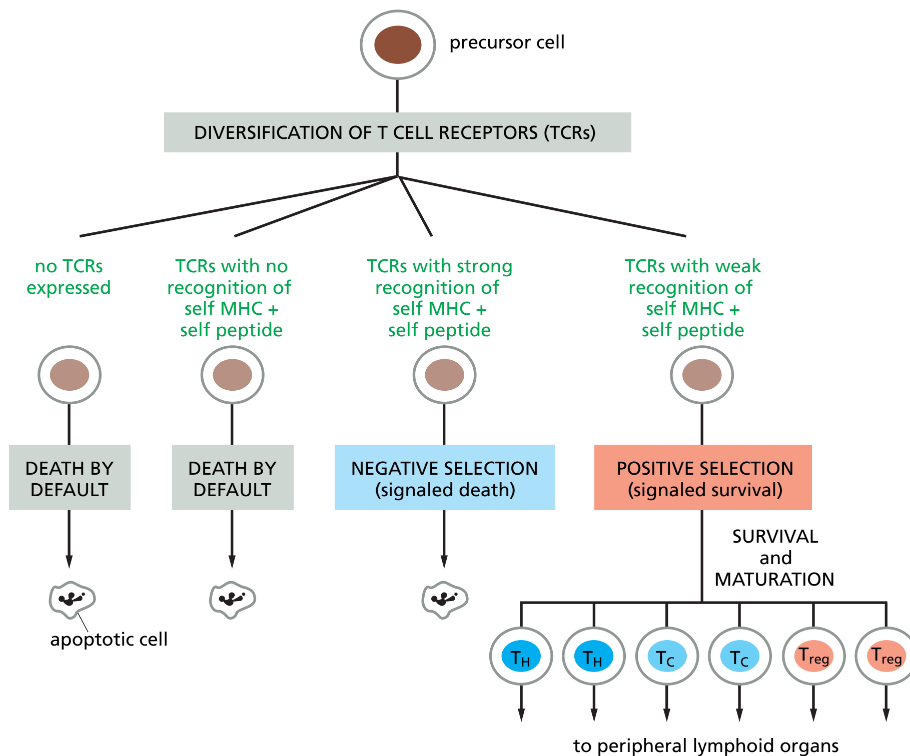


Figure 25–64 The result of positive and negative selection in the thymus. Cells with TCRs that would enable them to respond to foreign peptides in association with self MHC proteins (see Figure 25–63) are positively selected: they survive, mature, and migrate to peripheral lymphoid organs. All of the other cells undergo apoptosis—either because they do not express TCRs that recognize self MHC proteins with self peptides bound or because they recognize such complexes too well and undergo negative selection.

Although not shown, the cells undergoing positive selection initially express both CD4 and CD8 co-receptors. During the process of positive selection, helper T cells (T_H), cytotoxic T cells (T_C), and regulatory T cells (T_{reg}) diverge by a poorly understood mechanism. In this process, helper cells and regulatory cells develop that express CD4 but not CD8 and recognize peptides in association with class II MHC proteins, while cytotoxic cells develop that express CD8 but not CD4 and recognize peptides in association with class I MHC proteins.

organs. The overall goal can be achieved by (1) ensuring the death of cytotoxic and helper T cells that bind *strongly* to the self-peptide–MHC complexes in the thymus while (2) promoting the survival of those that bind weakly and (3) permitting the death of those that do not bind at all. Process 2 constitutes a major part of the positive selection we have just discussed. Process 1 is called **negative selection**, or clonal deletion in the thymus (see Figure 25–13). In both process 1 and process 3, the cells die by apoptosis (**Figure 25–64**).

The most convincing evidence for negative selection in the thymus derives once again from experiments with transgenic mice. The introduction of TCR transgenes encoding a receptor that recognizes a male-specific peptide antigen, for example, results in large numbers of mature T cells expressing the transgenic receptor in both the thymus and peripheral lymphoid organs of female mice, which do not express the peptide. Very few, however, are found in male mice, in which the cells die in the thymus before they have a chance to mature. Like positive selection, negative selection requires the interaction of a TCR and a CD4 or CD8 co-receptor with an appropriate MHC protein. Unlike positive selection of developing helper and cytotoxic T cells, however, which occurs mainly on the surface of epithelial cells in the thymus cortex, negative selection of these cells occur in the thymus medulla, mainly on the surface of dendritic cells that are descendants of cells that have migrated into the thymus from the bone marrow.

Some Organ-specific Proteins Are Ectopically Expressed in the Thymus Medulla

After the discovery of negative selection of developing T cells in the thymus, immunologists wondered how T cells avoid responses against self proteins that are not present in the thymus. One explanation is that some potentially self-reactive T cells are deleted or functionally inactivated after they leave the thymus. This occurs when the cells recognize self peptides bound to MHC proteins on the surface of dendritic cells that have not been activated by pathogens and therefore do not provide appropriate activating signals. It can also occur via regulatory T cells in the periphery that suppress the activity of some self-reactive effector T cells. These two mechanisms are examples of *peripheral tolerance*,

because, unlike T cell deletion in the thymus *(central tolerance)*, they occur after T cells leave the thymus (see Figure 25–13).

Recently, a third explanation has been discovered. A special class of epithelial cells in the thymus medulla ectopically expresses proteins previously thought to be expressed only outside the thymus in specific organs; insulin, for example, which is made by β cells in the pancreas, is also made by a small subset of medullary thymic epithelial cells. The ectopic expression of many of these proteins, including insulin, depends on a nuclear protein called *autimmune regulator (AIRE)*, which is specifically expressed in the same medullary thymic epithelial cells. Inactivation of the gene encoding AIRE in mice or humans results in a severe multi-organ autoimmune disease, indicating the importance of AIRE-dependent central tolerance to at least some "organ-specific" self proteins. It remains a mystery how AIRE promotes this ectopic expression of genes in the thymus medulla.

The Function of MHC Proteins Helps Explain Their Polymorphism

The role of MHC proteins in binding foreign peptides and presenting them to T cells helps explain the extensive polymorphism of these proteins. In the evolutionary war between pathogens and the adaptive immune system, pathogens tend to change their antigens to avoid associating with MHC proteins. When a pathogen succeeds, it is able to sweep through a population as an epidemic. In such circumstances, the few individuals that produce a new MHC protein that can associate with an antigen of the altered pathogen have a large selective advantage. In addition, individuals with two different alleles at any given MHC locus (heterozygotes) have a better chance of resisting infection than those with identical alleles at the locus, as they have a greater capacity to present peptides from a wide range of pathogens. Thus, this type of selection will tend to promote and maintain a large diversity of MHC proteins in the population. Support for the idea that infectious diseases have been a driving force for MHC polymorphism has come from studies in West Africa. In this region, individuals with a specific MHC allele have a reduced susceptibility to a severe form of malaria. Although the allele is rare elsewhere, it is found in 25% of the West African population where this form of malaria is common.

If greater MHC diversity means greater resistance to infection, why do we each have so few MHC genes encoding these molecules? Why have we not evolved strategies for increasing the diversity of MHC proteins—by alternative RNA splicing, for example, or by the genetic recombination mechanisms used to diversify antibodies and TCRs? One reason for the restricted diversity of MHC proteins in an individual may be that each time a new MHC protein is added to the repertoire, the T cells with TCRs that bind strongly to self peptides bound to the new MHC protein must be eliminated to maintain self tolerance. The elimination of these T cells would counteract the advantage of adding the new MHC protein. Thus, the number of MHC proteins we express is thought to represent a balance between the advantages of presenting a wide diversity of foreign peptides to T cells against the disadvantages of severely restricting the T cell repertoire during negative selection in the thymus. Computer modeling studies support this explanation.

Summary

There are three main functionally distinct classes of T cells. Cytotoxic T cells kill infected cells directly by inducing them to undergo apoptosis. Helper T cells help activate B cells to make antibody responses, cytotoxic T cells to kill their target cells, dendritic cells to stimulate T cell responses, and macrophages to destroy microorganisms that either invaded the macrophage or were ingested by it. Finally, regulatory T cells suppress the activity of effector T cells and dendritic cells and are crucial for self tolerance.

All T cells express cell-surface, antibodylike receptors (TCRs), which are encoded by genes that are assembled from multiple gene segments during T cell development

in the thymus. TCRs recognize fragments of foreign proteins that are displayed on the surface of host cells in association with MHC proteins. T cells are activated in peripheral lymphoid organs by antigen-presenting cells, which express peptide–MHC complexes, co-stimulatory proteins, and various cell–cell adhesion molecules on their cell surface. The most potent of these antigen-presenting cells are dendritic cells, which are specialized for antigen presentation and are required for the activation of naïve T cells.

Class I and class II MHC proteins have crucial roles in presenting foreign protein antigens to T cells: class I MHC proteins present antigens to cytotoxic T cells, while class II MHC proteins present antigens to helper and regulatory T cells. Whereas class I proteins are expressed on almost all vertebrate cells, class II proteins are normally restricted to those cell types that interact with helper T cells, such as dendritic cells, macrophages, and B lymphocytes.

