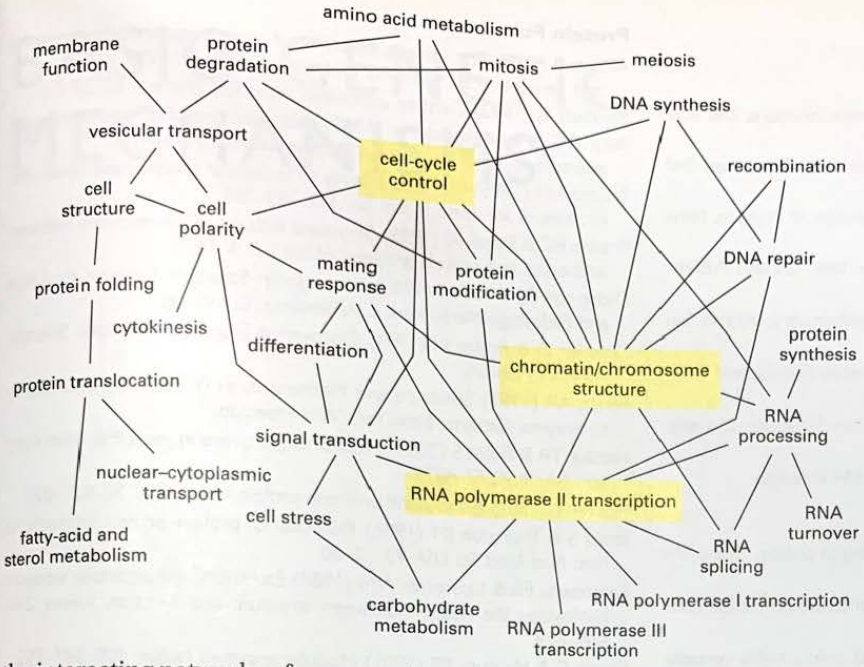

Figure 3–78 A map of the protein–protein interactions observed between different functional groups of proteins in the yeast *S. cerevisiae*. To produce this map, more than 1500 proteins were assigned to the indicated 31 functional groups. About 70 percent of the known protein–protein interactions were observed to occur between proteins assigned to the same functional group. However, as indicated, a surprisingly large number of interactions crossed between these groups. Each line in this diagram designates that more than 15 protein–protein interactions were observed between proteins in the two functional groups that are connected by that line. The three functional groups highlighted in *yellow* display at least 15 interactions with 10 or more of the 31 functional groups defined in the study. (From C.L. Tucker, J.F. Gera, and P. Vetz, *Trends Cell Biol.* 11:102–106, 2001.)

the interacting networks of macromolecules in cells, deciphering their full functional meaning may well keep scientists busy for centuries.

Summary

Proteins can form enormously sophisticated chemical devices, whose functions largely depend on the detailed chemical properties of their surfaces. Binding sites for ligands are formed as surface cavities in which precisely positioned amino acid side chains are brought together by protein folding. In the same way, normally unreactive amino acid side chains can be activated to make and break covalent bonds. Enzymes are catalytic proteins that greatly speed up reaction rates by binding the high-energy transition states for a specific reaction path; they also perform acid catalysis and base catalysis simultaneously. The rates of enzyme reactions are often so fast that they are limited only by diffusion; rates can be further increased if enzymes that act sequentially on a substrate are joined into a single multienzyme complex, or if the enzymes and their substrates are confined to the same compartment of the cell.

Proteins reversibly change their shape when ligands bind to their surface. The allosteric changes in protein conformation produced by one ligand affect the binding of a second ligand, and this linkage between two ligand-binding sites provides a crucial mechanism for regulating cell processes. Metabolic pathways, for example, are controlled by feedback regulation: some small molecules inhibit and other small molecules activate enzymes early in a pathway. Enzymes controlled in this way generally form symmetric assemblies, allowing cooperative conformational changes to create a steep response to changes in the concentrations of the ligands that regulate them.

Changes in protein shape can be driven in a unidirectional manner by the expenditure of chemical energy. By coupling allosteric shape changes to ATP hydrolysis, for example, proteins can do useful work, such as generating a mechanical force or moving for long distances in a single direction. The three-dimensional structures of proteins, determined by x-ray crystallography, have revealed how a small local change caused by nucleoside triphosphate hydrolysis is amplified to create major changes elsewhere in the protein. By such means, these proteins can serve as input–output devices that transmit information, as assembly factors, as motors, or as membrane-bound pumps. Highly efficient protein machines are formed by incorporating many different protein molecules into larger assemblies in which the allosteric movements of the individual components are coordinated. Such machines are now known to perform many of the most important reactions in cells.

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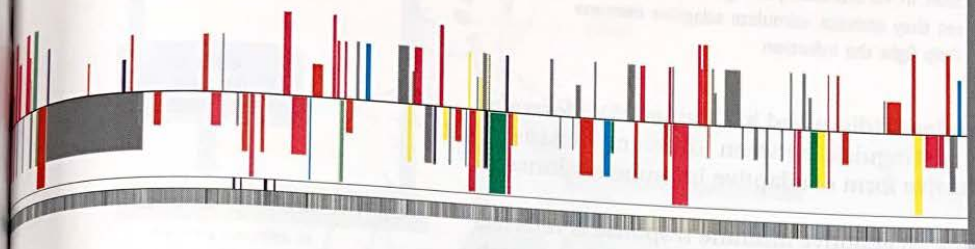


24

THE ADAPTIVE IMMUNE SYSTEM

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. Indeed, 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 25), but they can also mount much more sophisticated defenses, called **adaptive immune responses**. The innate responses call the adaptive immune responses into play, and both work together to eliminate the pathogens (Figure 24–1). Unlike innate immune responses, the adaptive responses are highly specific to the particular pathogen that induced them. They can also 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 mainly on adaptive immune responses, and, unless we indicate otherwise, the term immune responses refers to them. We discuss innate immune responses in detail in Chapter 25.

The function of adaptive immune responses is to destroy invading pathogens and any toxic molecules they produce. Because these responses are destructive, it is crucial that they be made only in response to molecules that are foreign to the host and not to the molecules of the host itself. The ability to distinguish what is *foreign* from what is *self* in this way is a fundamental feature of the adaptive immune system. Occasionally, the system fails to make this distinction and reacts destructively against the host's own molecules. Such *autoimmune diseases* can be fatal.

Of course, many foreign molecules that enter the body are harmless, and it would be pointless and potentially dangerous to mount adaptive immune responses against them. Allergic conditions such as hayfever and asthma are examples of deleterious adaptive immune responses against apparently harmless foreign molecules. Such inappropriate responses are normally avoided because the innate immune system calls adaptive immune responses into play only when it recognizes molecules characteristic of invading pathogens called

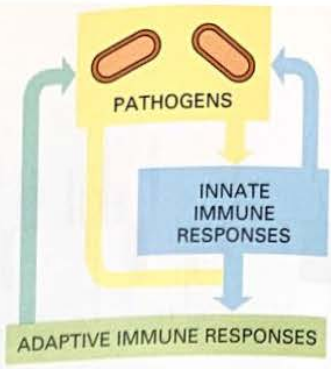


Figure 24–1 Innate and adaptive immune responses. Innate immune responses are activated directly by pathogens and defend all multicellular organisms against infection. In vertebrates, pathogens, together with the innate immune responses they activate, stimulate adaptive immune responses, which then help fight the infection.

pathogen-associated immunostimulants (discussed in Chapter 25). Moreover, the innate immune system can 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 process 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 that is specific to the administered 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.

Adaptive immune responses are carried out by white blood cells called **lymphocytes**. There are two broad classes of such responses—*antibody responses* and *cell-mediated immune responses*, and they are carried out by different classes of lymphocytes, called **B cells** and **T cells**, respectively. 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 24–2). 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 **cell-mediated immune responses**, the second class of adaptive immune response, activated T cells react directly against a foreign antigen that is presented to them on the surface of a host cell. 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 24–2). In other cases, the T cell produces signal molecules that activate macrophages to destroy the invading microbes that they have phagocytosed.

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 cell-mediated immune responses they are responsible for. Remarkably, T cells can detect microbes hiding inside host cells and either kill the infected cells or help other cells to eliminate the microbes.

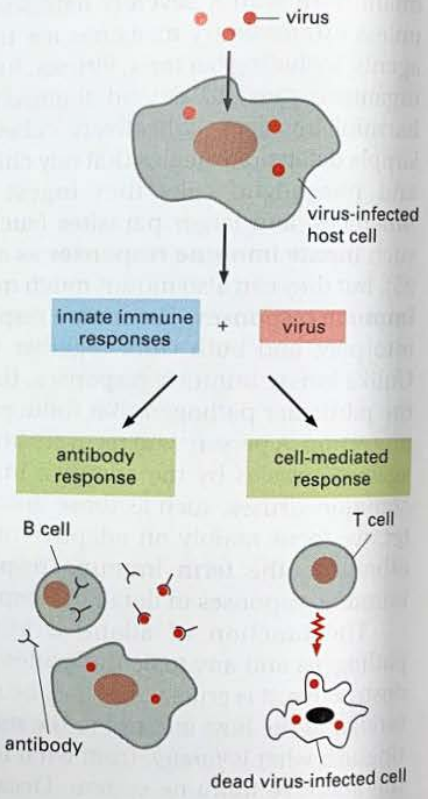


Figure 24–2 The two main classes of adaptive immune responses. Lymphocytes carry out both classes of responses. Here, the lymphocytes are responding to a viral infection. In one class of response, B cells secrete antibodies that neutralize the virus. In the other, a cell-mediated response, T cells kill the virus-infected cells.