Both classes of MHC proteins have a single peptide-binding groove, which binds small peptide fragments derived from proteins. Each MHC protein can bind a large set of peptides, which are constantly being produced intracellularly by normal protein degradation processes. However, class I MHC proteins mainly bind fragments produced in the cytosol, while class II MHC proteins mainly bind fragments produced in endocytic compartments. After they have formed inside the target cell, the peptide–MHC complexes are transported to the cell surface. Complexes that contain a peptide derived from a foreign protein are recognized by TCRs, which interact with both the peptide and the walls of the peptide-binding groove of the MHC protein. T cells also express CD4 or CD8 co-receptors, which simultaneously recognize nonpolymorphic regions of MHC proteins on the antigen-presenting cell or target cell: helper cells and regulatory cells express CD4, which recognizes class II MHC proteins, while cytotoxic T cells express CD8, which recognizes class I MHC proteins.

A combination of positive and negative selection processes operates during T cell development in the thymus to shape the TCR repertoire. These processes help to ensure that only T cells with potentially useful receptors survive and mature, while all of the others die by apoptosis. First, T cells that can respond to peptides complexed with self MHC proteins are positively selected; subsequently, the T cells in this group that can react strongly with self peptides complexed with self MHC proteins are eliminated. Helper and cytotoxic T cells that leave the thymus with receptors that could react with self antigens are eliminated, functionally inactivated, or actively suppressed when they recognize self antigens on nonactivated dendritic cells.

HELPER T CELLS AND LYMPHOCYTE ACTIVATION

Helper T cells are arguably the most important cells in adaptive immunity, as they are required for almost all adaptive immune responses. They help activate B cells to secrete antibodies, and they help macrophages to destroy ingested pathogens. They also help activate cytotoxic T cells to kill infected target cells, at least partly by stimulating dendritic cells to activate naïve cytotoxic cells more efficiently. As dramatically demonstrated in AIDS patients, without helper T cells we cannot even defend ourselves against many microbes that are normally harmless.

Helper T cells themselves, however, can function only when activated to become effector cells. Naïve helper cells are activated on the surface of dendritic cells, which become activated during the innate immune responses triggered by an infection. The innate responses, mainly via activated dendritic cells, also dictate what kind of effector cell a helper T cell will develop into, and they thereby determine the nature of the adaptive immune responses elicited.

In this final section, we discuss the multiple signals that help activate a T cell and how a helper T cell, once activated to become an effector cell, helps activate other cells. We also consider how innate immune responses determine the nature of adaptive responses by stimulating helper T cells to differentiate into different types of effector cells. Finally, we discuss the probable evolutionary origins of the Ig superfamily of proteins, which includes the MHC proteins, antibodies, and TCRs.

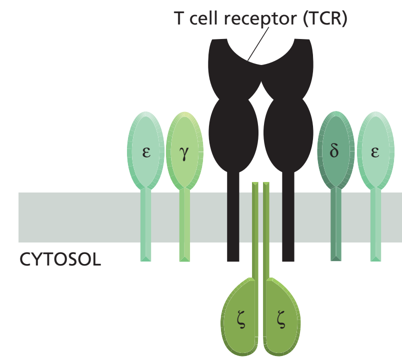


Figure 25–65 The TCR and its associated CD3 complex. All of the CD3 polypeptide chains (shown in *green*), except for the ζ (zeta) chains, have extracellular Ig-like domains and are therefore members of the Ig superfamily. All of the four types of CD3 polypeptide chains form heterodimers or homodimers (as shown) and are rapidly phosphorylated on tyrosines in their intracellular domains following TCR activation (not shown). Some of these phosphorylated tyrosines then serve as docking sites for intracellular signaling proteins, as shown in Figure 25–66.

Activated Dendritic Cells Use Multiple Mechanisms to Activate T Cells

When a dendritic cell is activated during an infection, it changes its shape and migratory behavior, increases the amount of MHC proteins displayed on its surface, activates its antigen-processing pathways, and starts producing both cell-surface-bound co-stimulatory proteins and secreted cytokines (including chemokines). The dramatic changes also enable the dendritic cell to migrate to a peripheral lymhoid organ and activate naïve T cells to become effector cells.

The dendritic cell initially signals to the T cell through the T cell receptors (TCRs), which bind to a foreign peptide complexed with a class II MHC protein on an opposing dendritic cell surface. The TCR, however, does not act on its own to transmit the signal into the T cell. It is helped by a complex of invariant transmembrane proteins called **CD3**, with which the TCR is associated (**Figure 25–65**). Moreover, the CD4 co-receptor on a helper or regulatory T cell and the CD8 co-receptor on a cytotoxic T cell bind to the same MHC protein as the TCR and also play a crucial part in transmitting the signal, as illustrated in **Figure 25–66**.

In addition to signaling through the TCR and its associated proteins and co-receptors, co-stimulatory proteins on the dendritic cell surface bind to other receptors on the T cell surface to provide further signals required to activate the T cell. Among these co-stimulatory proteins on the activated dendritic cell are the **B7 proteins**, which are recognized by the co-receptor protein **CD28** on the surface of the T cell. Once activated, a helper T cell itself expresses a co-stimulatory protein called *CD40 ligand*, which acts back on *CD40 receptors* on the dendritic cell surface to increase and sustain the activation of the dendritic cell, creating a positive feedback loop that amplifies the T cell response.

Once bound to the surface of a dendritic cell, a T cell increases the strength of the binding by activating an integrin adhesion protein, which then binds

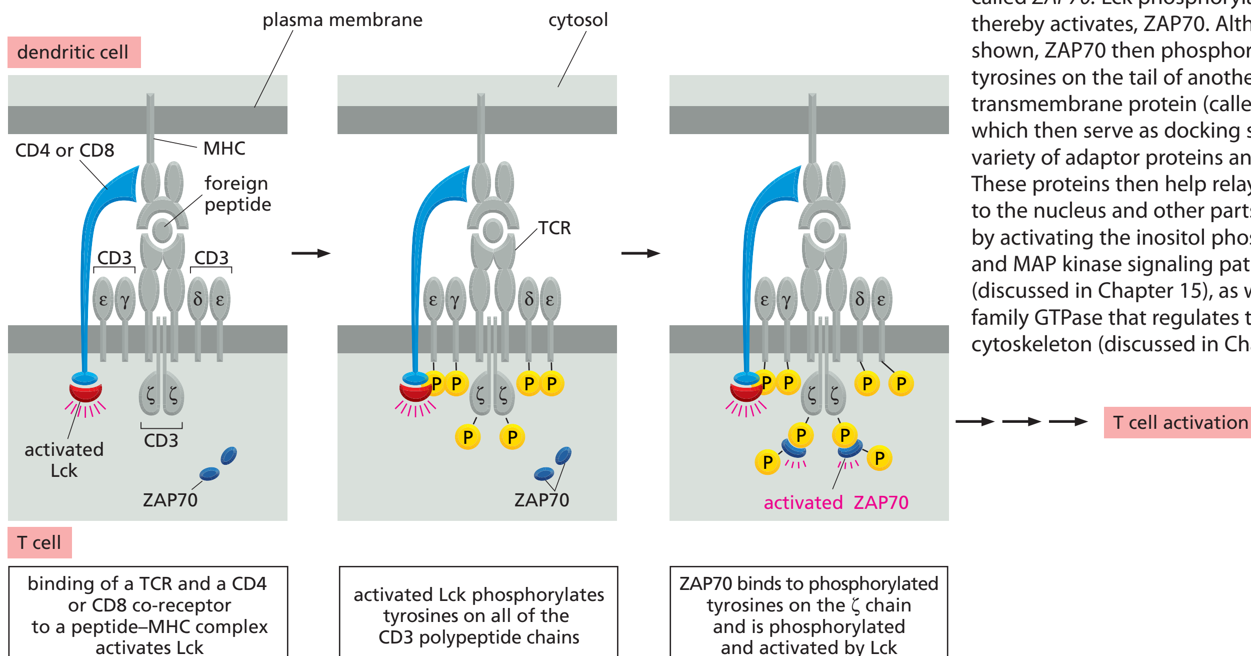


Figure 25–66 Signaling events initiated by the binding of peptide–MHC complexes to TCRs. When TCRs (and CD3) are clustered by binding to peptide–MHC complexes on an activated dendritic cell, CD4 molecules on helper cells or CD8 molecules on cytotoxic T cells are clustered with them, binding to invariant parts of the same class II or class I MHC proteins, respectively, on the dendritic cell. This brings the Src-like cytoplasmic tyrosine kinase Lck into the signaling complex and activates it. Lck activation also depends on a transmembrane protein tyrosine phosphatase on the T cell surface called CD45, which removes inhibitory phosphates from Lck (not shown).

Once activated, Lck initiates a tyrosine phosphorylation cascade by phosphorylating tyrosines on all of the chains of the CD3 complex. The phosphotyrosines on the CD3 ζ chain now serve as docking sites for yet another cytoplasmic tyrosine kinase called *ZAP70*. Lck phosphorylates, and thereby activates, ZAP70. Although not shown, ZAP70 then phosphorylates tyrosines on the tail of another transmembrane protein (called LAT), which then serve as docking sites for a variety of adaptor proteins and enzymes. These proteins then help relay the signal to the nucleus and other parts of the cell by activating the inositol phospholipid and MAP kinase signaling pathways (discussed in Chapter 15), as well as a Rho family GTPase that regulates the actin cytoskeleton (discussed in Chapter 16).

more strongly to its Ig-like ligand on the surface of the dendritic cell. This increased adhesion enables the T cell to remain bound to the antigen-presenting cell long enough to become activated.

This initial signaling through the TCR and associated proteins triggers the active assembly of an **immunological synapse** at the interface between the T cell and the dendritic cell. In these bull's-eye-like structures, TCRs and their associated CD3 subunits, co-receptors, and intracellular signaling proteins are clustered at the center, with cell–cell adhesion proteins forming a peripheral ring. Similar structures form when an effector helper or cytotoxic cell interacts with its target cell. Not all of the TCRs in the synapse are bound to a foreign peptide complexed with an MHC protein; some are bound to a self peptide bound to an MHC protein, and these TCRs also contribute to the activation of the T cell (recall that all T cells are initially positively selected in the thymus for their weak recognition of such self-peptide–MHC complexes).

The combined actions of the various signals just discussed stimulate helper T cells to proliferate and to begin to differentiate into effector cells by a curiously indirect mechanism. The signals cause the T cells to help stimulate their own proliferation and differentiation by inducing the cells to secrete a cytokine called **interleukin-2 (IL2)** and simultaneously to synthesize high-affinity cell-surface receptors that bind it. The binding of IL2 to the IL2 receptors activates intracellular signaling pathways that turn on genes that help the T cells to proliferate and differentiate into effector cells (**Figure 25–67**). Although some T cells do not make IL2, as long as they have been activated by their antigen and therefore express IL2 receptors they can be helped to proliferate and differentiate by IL2 made by neighboring activated T cells. IL2 also plays an important part in the development of regulatory T cells in the thymus, because without it these cells fail to develop.

Dendritic cells are not only important for activating T cells, they are also important for inactivating or eliminating self-reactive T cells. When T cells recognize self-peptide–MHC complexes on the surface of dendritic cells that have not been activated by a pathogen, they are either inactivated, so that they do not respond to the same peptide–MHC complexes even on activated dendritic cells, or they proliferate briefly and then die by apoptosis. Both of these mechanisms—clonal inactivation and clonal deletion—contribute to peripheral self tolerance. Dendritic cells also contribute to peripheral self tolerance by activating regulatory T cells, which then suppress the activity of self-reactive effector T cells, although the details of how dendritic cells selectively activate regulatory T cells are poorly understood (see Figure 25–13).

The Activation of T Cells Is Controlled by Negative Feedback

During the multi-step activation of a T cell, the cell starts to express a cell-surface protein called *CTLA4,* which inhibits intracellular signaling. This protein resembles CD28, and, like CD28, it binds to B7 proteins on the surface of the activating dendritic cell (see Figure 25–67). CTLA4 binds B7 with much higher affinity than does CD28 and, in doing so, it blocks the activating activity of CD28,

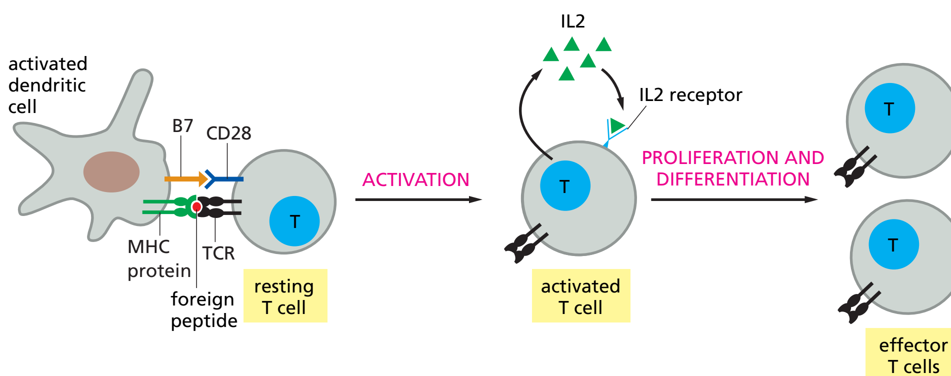


Figure 25–67 The stimulation of T cells by IL2. This model can apply to both helper and cytotoxic T cells, at least in culture. The combination of peptide–MHC complexes and a co-stimulatory B7 protein (either B7-1 or B7-2; also called CD80 and CD86, respectively) on the surface of an activated dendritic cell helps stimulate a resting T cell to make high affinity IL2 receptors and to secrete IL2. The binding of IL2 to its receptors then helps stimulate the T cells to proliferate and differentiate into effector cells. The various proteins associated with the TCRs (see Figure 25–65) are not shown.

Table 25–3 Some Accessory Proteins on the Surface of T Cells

PROTEIN*	SUPERFAMILY	EXPRESSED ON	LIGAND ON TARGET CELL	FUNCTIONS
CD3 complex	Ig (except for ζ)	all T cells	—	helps transduce signal when antigen–MHC complexes bind to TCRs; helps transport TCRs to cell surface
CD4	Ig	helper T cells, regulatory T cells	class II MHC	promotes adhesion to dendritic cells and target cells; signals T cell
CD8	Ig	cytotoxic T cells	class I MHC	promotes adhesion to dendritic cells and infected target cells; signals T cell
CD28	Ig	most T cells	B7 proteins (CD80 and CD86)	helps activate T cells
CTLA4	Ig	activated T cells	B7 proteins (CD80 and CD86)	inhibits T cell activation
CD40 ligand	Fas ligand family	effector helper T cells	CD40	co-stimulatory protein that helps activate macrophages, B cells, and dendritic cells

*CD stands for cluster of differentiation, as each of the CD proteins was originally defined as a blood cell "differentiation antigen" recognized by multiple monoclonal antibodies. Their identification depended on large-scale collaborative studies in which hundreds of such antibodies, generated in many laboratories, were compared and found to consist of relatively few groups (or "clusters"), each recognizing a single cell-surface protein. Since these initial studies, however, more than 240 CD proteins have been identified.

thereby providing negative feedback that holds the activation process in check. Thus, mice with a disrupted *Ctla4* gene die from a massive accumulation of activated T cells.

Table 25–3 summarizes some of the co-receptors and other accessory proteins found on the surface of T cells that we have discussed in this chapter.

Most of the T (and B) effector cells produced during an immune response must be eliminated after they have done their job. Although most of the cells die by apoptosis, the extracellular mechanisms responsible for their elimination are poorly understood. One possibility is that, as antigen levels fall and the response subsides, effector T cells are deprived of the antigen and cytokine stimulation that they need to survive, so that only memory cells and some long-lived effector cells survive. The death of effector T cells, however, does not occur solely through a lack of survival signals. In the case of effector cytotoxic T cells, for example, the cytokine *interferon-γ* (*IFNγ*) plays an important part in inducing the cell death; as effector cytotoxic T cells make IFNγ (see Figure 25–60), this is another form of negative feedback.

Before considering how effector helper T cells help activate macrophages and B cells, we need to discuss the two main functionally distinct subclasses of effector helper T cells, T_H1 and T_H2 cells, and how they are generated.

The Subclass of Effector Helper T Cell Determines the Nature of the Adaptive Immune Response

When an activated dendritic cell activates a naïve helper T cell in a peripheral lymphoid organ, the T cell usually differentiates into a T_H1 or T_H2 effector helper cell. The outcome depends on the affinity of the TCR for the peptide–MHC complex, on the density of the complex on the dendritic cell surface, and on the nature of the dendritic cell.

The two main subclasses of effector helper T cells can be distinguished by the cytokines they secrete. **T_H1 cells** mainly secrete *IFNγ* and *tumor necrosis factor-α (TNFα)*, which activate macrophages to kill microbes located within the macrophages' phagosomes. They also help activate cytotoxic T cells to kill infected cells. Thus, T_H1 cells mainly defend an animal against intracellular microbes. They also, however, stimulate B cells to secrete specific subclasses of IgG antibodies that can coat extracellular microbes and activate complement, thereby helping to eliminate some extracellular microbes as well.

T_H2 cells, by contrast, mainly defend an animal against extracellular pathogens, including microbes and multicellular parasites. They secrete a variety

of cytokines, including *interleukins 4 and 10 (IL4* and *IL10),* and they help stimulate B cells to make most classes of antibodies, including IgM, IgA, IgE, and some subclasses of IgG. Some of these antibodies bind to mast cells, basophils, and eosinophils; when activated by antigen binding, these cells release local mediators that cause sneezing, coughing, or diarrhea and thereby help expel extracellular microbes and larger parasites from epithelial surfaces of the body.