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) and in **lymphoid organs**, such as the thymus, lymph nodes, spleen, and appendix (Figure 24–3).

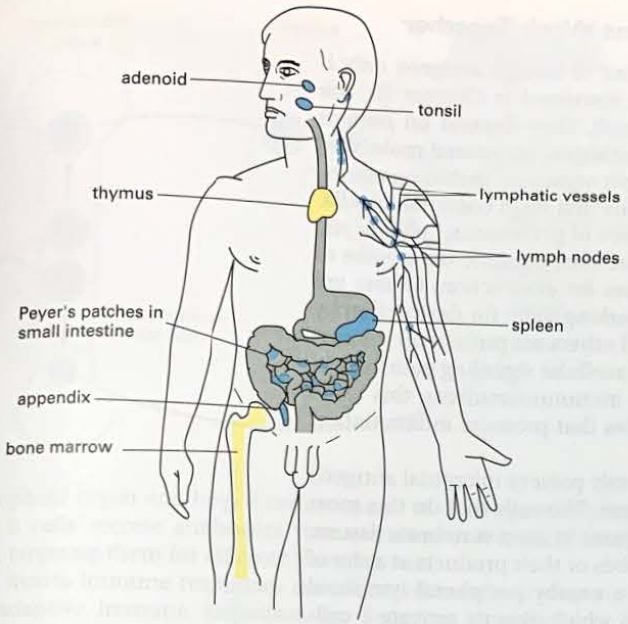


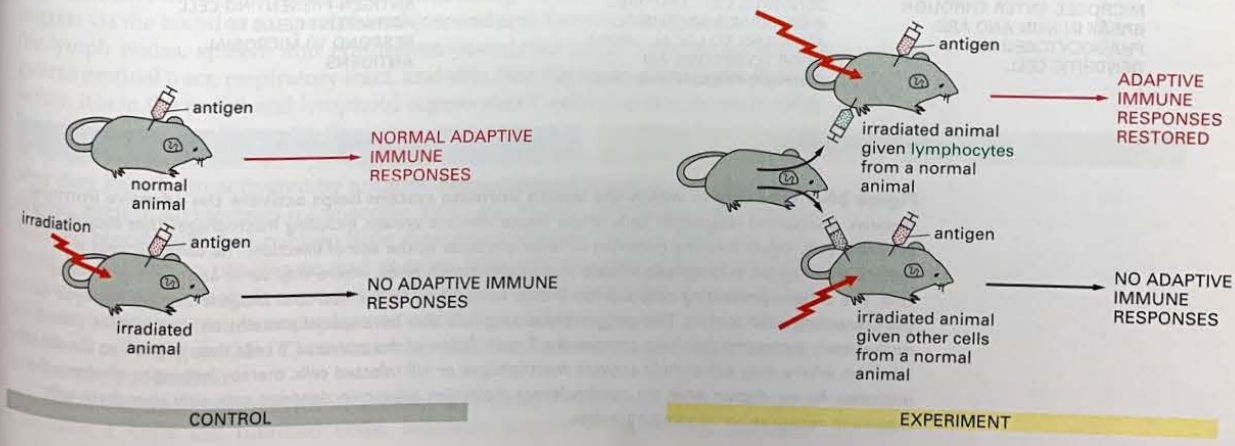
Figure 24–3 Human lymphoid organs. Lymphocytes develop in the thymus and bone marrow *(yellow)*, which are therefore called *central (or primary) lymphoid organs.* The newly formed lymphocytes migrate from these primary organs to *peripheral (or secondary) lymphoid organs (blue),* where they can react with foreign antigen. Only some of the peripheral lymphoid organs and lymphatic vessels 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).

In this section, we discuss the general properties of lymphocytes that apply to both B cells and T cells. We shall see that each lymphocyte is committed to respond to a specific antigen and that its response during its first encounter with an antigen ensures that a more rapid and effective response occurs on subsequent encounters with the same antigen. We consider how lymphocytes avoid responding to self antigens and how they continuously recirculate between the blood and lymphoid organs, ensuring that a lymphocyte will find its specific foreign antigen no matter where the anitgen enters the body.

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 brain. Despite their abundance, their central role in adaptive immunity was not demonstrated until 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. Only lymphocytes restored the adaptive immune responses of irradiated animals, indicating that lymphocytes are required for these responses (Figure 24–4).

Figure 24–4 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 cell-mediated adaptive immune responses, indicating that lymphocytes are required for both types of responses.



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 25, the innate immune responses to an infection are rapid. They depend on *pattern recognition receptors* that recognize patterns of pathogen-associated molecules (immunostimulants) that are not present in the host organism, including microbial DNA, lipids, and polysaccharides, and proteins that form bacterial flagella. Some of these receptors are present on the surface of professional phagocytic cells 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 the complement system. Still others are present on the surface of various types of host cells and activate intracellular signaling pathways in response to the binding of pathogen-associated immunostimulants; this leads to the production of extracellular signal molecules that promote inflammation and help activate adaptive immune responses.

Some cells of the innate immune system directly present microbial antigens to T cells to initiate an adaptive immune response. The cells that do this most efficiently are called *dendritic cells*, which are present in most vertebrate tissues. They recognize and phagocytose invading microbes or their products at a site of infection and then migrate with their prey to a nearby peripheral lymphoid organ. There they act as *antigen-presenting cells*, which directly activate T cells to respond to the microbial antigens. Once activated, some of the T cells then migrate to the site of infection, where they help other phagocytic cells, mainly macrophages, destroy the microbes (Figure 24–5). Other activated T cells remain

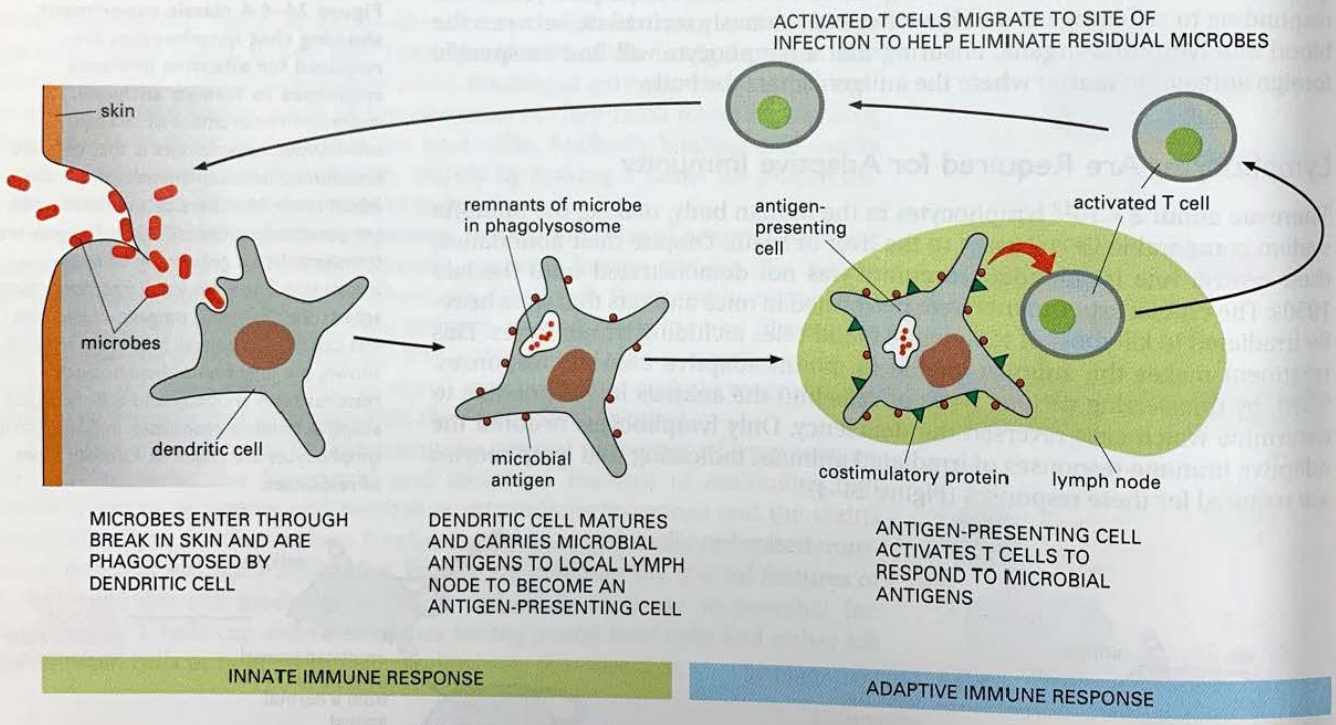


Figure 24–5 One way in which the innate immune system helps activate the adaptive immune system. Specialized phagocytic cells of the innate immune system, including macrophages (not shown) and dendritic cells ingest invading microbes or their products at the site of infection. The dendritic cells then mature and migrate in lymphatic vessels to a nearby lymph node, where they serve as antigen-presenting cells. The antigen-presenting cells activate T cells to respond to the microbial antigens that are displayed on the presenting cells' surface. The antigen-presenting cells also have special proteins on their surface (called *costimulatory molecules*) that help activate the T cells. Some of the activated T cells then migrate to the site of infection where they either help activate macrophages or kill infected cells, thereby helping to eliminate the microbes. As we discuss later, the costimulatory molecules appear on dendritic cells only after these cells mature in response to invading microbes.