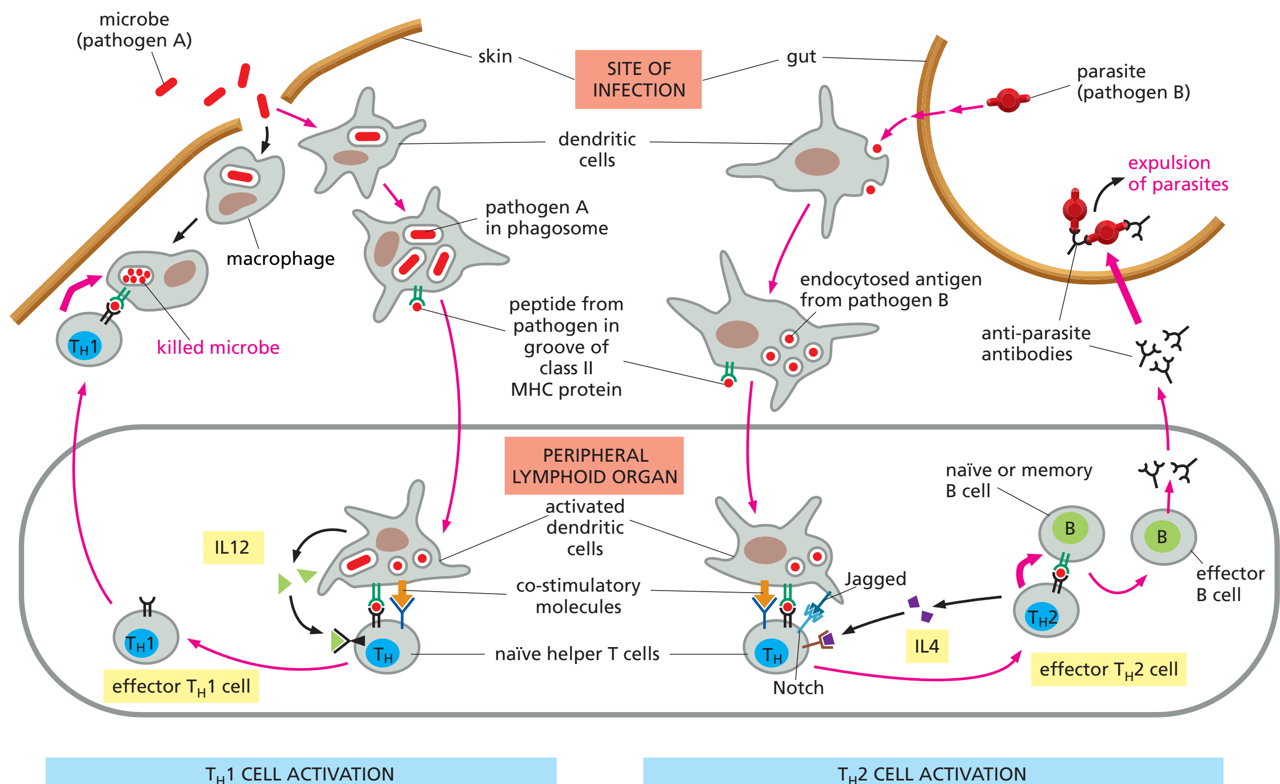
Thus, the decision of naïve helper T cells to differentiate into T_H1 or T_H2 effector cells influences the type of adaptive immune response that an animal mounts against the pathogen—whether it will be dominated by macrophage activation or by antibody production. The specific cytokines produced during the process of helper T cell activation have an important influence on the type of effector helper cell produced. Some intracellular bacteria, for example, stimulate dendritic cells to produce *IL12,* which induces T_H1 development, and thereby macrophage activation. As expected, mice that are deficient in either IL12 or its receptor are much more susceptible to these bacterial infections than are normal mice. Many parasitic protozoa and worms, by contrast, stimulate a dendritic cell to express the *Jagged protein* on its surface. Jagged is an activating ligand for *Notch receptors* (discussed in Chapters 15 and 22) on the T cell surface, and the resulting Notch signaling helps induce T_H2 development and the production of IL4. The T_H2 cells and IL4 help stimulate antibody production and eosinophil activation, leading to parasite expulsion (**Figure 25–68**). The IL4 also generates a positive feedback loop, as it is a potent inducer of T_H2 development.

Once a T_H1 or T_H2 effector cell develops, it inhibits the differentiation of the other type of helper T cell. IFNγ produced by T_H1 cells inhibits the development of T_H2 cells, while IL4 and IL10 produced by T_H2 cells inhibit the development of T_H1 cells. Thus, as the response proceeds, it reinforces the initial choice through its effect on the response of other T cells nearby.

Individuals infected with *Mycobacterium leprae,* the bacterium that causes leprosy, illustrate the importance of the T_H1/T_H2 decision. This bacterium replicates mainly within macrophages and causes either of two forms of disease, depending mainly on the genetic make-up of the infected individual. In some patients, the *tuberculoid* form of the disease occurs. Here, T_H1 cells develop and stimulate the infected macrophages to kill the bacteria. This produces a local inflammatory response, which damages skin and nerves. The result is a chronic

Figure 25–68 The activation of T_H1 and T_H2 cells. The differentiation of naïve helper T cells into either T_H1 or T_H2 effector cells determines the nature of the subsequent adaptive immune responses. Whether a naïve helper T cell becomes a T_H1 or T_H2 cell depends mainly on the signal proteins present when an activated dendritic cell in a peripheral lymphoid organ stimulates the helper T cell. The types of signal proteins produced depend on the local environment and the nature of the pathogen that activated the dendritic cell at the site of infection. IL12 secreted by activated dendritic cells promotes T_H1 cell development. By contrast, both the transmembrane Notch ligand Jagged on the surface of activated dendritic cells and the IL4 made by basophils, mast cells, and T_H2 cells promote T_H2 cell development.

In this figure, the effector T_H1 cell produced in the peripheral lymphoid organ migrates to the site of infection and helps a macrophage kill the microbes it has phagocytosed. The effector T_H2 cell remains in the lymphoid organ and helps activate a B cell to produce antibodies against the parasite. In addition to binding to the parasites, the antibodies bind to Fc receptors on mast cells, basophils, and eosinophils (see Figure 25–27), which then can help expel the parasite from the gut. Although not shown here, T_H1 cells also help activate B cells to make antibodies.



disease that progresses slowly but does not kill the host. In other patients, by contrast, the *lepromatous* form of the disease occurs. Here, T_H2 cells develop and stimulate the production of antibodies. As the antibodies cannot get through the plasma membrane to attack the intracellular bacteria, the bacteria proliferate unchecked and eventually kill the host. For unknown reasons, there is also a general depression of T-cell-mediated immunity to most antigens in the lepromatous form of the disease.

Naïve helper T cells can also develop into a recently described third type of effector cell called *T_H17 cells* because they secrete the pro-inflammatory interleukin *IL17*. T_H17 cells help defend against extracellular pathogens, but they also play an important part in many autoimmune diseases. They develop when some naïve helper T cells are activated by antigen in the presence of TGFβ and the interleukin IL6.

T_H1 Cells Activate Infected Macrophages and Stimulate An Inflammatory Response

Macrophages and dendritic cells ingest pathogens and their products at sites of infection. The dendritic cells become activated and carry microbial antigens to a peripheral lymphoid organ, where they preferentially induce the development of T_H1 cells. The T_H1 cells then migrate to the site of infection to help activate infected macrophages (see Figure 25–68).

T_H1 effector cells use two signals to activate the specific macrophages they recognize. They secrete IFNγ, which binds to IFNγ receptors on the macrophage surface, and they display the co-stimulatory protein CD40 ligand, which binds to CD40 on the macrophage (**Figure 25–69**). (We see later that CD40 ligand is also used by helper T cells to activate B cells.) Once activated, the macrophage can kill the microbes in their phagosomes: lysosomes can now fuse more readily with the phagosomes, unleashing a hydrolytic attack, and the activated macrophage makes oxygen radicals and nitric oxide, both of which are highly toxic to the microbes (discussed in Chapter 24). Because dendritic cells also express CD40, the T_H1 cells at sites of infection can also help activate them. As a result, the dendritic cells increase their production of class II MHC proteins, co-stimulatory proteins, and various cytokines, especially IL12. This makes them more effective at stimulating naïve helper T cells to differentiate into T_H1 effector cells in peripheral lymphoid organs, providing a positive feedback loop that increases the production of T_H1 cells and, thereby, the activation of macrophages.

T_H1 effector cells also stimulate an *inflammatory response* (discussed in Chapter 24) by recruiting more phagocytic cells into the infected site. They do so in three ways:

1. They secrete cytokines that act on the bone marrow to increase the production of monocytes (macrophage precursors that circulate in the blood) and neutrophils.
2. They secrete other cytokines that activate endothelial cells lining local blood vessels to express cell adhesion molecules that cause monocytes and neutrophils in the blood to adhere there.
3. They secrete chemokines that direct the migration of the adherent monocytes and neutrophils out of the bloodstream into the site of infection.

A T_H1 cell can also help activate a cytotoxic T cell in a peripheral lymphoid organ by producing chemokines that attract the cytotoxic cells to the site of the T_H1-cell–dendritic-cell interaction, while at the same time stimulating the dendritic cell to produce more co-stimulatory proteins. In addition, T_H1 cells can help effector cytotoxic T cells kill virus-infected target cells by secreting IFNγ, which increases the efficiency with which target cells process viral antigens for presentation to cytotoxic T cells (see Figure 25–60). An effector T_H1 cell can also directly kill some cells itself, including effector lymphocytes: by expressing *Fas ligand* on its surface, it can induce effector T or B cells that express cell-surface *Fas* to undergo apoptosis (see 25–47B).

Both T_H1 and T_H2 cells can also help stimulate B cells to proliferate and differentiate and to switch the class of antibody they make, from IgM and IgD to

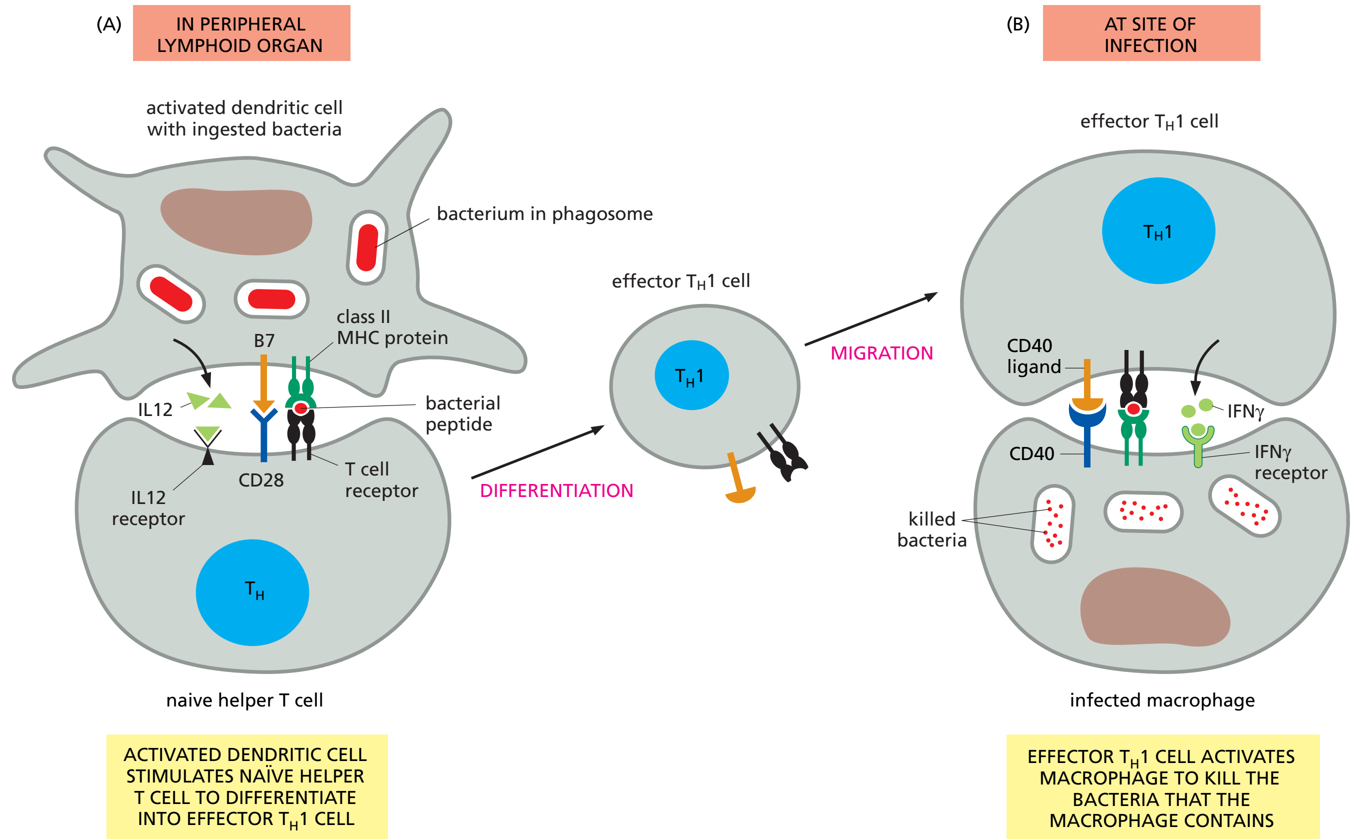


Figure 25–69 The differentiation of T_H1 cells and their activation of macrophages. (A) An activated dendritic cell that has ingested bacteria at a site of infection and migrated to a peripheral lymphoid organ activates a naïve helper T cell to differentiate into a T_H1 effector cell. The dendritic cell uses both cell-surface co-stimulatory proteins such as the B7 proteins and secreted IL12 to induce T_H1 cell differentiation. (B) The activated T_H1 effector cell then migrates from the peripheral lymphoid organ to the infected site, where it helps activate macrophages to kill the bacteria harbored within the macrophages' phagosomes. As indicated, it does this by means of secreted IFNγ and membrane-bound CD40 ligand, which binds to CD40 on the macrophage.

one of the secondary classes of antibody. Before considering how helper T cells do this, we need to discuss the role of the B cell antigen receptor in the activation of B cells.

Antigen Binding to B Cell Receptors (BCRs) Is Only One Step in B Cell Activation

Like T cells, most B cells require multiple extracellular signals to become activated. One signal is provided by antigen binding to the **B cell receptor** (**BCR**) for antigen, which, as discussed previously, is a membrane-bound antibody molecule. A helper T cell usually provides the other required signals. If a B cell receives the first signal only, it may be eliminated or functionally inactivated, which is one way in which B cells become tolerant to self antigens.

Signaling through the BCR works in much the same way as signaling through the TCR (see Figure 25–66). The receptor is associated with two invariant protein chains, Igα and Igβ, which help convert antigen binding to BCRs into intracellular signals. When antigen cross-links BCRs on the surface of a B cell, it causes them and their associated invariant chains to cluster into small aggregates. This aggregation leads to the assembly of an intracellular signaling complex at the site of the clustered receptors and to the initiation of a tyrosine phosphorylation cascade (**Figure 25–70**).

A co-receptor complex that binds complement proteins greatly enhances the efficiency of signaling through the BCR and its associated invariant chains.

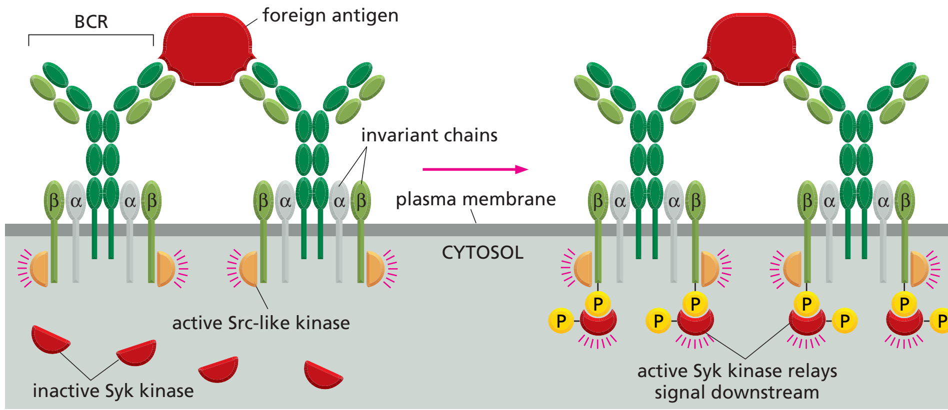


Figure 25–70 Early signaling events in B cells activated by the binding of antigen to BCRs. The antigen cross-links adjacent BCRs, which are transmembrane antibody molecules. The cross-linking causes the BCRs and their associated invariant chains (heterodimers of Igα and Igβ) to cluster. A Src-like cytoplasmic tyrosine kinase, which can be *Fyn*, *Blk*, or *Lyn*, is associated with the cytosolic tail of Igβ. It joins the cluster and phosphorylates Igα and Igβ (for simplicity, only the phosphorylation on Igβ is shown). As in the case of TCR activation, the protein tyrosine phosphatase CD45 is also required to activate these Src-like kinases (not shown). The resulting phosphotyrosines on Igα and Igβ serve as docking sites for another Src-like tyrosine kinase called *Syk*, which is homologous to ZAP70 in T cells (see Figure 25–66). Like ZAP70, Syk becomes phosphorylated and thereby activated, and it then relays the signal downstream.

If a microbe directly activates the complement system (discussed in Chapter 24), complement proteins are often deposited on the microbe surface, greatly increasing the B cell response to the microbe. Now, when the microbe clusters BCRs on a B cell, the *complement-binding co-receptor complexes* are brought into the cluster, increasing the strength of signaling by activating PI 3-kinase (discussed in Chapter 15) (**Figure 25–71**A). As expected, antibody responses are greatly reduced in mice lacking either one of the required complement components or one of the complement-binding co-receptor subunits on B cells.

Later in the immune response, by contrast, when IgG antibodies are present on the surface of the microbe, a different co-receptor dampens down the B cell response. These are *Fc receptors*, which bind the tails of the IgG antibodies. They recruit both lipid and protein phosphatase enzymes into the signaling complex that decrease the strength of signaling (Figure 25–71B). In this way, the Fc receptors on B cells act as inhibitory co-receptors, just as the CTLA4 proteins do on T cells. Thus, the co-receptors on a T cell or B cell allow the cell to gain additional information about the antigen bound to its receptors and thereby make a more informed decision as to how to respond.

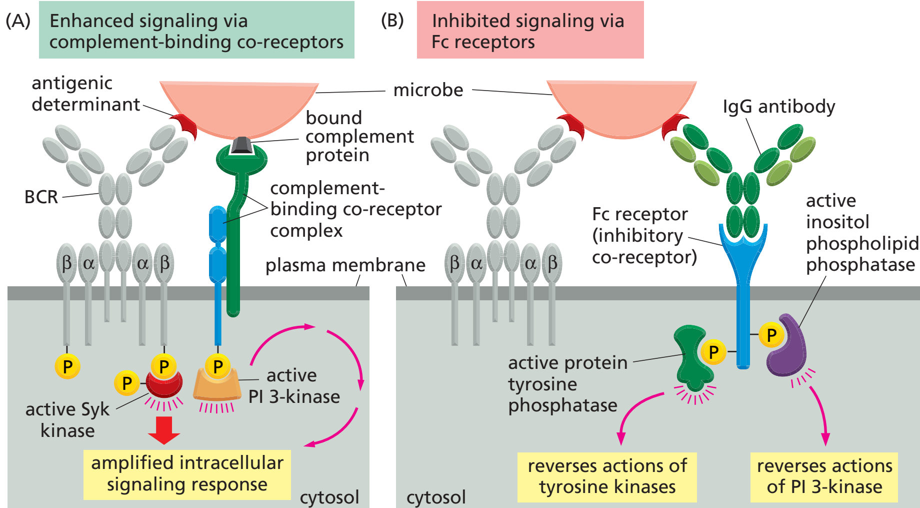


Figure 25–71 The influence of B cell co-receptors on the effectiveness of signaling through BCRs. (A) The binding of microbe–complement complexes to BCRs cross-links the BCRs to complement-binding, co-receptor complexes. The cytosolic tail of one component of the co-receptor complex becomes phosphorylated on tyrosines, which then serve as docking sites for PI 3-kinase. As discussed in Chapter 15, PI 3-kinase is activated to phosphorylate specific inositol phospholipids in the plasma membrane, which then act as docking sites to recruit intracellular signaling proteins (not shown). These signaling proteins act together with the signals generated by the Syk kinase to amplify the response. (B) When IgG antibodies bind to foreign antigen, usually late in a response, the Fc regions of the antibodies bind to Fc receptors on the B cell surface and are thus recruited into the signaling complex. The Fc receptors become phosphorylated on tyrosines; these then serve as docking sites for two types of phosphatase enzymes: (1) an inositol phospholipid phosphatase, which dephosphorylates the inositol phospholipid docking sites in the plasma membrane generated by PI 3-kinase, thereby reversing the activating effects of PI 3-kinase; (2) protein tyrosine phosphatases, which inhibit signaling by the activated tyrosine kinases.