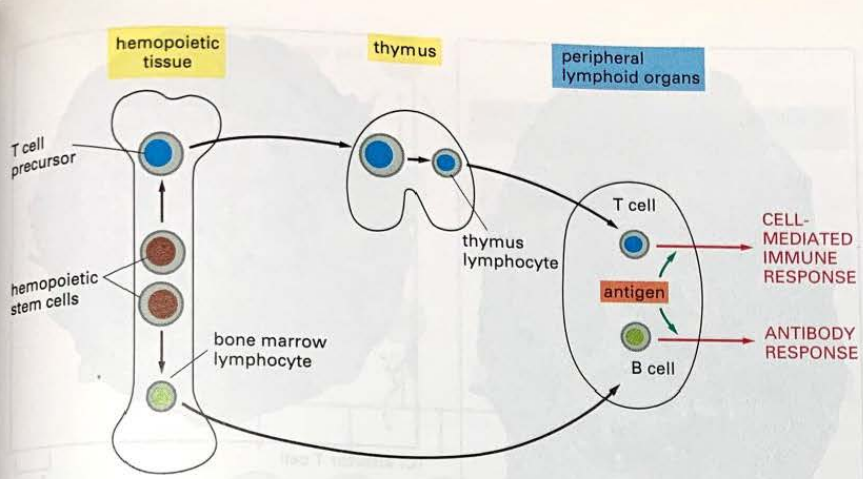


Figure 24–6 The development and activation of T and B cells. The central lymphoid organs, where lymphocytes develop from precursor cells, are labeled in *yellow boxes*. Lymphocytes respond to antigen in peripheral lymphoid organs, such as lymph nodes or spleen.

in the lymphoid organ and help B cells respond to the microbial antigens. The activated B cells secrete antibodies that circulate in the body and coat the microbes, targeting them for efficient phagocytosis.

Thus, innate immune responses are activated mainly at sites of infection, whereas adaptive immune responses are activated in peripheral lymphoid organs. The two types of responses work together to eliminate invading pathogens.

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.

Despite their different origins, both T and B cells develop from the same *pluripotent 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 22) are located primarily in *hemopoietic* tissues—mainly the liver in fetuses and the bone marrow in adults. T cells develop in the thymus from precursor cells that migrate there from the hemopoietic tissues via the blood. In most mammals, including humans and mice, B cells develop from stem cells in the hemopoietic tissues themselves (Figure 24–6). 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 24–3).

As we discuss later, most lymphocytes die in the central lymphoid organ 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 24–3). As mentioned earlier, it is in the peripheral lymphoid organs that T cells and B cells react with foreign antigens (see Figure 24–6).

T and B cells become morphologically distinguishable from each other only after they have been activated by antigen. Nonactivated 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 24–7A). Both are activated by antigen to 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 (Figure 24–7B). In contrast, effector T cells (Figure 24–7C) contain very little endoplasmic reticulum and do not secrete antibodies.

There are two main classes of T cells—*cytotoxic T cells* and *helper T cells.* Cytotoxic T cells kill infected cells, whereas helper T cells help activate

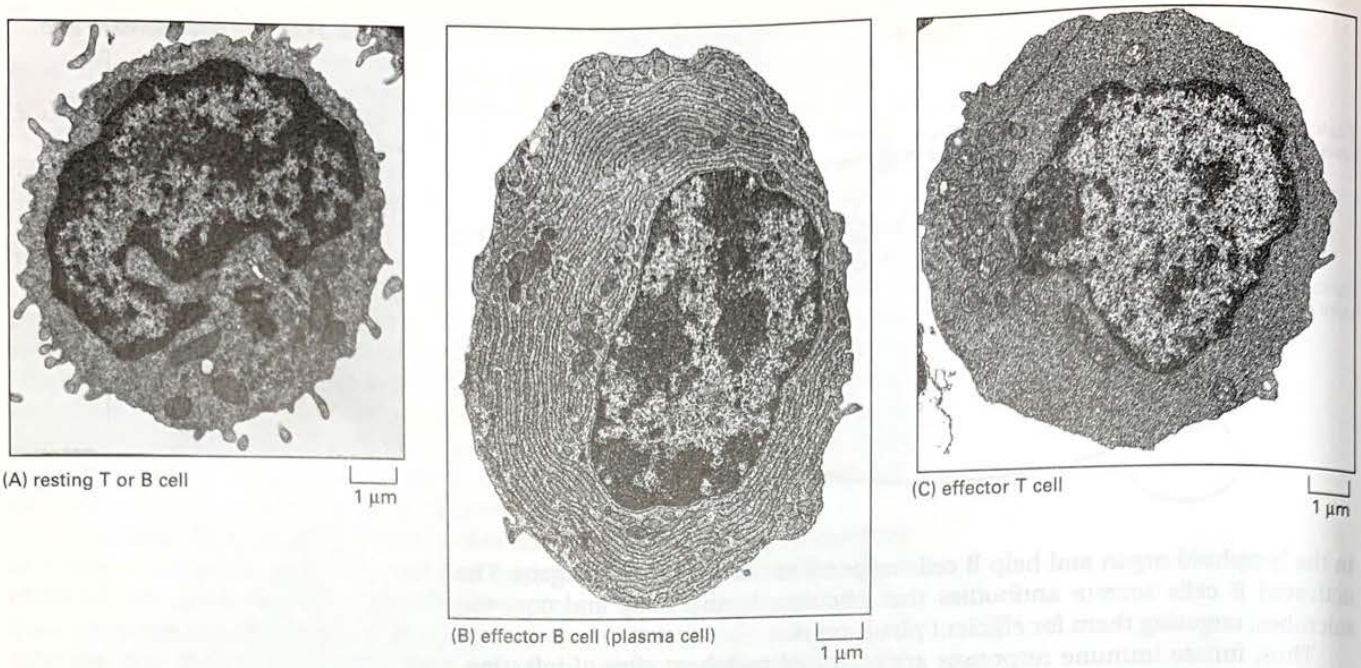


Figure 24–7 Electron micrographs of nonactivated and activated lymphocytes. (A) A resting lymphocyte, which could be a T cell or a B cell, as these cells are difficult to distinguish morphologically until they have been activated to become effector cells. (B) An effector B cell (a plasma cell). It is 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. Note that 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 edn. Milan, Italy: Edi. Ermes, 1988; C, courtesy of Stefanello de Petris.)

macrophages, B cells, and cytotoxic T cells. Effector helper T cells secrete a variety of signal proteins called **cytokines**, which act as local mediators. They also display a variety of costimulatory proteins on their surface. By means of these cytokines and membrane-bound costimulatory proteins, they can influence the behavior of the various cell types they help. Effector cytotoxic T cells kill infected target cells also by means of proteins that they either secrete or display on their surface. Thus, whereas B cells can act over long distances by secreting antibodies that are distributed by the bloodstream, T cells can migrate to distant sites, but 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. B cells, for example, make antibodies 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 those lymphocytes that can react against the foreign antigens that the animal actually encounters are specifically selected for action. 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 fit 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 both to proliferate and to differentiate into an effector cell. An antigen therefore selectively stimulates those cells that express complementary antigen-specific receptors and are thus already committed to respond to it. This arrangement is what makes adaptive immune responses antigen-specific.

The term "clonal" in clonal selection theory derives from the postulate that the adaptive immune system is composed of millions of different families, or clones, of lymphocytes, each consisting of T or B cells descended from a common ancestor. Each ancestral cell was already committed to make one particular antigen-specific receptor protein, and so all cells in a clone have the same antigen specificity (Figure 24–8). According to the clonal selection theory, then, the immune system functions on the "ready-made" principle rather than the "made-to-order" one.

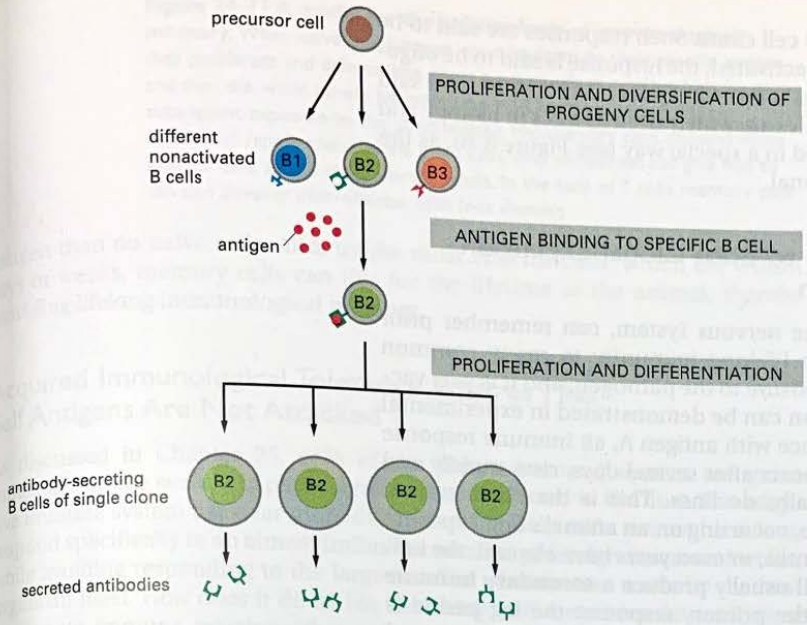


Figure 24–8 The clonal selection theory. An antigen activates only those lymphocyte clones (represented here by single cells) that are already committed to respond to it. A cell committed to respond to a particular antigen displays cell-surface receptors that specifically recognize the antigen, and all cells within a clone display the same receptor. The immune system is thought to consist of millions of different lymphocyte clones. A particular antigen may activate hundreds of different clones. Although only B cells are shown here, T cells operate in a similar way.