Antigen-Specific Helper T Cells Are Essential for Activating Most B Cells

Whereas antigen-presenting cells such as dendritic cells and macrophages are omnivorous and ingest and present antigens on their MHC proteins nonspecifically, a B cell generally presents only peptides derived from an antigen that it specifically recognizes using its BCRs. Thus, BCRs do more than just bind antigen to begin the process of B cell activation; they also play a crucial part in recruiting T cell help. Through endocytosis, they deliver their bound protein antigen to an endosomal compartment, where the antigen is degraded into peptides; many of these peptides are returned to the B cell surface bound to class II MHC proteins (see Figure 25–61). These peptide–class-II-MHC complexes are recognized by antigen-specific helper T cells, which then deliver further signals to the B cell that are required for its proliferation and antibody secretion (**Figure 25–72**).

How do the antigen-specific T cells required for B cell activation originate? As discussed previously, during primary antibody responses, naïve helper T cells are activated in a peripheral lymphoid organ by binding to a foreign peptide bound to a class II MHC protein on the surface of an activated dendritic cell. The effector helper T cells that results from this activation can then activate a B cell that displays the same complex of foreign peptide and class II MHC protein on its surface. Thus, the helper T cell activates only those B cells with BCRs that specifically recognize the antigen that initially activated the T cell, even though the TCRs and BCRs usually recognize distinct antigenic determinants on the antigen (see Figure 25–72). This requirement for *linked recognition* of antigen by a T cell and a B cell helps avoid autoimmune B cell responses, which would require the simultaneous presence of both helper T cells and B cells that recognize the same self antigen.

In secondary antibody responses, memory B cells themselves can act as antigen-presenting cells and activate helper T cells, as well as being the subsequent targets of the effector helper T cells. The mutually reinforcing actions of helper T cells and B cells lead to an antibody response that is both intense and highly specific.

Once a helper T cell has been activated to become an effector cell and contacts a B cell, the contact initiates an internal rearrangement of the helper cell's cytoplasm. The T cell orients its centrosome and Golgi apparatus toward the B cell, as described previously for an effector cytotoxic T cell contacting its target cell (see Figure 25–46). In this case, however, the orientation is thought to enable the effector helper T cell to direct both membrane-bound and secreted cytokines onto the B cell surface (see Figure 25–72). One crucial membrane-bound signal molecule is CD40 ligand, which we encountered earlier. It is expressed on the surface of effector helper T cells, but not on nonactivated naïve or memory helper T cells, and it is recognized by the CD40 protein on the B cell surface. This interaction between CD40 ligand and CD40 is required for helper T cells to activate B cells to proliferate and differentiate into memory or antibody-

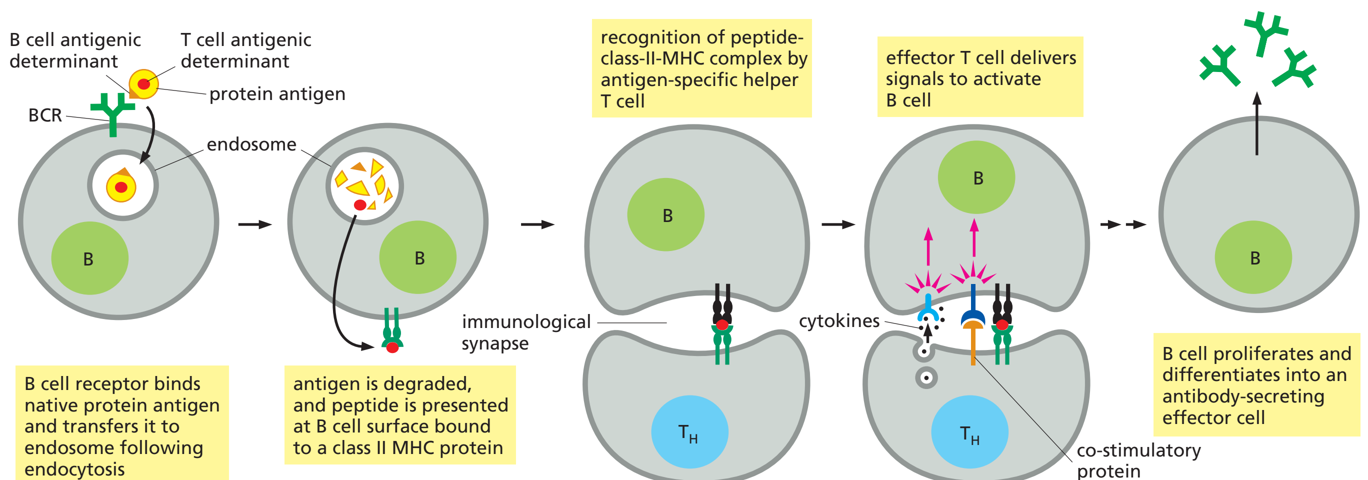


Figure 25–72 The activation of a B cell by a protein antigen and an effector helper T cell. Note that the B cell and T cells recognize different antigenic determinants on the antigen and that the effector helper T cell uses both secreted and membrane-bound co-stimulatory molecules to help activate the B cell.

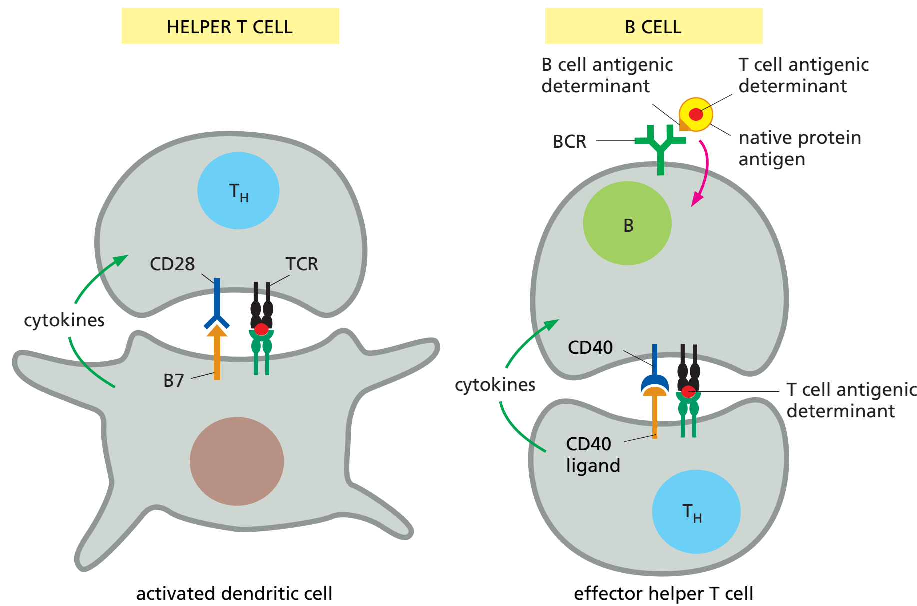


Figure 25–73 Comparison of the signals required to activate a helper T cell and a B cell to the same protein antigen. Note that in both cases secreted and membrane-bound molecules cooperate in the activation process. The *red arrow* indicates the endocytosis of the protein antigen. Although not shown, CD40 ligand is also used by effector helper T cells to increase and maintain the activation of dendritic cells, which express CD40, thereby creating a positive feedback loop.

The antigenic determinant recognized by the helper T cell is presented on the surface of both the dendritic cell and the B cell as a peptide fragment of the protein antigen bound to a class II MHC protein. By contrast, the B cell recognizes a different antigenic determinant on the surface of the folded (native) protein.

secreting effector cells. Individuals that lack CD40 ligand are severely immunodeficient. They are susceptible to the same infections that affect AIDS patients whose helper T cells have been destroyed.

Helper T cells also secrete cytokines to help B cells proliferate and differentiate and, in some cases, to switch the class of antibody they produce. The cytokines include the interleukins IL2 and IL4. IL4, for example, is produced by T_H2 cells and collaborates with CD40 ligand in stimulating B cell proliferation and differentiation; it also promotes switching to IgE antibody production. Mice deficient in IL4 production are severely impaired in their ability to make IgE.

Figure 25–73 compares the signals required for T and B cell activation; **Table 25–4** lists some of the cytokines discussed in this chapter.