There is compelling evidence to support the main tenets of the clonal selection theory. For example, when lymphocytes from an animal that has not been immunized are incubated in a test tube with a number of radioactively labeled antigens, only a very small proportion (less than 0.01%) bind each antigen, suggesting that only a few cells are committed to respond to these antigens. Moreover, when one antigen is made so highly radioactive that it kills any cell that it binds to, the remaining lymphocytes can no longer produce an immune response to that particular antigen, even though they can still respond normally to other antigens. Thus, the committed lymphocytes must have receptors on their surface that specifically bind that antigen. Although most experiments of this kind have involved B cells and antibody responses, other experiments indicate that T cells, like B cells, operate by clonal selection.

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 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 unique form of genetic recombination that occurs early in a lymphocyte's development, before it has encountered antigen. This assembly process generates the enormous diversity of receptors and lymphocytes, thereby enabling the immune system to respond to an almost unlimited diversity of antigens.

Most Antigens Activate Many Different Lymphocyte Clones

Most large molecules, including virtually all proteins and many polysaccharides, can serve as antigens. Those parts of an antigen that combine with 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 said to be *immunodominant*.

The diversity of lymphocytes is such that even a single antigenic determinant is likely to activate many 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 24–9, 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

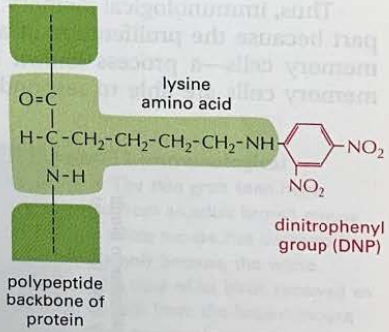


Figure 24–9 The dinitrophenyl (DNP) group. Although it is too small to induce an immune response on its own, when it is coupled covalently to a lysine side chain on a protein, as illustrated, DNP stimulates the production of hundreds of different species of antibodies that all bind specifically to it.

antibodies, each made by a different B cell clone. Such responses are said to be *polyclonal*. When only a few clones are activated, the response is said to be *oligoclonal;* and when the response involves only a single B or T cell clone, it is said to be *monoclonal*. Monoclonal antibodies are widely used as tools in biology and medicine, but they have to be produced in a special way (see Figure 8–6), as the responses to most antigens are polyclonal.

Immunological Memory Is Due to 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 (either antibody or cell-mediated) appears after several days, rises rapidly and 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 reinjected with antigen A, it will usually produce a **secondary immune response** that is very different from the primary response: the lag period is shorter, and the response is greater. 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 injection of 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 24–10).

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 cells in at least three stages of maturation: *naïve cells, effector cells* and *memory cells*. When **naïve cells** encounter antigen for the first time, some of them are stimulated to proliferate and differentiate into **effector cells**, which are actively engaged in making a response (effector B cells secrete antibody, while effector T cells kill infected cells or help other cells fight the infection). Instead of becoming effector cells, some naïve cells are stimulated to multiply and differentiate into **memory cells**—cells that are not themselves engaged in a response but are more easily and more quickly induced to become effector cells by a later encounter with the same antigen. Memory cells, like naïve cells, give rise to either effector cells or more memory cells (Figure 24–11).

Thus, immunological memory is generated during the primary response in part because the proliferation of antigen-stimulated naïve cells creates many memory cells—a process known as *clonal expansion*—and in part because memory cells are able to respond more sensitively and rapidly to the same

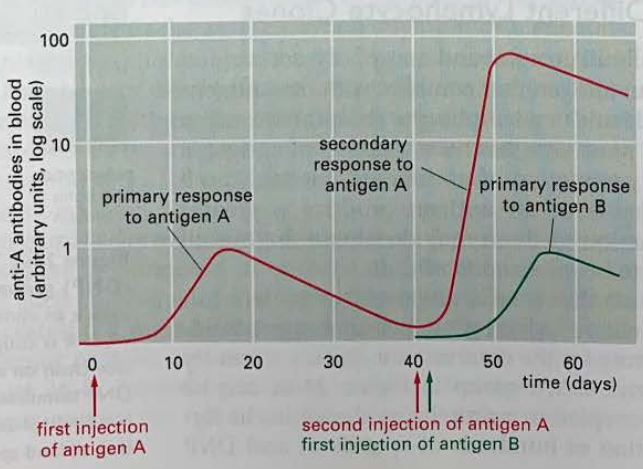


Figure 24–10 Primary and secondary antibody responses. The secondary response induced by a second exposure to antigen A is faster and greater than the primary response and is specific for A, indicating that the adaptive immune system has specifically remembered encountering antigen A before. The same type of immunological memory is observed in T-cell-mediated responses.

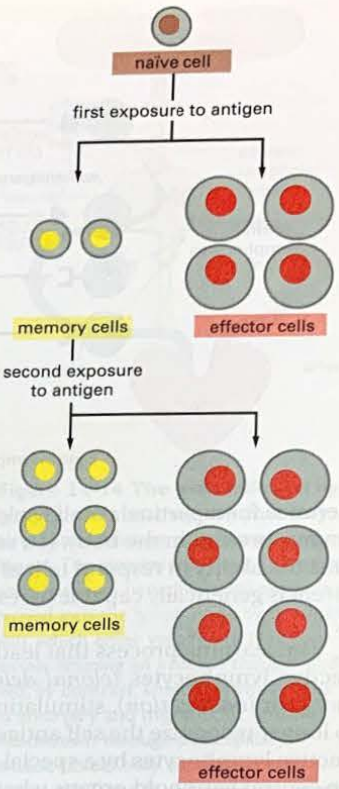


Figure 24–11 A model for the cellular basis of immunological memory. When naïve lymphocytes are stimulated by their specific antigen, they proliferate and differentiate. Most become effector cells which function and then die, while others become long-lived memory cells. During a subsequent exposure to the same antigen, the memory cells respond more readily and rapidly than did the naïve cells: they proliferate and give rise to effector cells and to more memory cells. In the case of T cells, memory cells can also develop from effector cells (not shown).

antigen than do naïve cells. And, unlike most effector cells, which die within days or weeks, memory cells can live for the lifetime of the animal, thereby providing lifelong immunological memory.

Acquired Immunological Tolerance Ensures That Self Antigens Are Not Attacked

As discussed in Chapter 25, cells of the innate immune system recognize molecules on the surface of pathogens that are not found in 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 does it do it? For one thing, self molecules do not induce the innate immune reactions that are required to activate adaptive immune responses. But even when an infection triggers innate reactions, self molecules still do not normally induce adaptive immune responses. Why not?

One answer is that the adaptive immune system "learns" 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, as long as 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 or an adjuvant.) If, however, cells from one strain of mouse are introduced into a neonatal 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 some 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 24–12).

The unresponsiveness of an animal's adaptive immune system to its own macromolecules *(natural immunological 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 25). Mutant mice, however, 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. Natural immunological

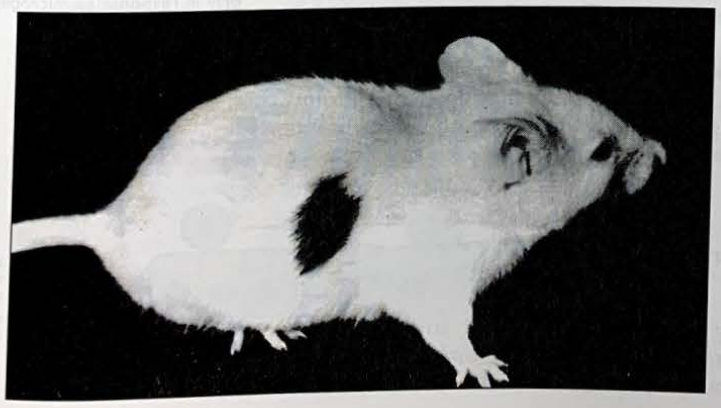


Figure 24–12 Immunological tolerance. The skin graft seen here, transplanted from an adult brown mouse to an adult white mouse, has survived for many weeks only because the white mouse, at the time of its birth, received an injection of cells from the brown mouse and therefore became immunologically tolerant to them. The cells 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 edn. Oxford, UK: Blackwell Scientific, 1988.)