A Special Class of B Cells Recognize T-cell-independent Antigens

Some antigens can stimulate B cells to proliferate and differentiate into antibody-secreting effector cells without help from T cells. Most of these *T-cell-independent antigens* are microbial polysaccharides that do not activate helper T cells. Some activate B cells directly by providing both the antigen signal and the accessory signals normally provided by helper T cells. Others are large polymers with repeating, identical antigenic determinants (see Figure 25–29B); their multipoint binding to BCRs can generate a strong enough signal on its own to activate the B cell directly, without additional signals.

Table 25–4 Properties of Some Cytokines

CYTOKINE	SOME SOURCES	SOME ACTIONS
IL2	all helper T cells; some cytotoxic T cells	stimulates proliferation and differentiation of activated T cells; required for regulatory T cell development in thymus
IL4	T_H2 cells, basophils, and mast cells	stimulates B cell proliferation, differentiation, and class switching to IgE and IgG1; promotes T_H2 and inhibits T_H1 cell development
IL7	many non-T cells	promotes memory T cell survival
IL10	T_H2 cells, macrophages, and dendritic cells	inhibits macrophages and T_H1 cell development
IL12	B cells, macrophages, dendritic cells, and granulocytes	induces T_H2 cell development and inhibits T_H1 cell development
IL15	many non-T cells	promotes memory T cell survival
IL17	some effector helper T cells	stimulates inflammatory responses
IFNγ	T_H1 cells and cytotoxic T cells	activates macrophages; increases MHC expression in many cell types
TGFβ	regulatory T cells	suppresses effector T cell activity, dendritic cells, and macrophages
TNFα	T_H1 cells and macrophages	activates endothelial cells and macrophages

Because T-cell-independent antigens do not activate helper T cells, they fail to induce B cell memory, affinity maturation, or class switching, all of which require help from T cells. They therefore mainly stimulate the production of low-affinity (but high-avidity) IgM antibodies. Most B cells that make antibodies without T cell help belong to a distinct B cell lineage. They are called *B1 cells* to distinguish them from *B2 cells,* which require T cell help. B1 cells seem to be especially important in defense against intestinal pathogens.

Immune Recognition Molecules Belong to the Ancient Ig Superfamily

Most of the proteins that mediate cell–cell recognition or antigen recognition in the immune system contain Ig or Ig-like domains, suggesting that they have a common evolutionary history. Included in this **Ig superfamily** are antibodies, TCRs, MHC proteins, the CD4, CD8, and CD28 co-receptors, the B7 co-stimulatory proteins, and most of the invariant polypeptide chains associated with TCRs and BCRs, as well as the various Fc receptors on lymphocytes and other white blood cells. All of these proteins contain one or more Ig or Ig-like domains. In fact, about 15% of the 250 or so proteins that have been characterized on the surface of white blood cells belong to this superfamily. Many of these molecules are dimers or higher oligomers in which Ig or Ig-like domains of one chain interact with those in another (**Figure 25–74**).

A separate exon usually encodes the amino acids in each Ig-like domain. It seems likely that the entire gene superfamily evolved from a gene coding for a single Ig-like domain—similar to that encoding β_2-microglobulin (see Figures 25–50A and 25–52) or the Thy-1 protein (see Figure 25–74)—that may have mediated cell–cell interactions. There is evidence that such a primordial gene arose before vertebrates diverged from their invertebrate ancestors about 400 million years ago. New family members presumably arose by exon and gene duplications.

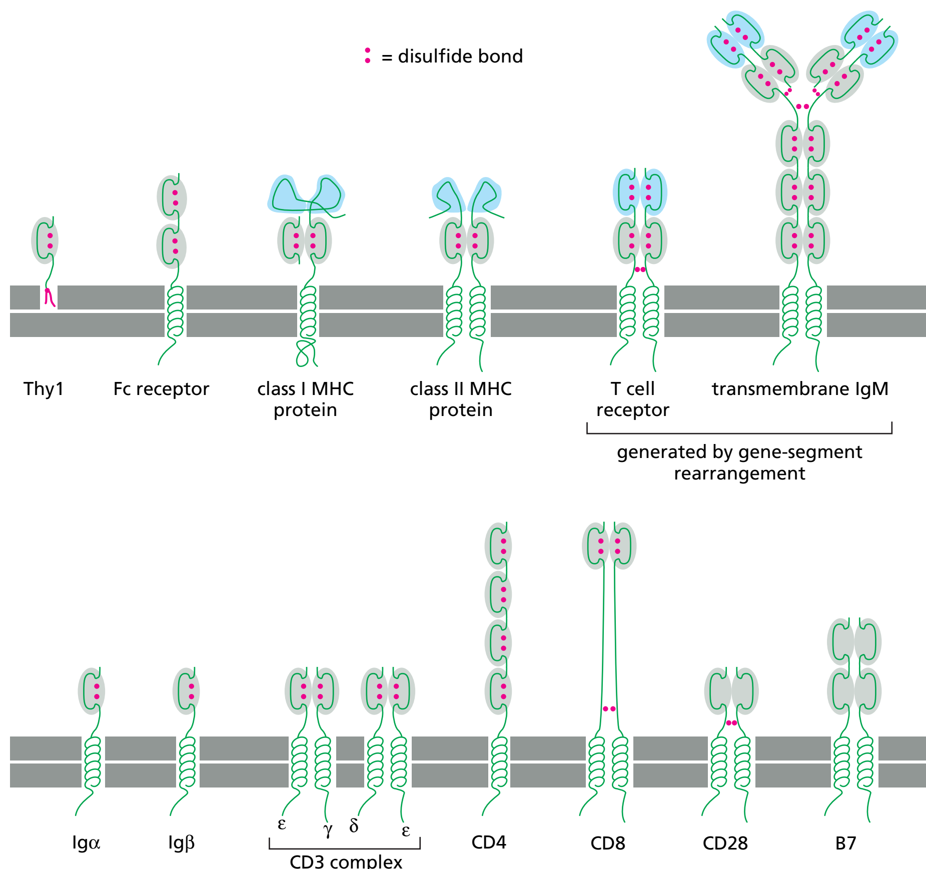


Figure 25–74 Some of the cell-surface proteins discussed in this chapter that belong to the Ig superfamily. The Ig and Ig-like domains are shaded in *gray,* except for the antigen-binding domains (not all of which are Ig domains), which are shaded in *blue*. The function of Thy1 is unknown, but it is held in the plasma membrane by a glycosylphosphatidylinositol (GPI) anchor and is widely used to identify T cells in mice. The Ig superfamily also includes many cell-surface proteins involved in cell–cell interactions outside the immune system, such as the neural cell adhesion molecule (NCAM) discussed in Chapter 19 and the receptors for various protein growth factors discussed in Chapter 15 (not shown). There are more than 750 members of the Ig superfamily in humans.

The multiple gene segments that encode antibodies and TCRs may have arisen when a transposable element, or transposon (discussed in Chapter 5), inserted into an exon of a gene encoding an Ig family member in an ancestral lymphocyte-like cell. The transposon may have contained the ancestors of the *Rag* genes, which, as discussed earlier, encode the proteins that initiate V(D)J recombination; the finding that the RAG proteins can act as transposons in a test tube strongly supports this view. Once the transposon had inserted into the exon, the gene could be expressed only if the transposon was excised by the RAG proteins and the two ends of the exon were rejoined, much as occurs when the *V* and *J* gene segments of an Ig light chain gene are assembled (see Figure 25–38). A second insertion of the transposon into the same exon may then have divided the gene into three segments, equivalent to the present-day *V, D,* and *J* gene segments. Subsequent duplication of either the individual gene segments or the entire split gene may then have generated the arrangements of gene segments that characterize the adaptive immune systems of present-day vertebrates.

Summary

The production of an effector helper T cell from of a naïve helper T cell requires multiple signals from an activated dendritic cell. MHC–peptide complexes on the dendritic cell surface provide one signal, by binding to both TCRs and a CD4 co-receptor on the T cell. Co-stimulatory proteins on the dendritic cell surface, including CD28, and secreted cytokines are the other signals. When naïve helper T cells are initially activated on a dendritic cell, most differentiate into either T_H1 or T_H2 effector cells, depending mainly on the signal proteins in their environment. T_H1 cells activate macrophages, cytotoxic T cells, and B cells, while T_H2 cells activate mainly B cells. In both cases, the effector helper T cells recognize the same complex of foreign peptide and class II MHC protein on the target cell surface as they initially recognized on the dendritic cell that activated them. They activate their target cells by a combination of membrane-bound and secreted co-stimulatory proteins. One membrane-bound signal protein used by both T_H1 and T_H2 cells is CD40 ligand.

Like T cells, B cells require multiple signals for activation. Antigen binding to the B cell antigen receptors (BCRs) provides one signal, while antigen-specific effector helper T cells provide the other signals. The requirement for multiple signals to activate either a T cell or a B cell helps to prevent inappropriate and dangerous activation of lymphocytes, including self-reactive lymphocytes.

Most of the proteins involved in cell–cell recognition and antigen recognition in the immune system, including antibodies, TCRs, and MHC proteins, as well as the various co-receptors discussed in this chapter, belong to the ancient Ig superfamily. This superfamily is thought to have evolved from a primordial gene encoding a single Ig-like domain. The mechanisms for diversifying antibodies and T cell receptors by recombining gene segments may have arisen when a transposon inserted into an exon of a gene encoding an Ig family member.

REFERENCES

General

Abbas AK, Lichtman AH & Pober JS (2005) Cellular and Molecular Immunology 3rd ed. Philadelphia: WB Saunders.

Janeway CA Jr, Travers P, Walport M & Shlomchik M (2005) Immunobiology: The Immune System in Health and Disease 6th ed. London: Garland Science.