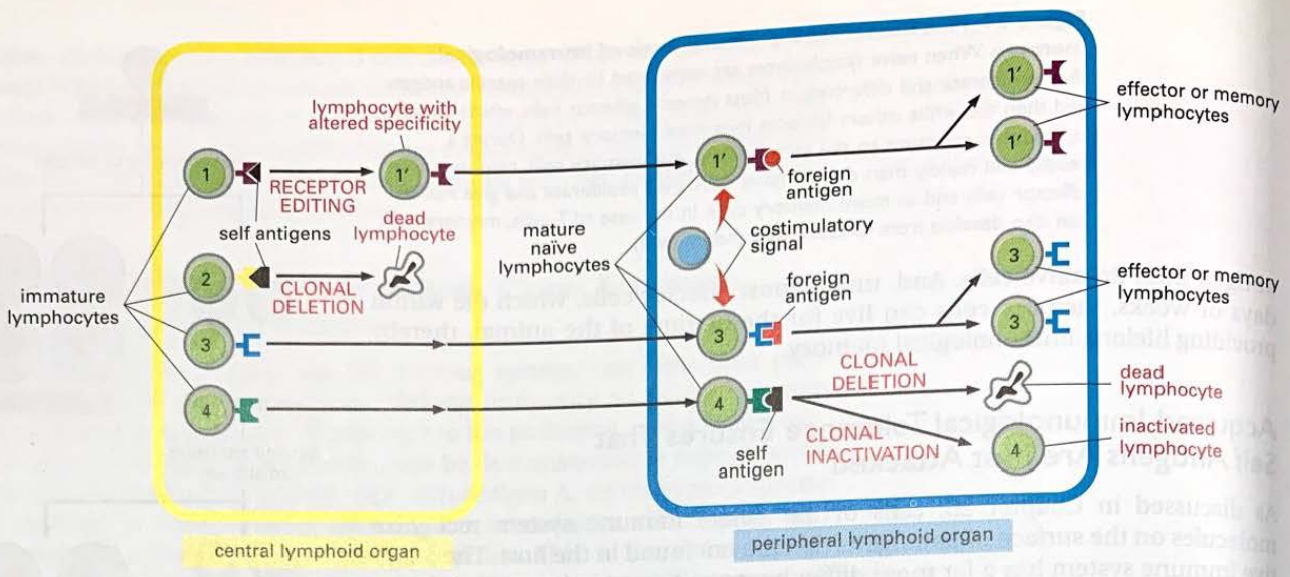


Figure 24–13 Induction of immunological tolerance to self antigens in central and peripheral lymphoid organs. When a self-reactive immature lymphocyte binds its self antigen in the central lymphoid organ where the cell is produced, it may be induced to alter the receptor it makes so that it is no longer self-reactive. This process is called receptor editing and seems to occur only in developing B cells. Alternatively, the cell may die by apoptosis, a process called clonal deletion. When a self-reactive naïve lymphocyte escapes tolerance in the central lymphoid organ and binds its self antigen in a peripheral lymphoid organ, it may either die by apoptosis or be inactivated, as the binding usually occurs in the absence of a costimulatory signal. Although not shown, some self-reactive cells survive and are suppressed by special regulatory T cells.

When a naïve lymphocyte binds a foreign antigen in a peripheral lymphoid organ in the presence of a costimulatory signal, it is stimulated to proliferate and differentiate into an effector or memory cell. As microbes are usually responsible for inducing costimulatory signals, most adaptive immune reactions normally occur only in response to microbes.

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 removed, an 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.

The learning process that leads to self-tolerance can involve killing the self-reactive lymphocytes *(clonal deletion)*, functionally inactivating them *(clonal anergy* or *inactivation)*, stimulating the cells to produce modified receptors that no longer recognize the self antigen *(receptor editing)*, or the suppression of self-reactive lymphocytes by a special type of regulatory T cell. The process begins in the central lymphoid organs when newly formed self-reactive lymphocytes first encounter their self antigen. Instead of being activated by binding antigen, the immature lymphocytes are induced to either alter their receptors or die by apoptosis. Lymphocytes that could potentially respond to self antigens that are not present in the central lymphoid organs often die or are either inactivated or suppressed after they have matured and migrated to peripheral lymphoid organs.

Why does the binding of self antigen lead to tolerance rather than activation? As we discuss later, for a lymphocyte to be activated in a peripheral lymphoid organ, it must not only bind its antigen but must also receive a *costimulatory signal*. The latter signal is provided by a helper T cell in the case of a B lymphocyte and by an antigen-presenting cell in the case of a T lymphocyte. The production of costimulatory signals usually depends on exposure to pathogens, and so a self-reactive lymphocyte normally encounters its antigen in the absence of such signals. Without a costimulatory signal, an antigen tends to kill or inactivate a lymphocyte rather than activate it (Figure 24–13).

Tolerance to self antigens sometimes breaks 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. 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 may help trigger certain anti-self responses in genetically susceptible individuals.

Lymphocytes Continuously Circulate Through Peripheral Lymphoid Organs

Pathogens generally enter the body through an epithelial surface, usually through the skin, gut, or respiratory tract. How do the microbial antigens travel from these entry points to a peripheral lymphoid organ, such as a lymph node

or the spleen, where lymphocytes are activated (see Figure 24–6)? The route and destination depend on the site of entry. Antigens that enter through the skin or respiratory tract are carried via the lymph to local lymph nodes; those that enter through the gut end up in gut-associated peripheral lymphoid organs such as Peyer's patches; and those that enter the blood are filtered out in the spleen. In most cases, dendritic cells carry the antigen from the site of infection to the peripheral lymphoid organ, where they become antigen-presenting cells (see Figure 24–5), specialized for activating T cells (as we discuss later).

But the lymphocytes that can recognize a particular microbial antigen in a peripheral lymph organ are only a tiny fraction of the total lymphocyte population. How do these rare cells find an antigen-presenting cell displaying their antigen? The answer is that they continuously circulate between the lymph and blood until they encounter their antigen. 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 24–3). 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 24–14). This continuous recirculation between the blood and lymph ends only if a lymphocyte encounters its specific antigen (and a costimulatory signal) on the surface of an antigen-presenting cell in a peripheral lymphoid organ. Now the lymphocyte is retained in the peripheral lymphoid organ, where it proliferates and differentiates into effector cells. Some of the effector T cells then leave the organ via the lymph and migrate through the blood to the site of infection (see Figure 24–5).

Lymphocyte recirculation depends on specific interactions between the lymphocyte cell surface and the surface of the specialized endothelial cells lining the postcapillary venules in the peripheral lymphoid organs. Many cell types in the blood come into contact with these endothelial cells, but only lymphocytes adhere and then migrate out of the bloodstream. 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, for example, depends on a homing receptor protein called *L-selectin*, a member of the selectin family of cell-surface lectins discussed in Chapter 19. This protein binds to specific sugar groups on a counterreceptor that is expressed exclusively on the surface of the specialized endothelial cells 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 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 (discussed in Chapter 19), which become activated on the lymphocyte surface. Now the lymphocytes stop rolling and crawl out of the blood vessel into the lymph node (Figure 24–15).

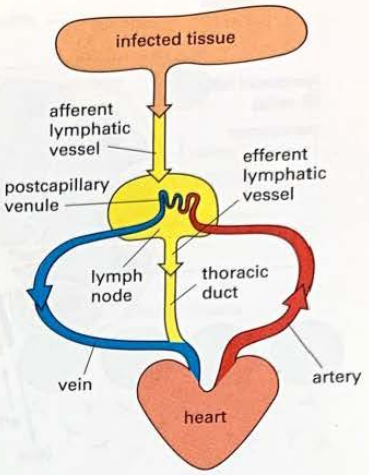


Figure 24–14 The path followed by lymphocytes as they continuously circulate between the lymph and blood. The circulation through a lymph node is shown here. Microbial antigens are carried into the lymph node by dendritic cells, which enter via afferent lymphatic vessels draining an infected tissue. 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. A typical circulation cycle takes about 12–24 hours.

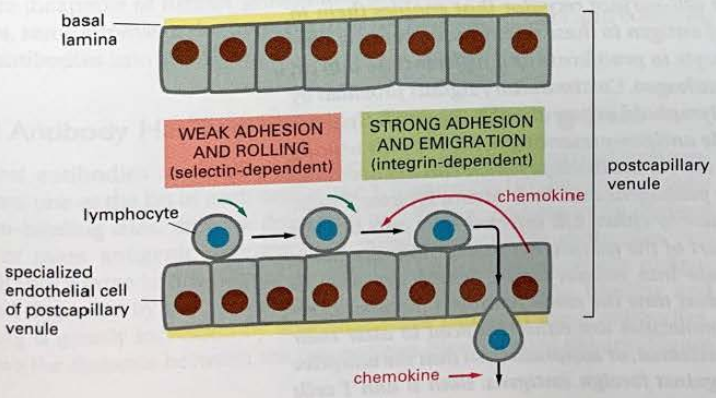


Figure 24–15 Migration of a lymphocyte out of the bloodstream into a lymph node. A circulating lymphocyte adheres weakly to the surface of the specialized endothelial cells lining a postcapillary venule in a lymph node. This initial adhesion is mediated by L-selectin on the lymphocyte surface. The adhesion 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, the lymphocyte rapidly activates a stronger adhesion system, mediated by an integrin. This strong adhesion enables the cell to stop rolling and migrate out of the venule between the endothelial cells. The subsequent migration of the lymphocytes in the lymph node also depends on chemokines, which are produced within the node. The migration of other white blood cells out of the bloodstream into sites of infection occurs in a similar way.

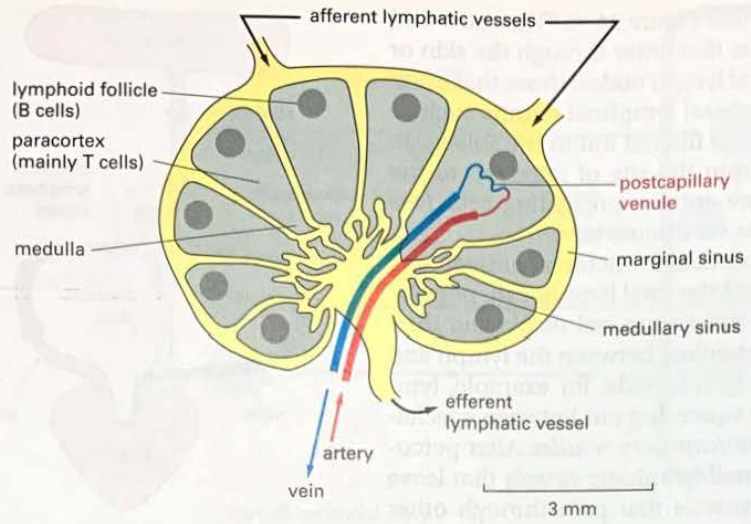


Figure 24–16 A simplified drawing of a human lymph node. B cells are primarily clustered in structures called lymphoid follicles, whereas T cells are found mainly in the paracortex. Both types of lymphocytes are attracted by chemokines to enter the lymph node from the blood via postcapillary venules. They 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 secondary lymphoid organ (see Figure 24–14).

Chemokines are small, secreted, positively charged proteins that have a central role in guiding the migrations of various types of white blood cells. They are all structurally related and bind to the surface of endothelial cells, and to negatively charged proteoglycans of the extracellular matrix in organs. By binding to G-protein-linked 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. (The AIDS virus (HIV) also binds to chemokine receptors, which allows the virus to infect white blood cells.) T and B cells initially enter the same region of a lymph node but are then attracted by different chemokines to separate regions of the node—T cells to the *paracortex* and B cells to *lymphoid follicles* (Figure 24–16). Unless they encounter their antigen, both types of cells soon leave the lymph node via lymphatic vessels. If they encounter their antigen, however, they remain in the node, proliferate, and differentiate into either effector cells or memory cells. Most 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 microbe-specific molecules associated with invading pathogens. In addition to fighting infection directly, these responses help activate adaptive immune responses in peripheral lymphoid organs. Unlike innate immune responses, adaptive responses provide specific and long-lasting protection against the particular pathogen that induced them.

The adaptive immune system is composed of 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. Costimulatory signals provided by another specialized cell in a peripheral lymphoid organ are also required. Helper T cells provide such signals for B cells, while antigen-presenting 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 at sites of infection to either kill infected host cells or help other cells to eliminate pathogens. 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. Lymphocytes that would react against self molecules are either induced to alter their receptors, induced to kill themselves, inactivated, or suppressed, so that the adaptive immune system normally reacts only against foreign antigens. Both B and T cells

circulate continuously between 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.

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 24–2). The binding of antibodies to invading pathogens also recruits various types of white blood cells and a system of blood proteins, collectively called *complement* (discussed in Chapter 25). The white blood cells and activated 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 and a different antigen-binding site. 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 Receptors and Secreted Molecules

As predicted by the clonal selection theory, 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. Instead, they are 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 binds to the receptor.

Each B cell produces a single species of antibody, each with a unique antigen-binding site. When a naïve or memory B cell is activated by antigen (with the aid of a helper T cell), it proliferates and differentiates into an antibody-secreting effector cell. Such 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 24–17). 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 24–7B), which continuously secretes antibodies at the astonishing rate of about 2000 molecules per second. Plasma cells seem to have committed so much of their protein-synthesizing machinery to making antibody that they are incapable of further growth and division. Although many die after several days, some survive in the bone marrow for months or years and continue to secrete antibodies into the blood.

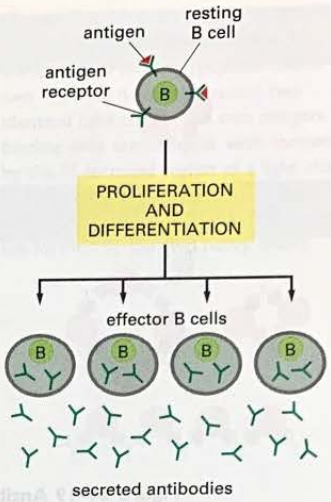


Figure 24–17 B cell activation. When naïve or memory B cells are activated by antigen (and helper T cells—not shown), they proliferate and differentiate into effector cells. The effector cells produce and secrete antibodies with a unique antigen-binding site, which is the same as that of their original membrane-bound antibodies that served as 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 24–18). 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 24–19). This lattice can be rapidly phagocytosed and degraded by macrophages. The efficiency of antigen binding and cross-linking is greatly increased by a flexible *hinge region* in most antibodies, which allows the distance between the two antigen-binding sites to vary (Figure 24–20).

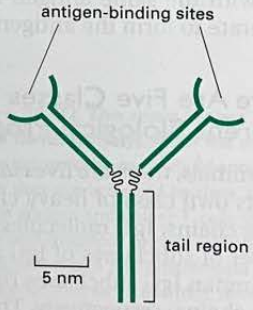


Figure 24–18 A simple representation of an antibody molecule. Note that its two antigen-binding sites are identical.

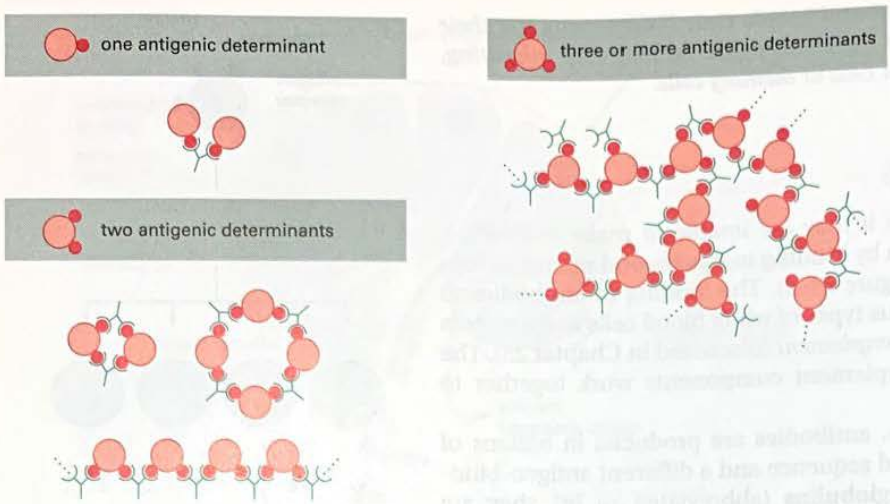


Figure 24–19 Antibody–antigen interactions. Because antibodies have two identical antigen-binding sites, they can cross-link antigens. The types of antibody–antigen complexes that form depend on the number of antigenic determinants on the antigen. Here a single species of antibody (a monoclonal antibody) is shown binding to antigens containing one, two, or three copies of a single type of antigenic determinant. Antigens with two antigenic determinants can form small cyclic complexes or linear chains with antibody, while antigens with three or more antigenic determinants can form large three-dimensional lattices that readily precipitate out of solution. Most antigens have many different antigenic determinants (see Figure 24–29A), and different antibodies that recognize different determinants can cooperate in cross-linking the antigen (not shown).

The protective effect of antibodies is not due simply to their ability to bind antigen. They engage in a variety of activities that are mediated by the tail of the Y-shaped molecule. 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.

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 acids). The four chains are held together by a combination of noncovalent and covalent (disulfide) bonds. 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 24–21).

There Are Five Classes of 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 made by a developing B cell, although many B cells eventually switch to making other

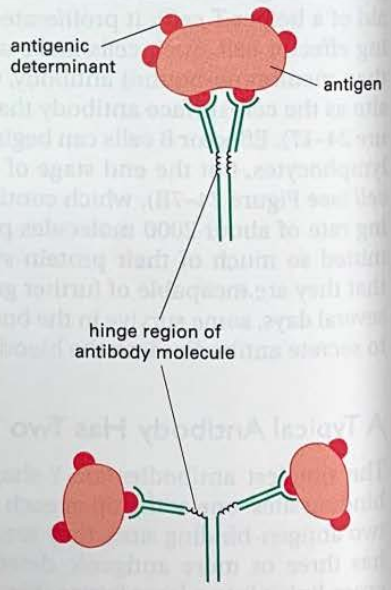


Figure 24–20 The hinge region of an antibody molecule. Because of its flexibility, the hinge region improves the efficiency of antigen binding and cross-linking.