Lymphocytes and the Cellular Basis of Adaptive Immunity

Ahmed R & Rousse BT (eds) (2006) Immunological memory. *Immunol Rev* 211:5–337.

Ebert LM, Schaerli P & Moser B (2005) Chemokine-mediated control of T cell traffic in lymphoid and peripheral tissues. *Mol Immunol* 42:799–809.

Goodnow CC, Sprent J, Fazekas de St Groth B & Vinuesa CG (2005) Cellular and genetic mechanisms of self tolerance and autoimmunity. *Nature* 435:590–597.

Gourley TS, Wherry EJ, Masopust D & Ahmed R (2004) Generation and maintenance of immunological memory. *Semin Immunol* 16:323–333.

Janeway CA Jr & Medzhitov R (2002) Innate immune recognition. *Annu Rev Immunol* 20:197–216.

Kyewski B & Klein L (2006) A central role for central tolerance. *Annu Rev Immunol* 24:571–606.

Singh NJ & Schwartz RH (2006) Primer: mechanisms of immunologic tolerance. *Nature Clin Pract Rheumatol* J2:44–52.

Steinman RM & Hemmi H (2006) Dendritic cells: translating innate to adaptive immunity. *Curr Top Microbiol Immunol* 311:17–58.

Wiedle G, Dunon D & Imhof BA (2001) Current concepts in lymphocyte homing and recirculation. *Crit Rev Clin Lab Sci* 38:1–31.

B Cells and Antibodies

Burton DR & Woof JM (1992) Human antibody effector function. *Adv Immunol* 51:1–84.
Davies DR & Cohen GH (1996) Interactions of protein antigens with antibodies. *Proc Natl Acad Sci USA* 93:7–12.
Davies DR, Padlan EA & Sheriff S (1990) Antibody-antigen complexes. *Annu Rev Biochem* 59:439–473.
Jimenez R, Salazar G, Baldridge KK & Romesberg FE (2003) Flexibility and molecular recognition in the immune system. *Proc Natl Acad Sci USA* 100:92–97.
Padlan EA (1994) Anatomy of the antibody molecule. *Mol. Immunol.* 31:169–217.
Zhang M, Srivastava G & Lu L (2004) The pre-B cell receptor and its function during B cell development. *Cell Mol Immunol* 1:89–94.

The Generation of Antibody Diversity

Besmer E, Gourzi P & Papavasiliou FN (2004) The regulation of somatic hypermutation. *Curr Opin Immunol* 16:241–245.
Corcoran AE (2005) Immunoglobulin locus silencing and allelic exclusion. *Semin Immunol* 17:141–154.
Dudley DD, Chaudhuri J, Bassing CH & Alt FW (2005) Mechanism and control of V(D)J recombination versus class switch recombination: similarities and differences. *Adv Immunol* 86:43–112.
Honjo T, Nagaoka H, Shinkura R & Muramatsu M (2005) AID to overcome the limitations of genomic information. *Nature Immunol* 6:655–61.
Jung D & Alt FW (2004) Unraveling V(D)J recombination; insights into gene regulation. *Cell* 116:299–311.
Jung D, Giallourakis C, Mostoslavsky R & Alt FW (2006) Mechanism and control of V(D)J recombination at the immunoglobulin heavy chain locus. *Annu Rev Immunol* 24:541–570.
Longerich S, Basu U, Alt F & Storb U (2006) AID in somatic hypermutation and class switch recombination. *Curr Opin Immunol* 18:164–174.
Maizels N (2005) Immunoglobulin gene diversification. *Annu Rev Genet* 39:23–46.
Neuberger M & Di Noia JM (2007) Molecular mechanisms of antibody somatic hypermutation. *Annu Rev Biochem* 76:in press.
Neuberger MS, Harris RS, Di Noia JM & Petersen-Mahrt SK (2003) Immunity through DNA deamination. *Trends Biochem Sci* 28:305–312.
Selsing E (2006) Ig class switching: targeting the recombinational mechanism. *Curr Opin Immunol* 18:249–254.

T cells and MHC Proteins

Coutinho A, Caramalho I, Seixas E & Demengeot J (2005) Thymic commitment of regulatory T cells is a pathway of TCR-dependent selection that isolates repertoires undergoing positive or negative selection. *Curr Top Microbiol Immunol* 293:43–71.
Krogsgaard M & Davis MM (2005) How T cells 'see' antigen. *Nature Immunol* 6:239–245.
Loureiro J & Ploegh HL (2006) Antigen presentation and the ubiquitin-proteasome system in host-pathogen interactions. *Adv Immunol* 92:225–305.
McDevitt HO (2000) Discovering the role of the major histocompatibility complex in the immune response. *Annu. Rev. Immunol.* 18:1–17.
Mellman I & Steinman RM (2001) Dendritic cells: specialized and regulated antigen processing machines. *Cell* 106:255–258.
Pulendran B (2004) Immune activation: death, danger and dendritic cells. *Curr Biol* 14:R30–32.
Reis e Sousa C (2006) Dendritic cells in a mature age. *Nature Rev Immunol* 6:476–483.
Rudolph MG, Stanfield RL & Wilson IA (2006) How TCRs bind MHCs, peptides, and coreceptors. *Annu Rev Immunol* 24:419–466.
Russell JH & Ley TJ (2002) Lymphocyte-mediated cytotoxicity. *Annu Rev Immunol* 20:323–370.
Sallusto F, Geginat J & Lanzavecchia A (2004) Central memory and effector memory T cell subsets: function, generation, and maintenance. *Annu Rev Immunol* 22:745–763.
Sakaguchi S, Ono M, Setoguchi R et al (2006) $Foxp3^+$ $CD25^+$ $CD4^+$ natural regulatory T cells in dominant self-tolerance and autoimmune disease. *Immunol Rev* 212:8–27.
Starr TK, Jameson SC & Hogquist KA (2003) Positive and negative selection of T cells. *Annu Rev Immunol* 21:139–176.
Steinman RM, Bonifaz L, Fujii S et al (2005) The innate functions of dendritic cells in peripheral lymphoid tissues. *Adv Exp Med Biol* 560:83–97.
Trombetta ES & Mellman I (2005) Cell biology of antigen processing *in vitro* and *in vivo*. *Annu Rev Immunol* 23:975–1028.
Villasenor J, Benoist C & Mathis D (2005) AIRE and APECED: molecular insights into an autoimmune disease. *Immunol Rev* 204:156–164.
von Boehmer H (2004) Selection of the T-cell repertoire: receptor-controlled checkpoints in T-cell development. *Adv Immunol* 84:201–238.
Watanabe N, Wang YH, Lee HK et al (2005) Hassall's corpuscles instruct dendritic cells to induce $CD4^+CD25^+$ regulatory T cells in human thymus. *Nature* 436:1181–1185.

Helper T Cells and Lymphocyte Activation

Abbas AK (2003) The control of T cell activation vs. tolerance. *Autoimmun Rev* 2:115–118.
Alegre ML, Frauwirth KA & Thompson CB (2001) T-cell regulation by CD28 and CTLA-4. *Nature Rev Immunol* 1:220–228.
Barclay AN (2003) Membrane proteins with immunoglobulin-like domains—a master superfamily of interaction molecules. *Semin Immunol* 15:215–223.
Castellino F & Germain RN (2006) Cooperation between $CD4^+$ and $CD8^+$ T cells: when, where, and how. *Annu Rev Immunol* 24:519–540.
Deane JA & Fruman DA (2004) Phosphoinositide 3-kinase: diverse roles in immune cell activation. *Annu Rev Immunol* 22:563–598.
Dustin ML, Tseng SY, Varma R & Campi G (2006) T cell-dendritic cell immunological synapses. *Curr Opin Immunol* 18:512–516.
Eason DD, Cannon JP, Haire RN et al (2004) Mechanisms of antigen receptor evolution. *Semin Immunol* 16:215–226.
Matthews SA & Cantrell DA (2006) The role of serine/threonine kinases in T-cell activation. *Curr Opin Immunol* 18:314–320.
McHeyzer-Williams LJ, Malherbe LP & McHeyzer-Williams MG (2006) Helper T cell-regulated B cell immunity. *Curr Top Microbiol Immunol* 311:59–83.
Mills DM & Cambier JC (2003) B lymphocyte activation during cognate interactions with $CD4^+$ T lymphocytes: molecular dynamics and immunologic consequences. *Semin Immunol* 15:325–329.
Murphy KM & Reiner SL (2002) The lineage decisions of helper T cells. *Nature Rev Immunol* 2:933–944.
Peggs KS & Allison JP (2005) Co-stimulatory pathways in lymphocyte regulation: the immunoglobulin superfamily. *B J Haematol* 130:809–824.
Reinhardt RL, Kang SJ, Liang HE & Locksley RM (2006) T helper cell effector fates—who, how and where? *Curr Opin Immunol* 18:271–277.
Reth M & Brummer T (2004) Feedback regulation of lymphocyte signalling. *Nature Rev Immunol* 4:269–277.
Samelson LE (2002) Signal transduction mediated by the T cell antigen receptor: the role of adapter proteins. *Annu Rev Immunol* 20:371–394.
Steinman RM, Hawiger D & Nussenzweig MC (2003) Tolerogenic dendritic cells. *Annu Rev Immunol* 21:685–711.
Villiers MB, Perrin-Cocon L, Marche PN & Villiers C (2004) Complement receptors and B lymphocytes. *Crit Rev Immunol* 24:465–478.