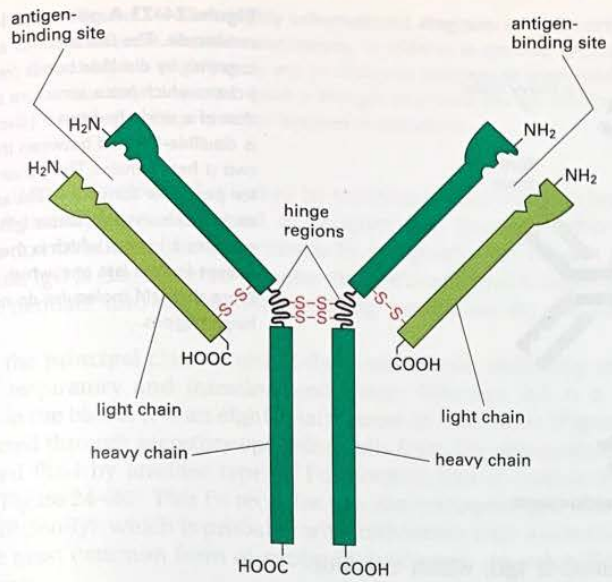


Figure 24–21 A schematic drawing of a typical antibody molecule. It is composed of four polypeptide chains—two identical heavy chains and two identical light chains. The two antigen-binding sites are identical, each formed by the N-terminal region of a light chain and the N-terminal region of a heavy chain. Both the tail (Fc) and hinge region are formed by the two heavy chains.

classes of antibody (discussed below). The immediate precursor of a B cell, called a **pre-B cell**, initially makes μ chains, which associate with so-called *surrogate light chains* (substituting for genuine light chains) and insert into the plasma membrane. The complexes of μ chains and surrogate light chains are 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 24–22).

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 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 24–23).

The binding of an antigen to a single secreted pentameric IgM molecule can activate the complement system. As discussed in Chapter 25, 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.

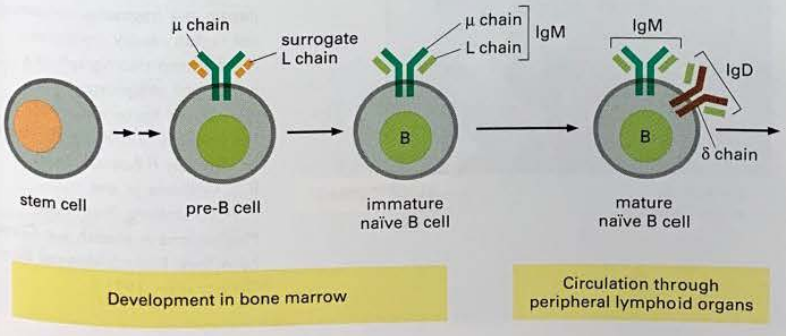


Figure 24–22 The main stages in B cell development. All of the stages shown occur independently of antigen. When they are activated by their specific foreign antigen and helper T cells in peripheral lymphoid organs, mature naïve B cells proliferate and differentiate into effector or memory cells (not shown).

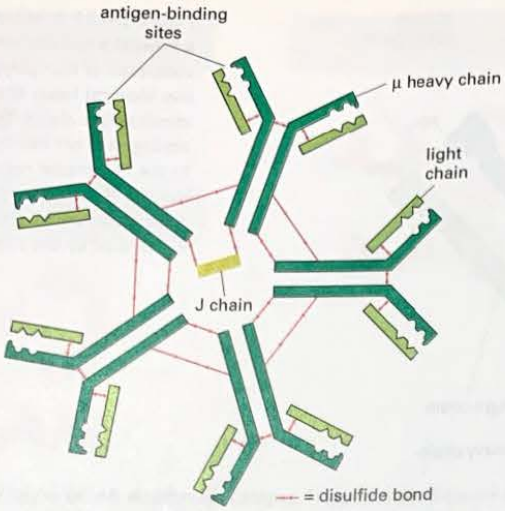


Figure 24–23 A pentameric IgM molecule. The five subunits are held together by disulfide bonds *(red)*. A single J chain, which has a structure similar to that of a single Ig domain (discussed later), is disulfide-bonded between the tails of two μ heavy chains. The J chain is required for pentamer formation. The addition of each successive four-chain IgM subunit 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.

The major class of immunoglobulin in the blood is **IgG**, which is a four-chain monomer produced in large quantities during *secondary* immune responses. Besides activating complement, the tail region of an IgG molecule binds to specific receptors on macrophages and neutrophils. 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 24–24).

IgG molecules 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 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

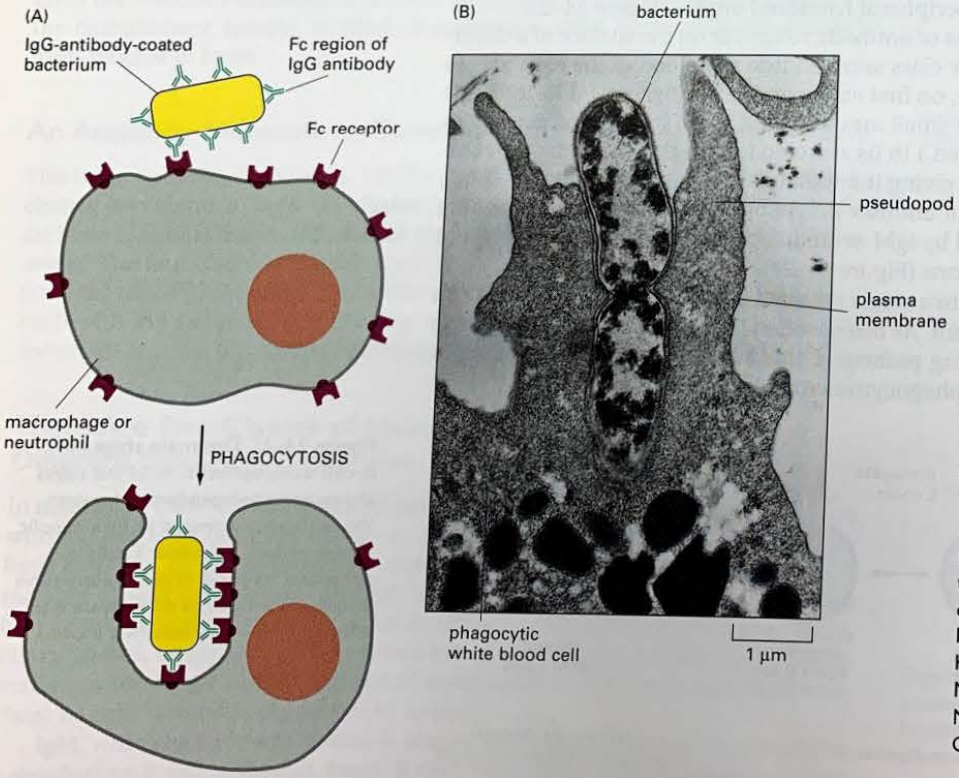
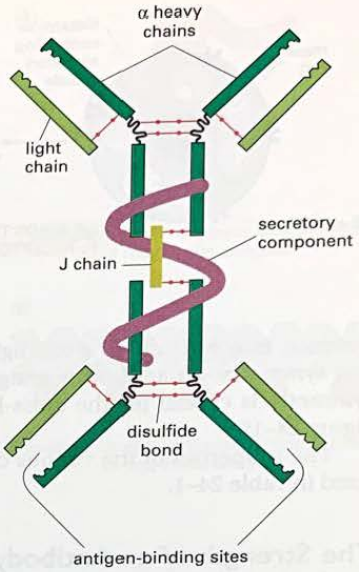


Figure 24–24 Antibody-activated phagocytosis. (A) An IgG-antibody-coated bacterium is efficiently phagocytosed by a macrophage or neutrophil, which has cell-surface receptors that bind the tail (Fc) region of IgG molecules. The binding of the antibody-coated bacterium to these Fc receptors activates 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. (B, courtesy of Dorothy F. Bainton, from R.C. Williams, Jr. and H.H. Fudenberg, Phagocytic Mechanisms in Health and Disease. New York: Intercontinental Book Corporation, 1971.)

Figure 24–25 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 thought to protect the IgA molecules from digestion by proteolytic enzymes in secretions.



across the cell in vesicles and released by exocytosis into the fetal blood (a process called *transcytosis*, discussed in Chapter 13). Because other classes of antibodies do not bind to these particular Fc receptors, they cannot pass across the placenta. IgG is also secreted into the mother's milk and is taken up from the gut of the neonate into the blood, 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. Whereas IgA is a four-chain monomer in the blood, it is an eight-chain dimer in secretions (Figure 24–25). It is transported through secretory epithelial cells from the extracellular fluid into the secreted fluid by another type of Fc receptor that is unique to secretory epithelia (Figure 24–26). 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 ($K_a \sim 10^{10}$ 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 24–27). These molecules cause blood vessels to dilate and become leaky, which in turn helps white blood cells, antibodies, and complement components to enter sites of infection. The same molecules are also 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*. These cells also have Fc receptors that bind IgE molecules and can kill various types of parasites, especially if the parasites 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

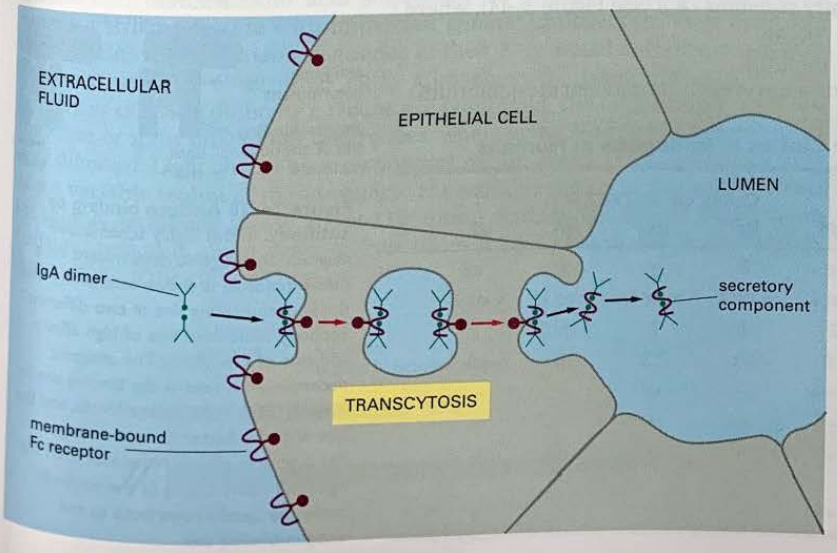


Figure 24–26 The mechanism of transport of a dimeric IgA molecule across an epithelial cell. The IgA molecule, as a J-chain-containing dimer, binds to a transmembrane receptor protein on the nonlumenal surface of a secretory epithelial cell. The receptor–IgA complexes are ingested by receptor-mediated 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 24–25. The J chain is not shown.

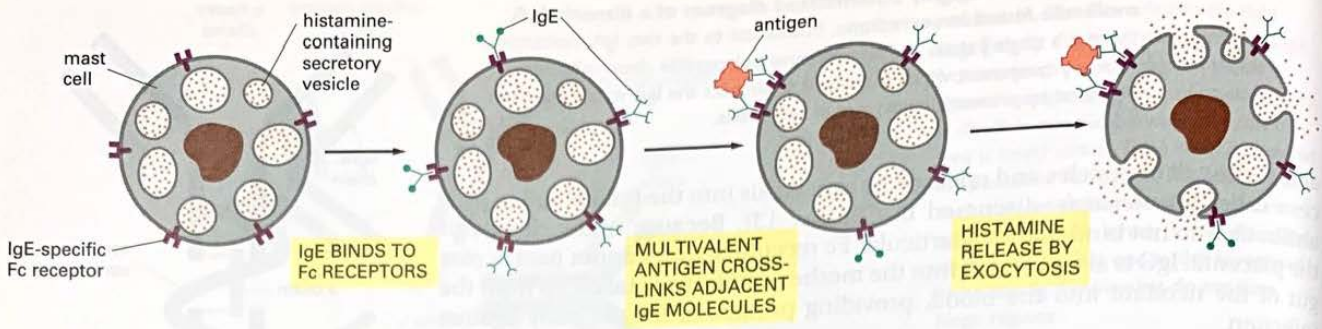


Figure 24–27 The role of IgE in histamine secretion by mast cells. A mast cell (or a basophil) binds IgE molecules after they are secreted by activated B cells. The soluble IgE antibodies bind to Fc receptor proteins on the mast cell surface that specifically recognize the Fc region of these antibodies. 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.

instance, may have either κ or λ light chains, but not one of each. As a result of this symmetry, an antibody's antigen-binding sites are always identical. Such symmetry is crucial for the cross-linking function of secreted antibodies (see Figure 24–19).

The properties of the various classes of antibodies in humans are summarized in Table 24–1.

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 antibody, like the binding of a substrate to an enzyme, is reversible. It is mediated by the sum of many relatively weak noncovalent forces, including hydrogen bonds and hydrophobic van der Waals forces, and ionic interactions. 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 24–28). Most antigenic macromolecules have many different antigenic determinants and are said to be *multivalent;* if two or more of them are identical (as in a polymer with a repeating structure), the antigen is said to be *polyvalent* (Figure 24–29).

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 3–44), where

$$K_a = \frac{[AgAb]}{[Ag][Ab]}$$

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

TABLE 24–1 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	–	–	–	–	+

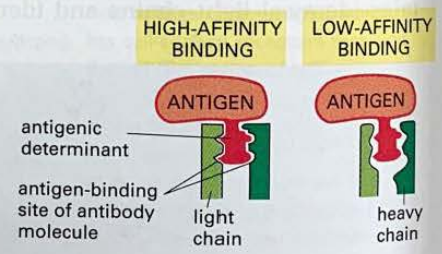


Figure 24–28 Antigen binding to antibody. In this highly schematized diagram, an antigenic determinant on a macromolecule is shown interacting with the antigen-binding site of two different antibody molecules, one of high affinity and one of low affinity. The antigenic determinant is held in the binding site by various weak noncovalent forces, 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.

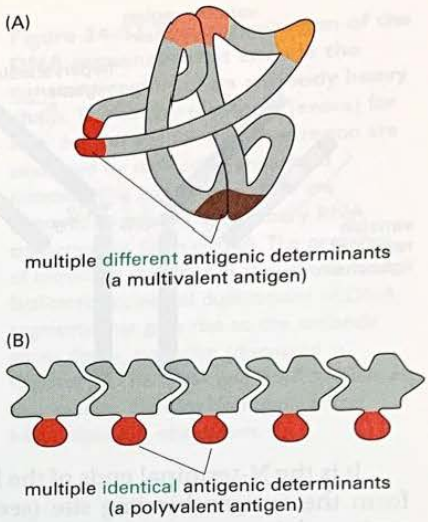


Figure 24–29 Molecules with multiple antigenic determinants. (A) A globular protein is shown with a number of different antigenic determinants. Different regions of a polypeptide chain usually come together in the folded structure to form each antigenic determinant on the surface of the protein. (B) A polymeric structure is shown with many *identical* antigenic determinants.

The affinity constant, sometimes called the association constant, can be determined 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 sites. When, however, a polyvalent antigen, carrying multiple copies of the same antigenic determinant, combines with a polyvalent antibody, the binding strength is greatly increased because all of the antigen-antibody bonds must be broken simultaneously before the antigen and antibody can dissociate. As a result, a typical 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 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 multivalent 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 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.

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 the amino acid sequences of many different κ chains are compared, the C-terminal halves are the same or show only minor differences, whereas the N-terminal halves are all very different. Light chains have a **constant region** about 110 amino acids long and a **variable region** of the same size. The variable region of the heavy chains (at their N-terminus) is also about 110 amino acids long, but the heavy-chain constant region is about three or four times longer (330 or 440 amino acids), depending on the class (Figure 24–30).

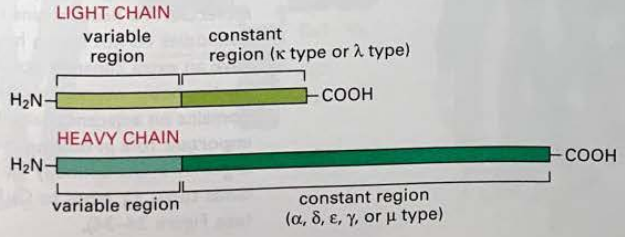


Figure 24–30 Constant and variable regions of immunoglobulin chains. Both light and heavy chains of an antibody molecule have distinct constant and variable regions.

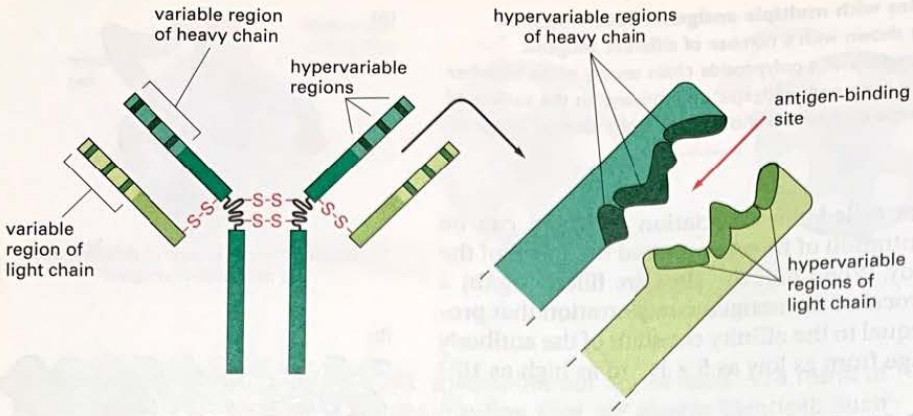


Figure 24–31 Antibody hypervariable regions. Highly schematized drawing of how the three hypervariable regions in each light and heavy chain together form the antigen-binding site of an antibody molecule.

It is the N-terminal ends of the light and heavy chains that come together to form the antigen-binding site (see Figure 24–21), and the variability of their amino acid sequences provides the structural basis for the diversity of antigen-binding sites. The diversity in the variable regions of both light and heavy chains is for the most part restricted to three small **hypervariable regions** in each chain; the remaining parts of the variable region, known as *framework regions*, are relatively constant. Only the 5–10 amino acids in each hypervariable region form the antigen-binding site (Figure 24–31). As a result, the size of the antigenic determinant that an antibody recognizes is generally comparably small. It can consist of fewer than 25 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. These repeating segments fold independently to form compact functional units called **immunoglobulin (Ig) domains**. As shown in Figure 24–32, 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 24–30). These domains pair with the variable (V_H) and first constant (C_H1) domain of the heavy chain to form the antigen-binding region. 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.

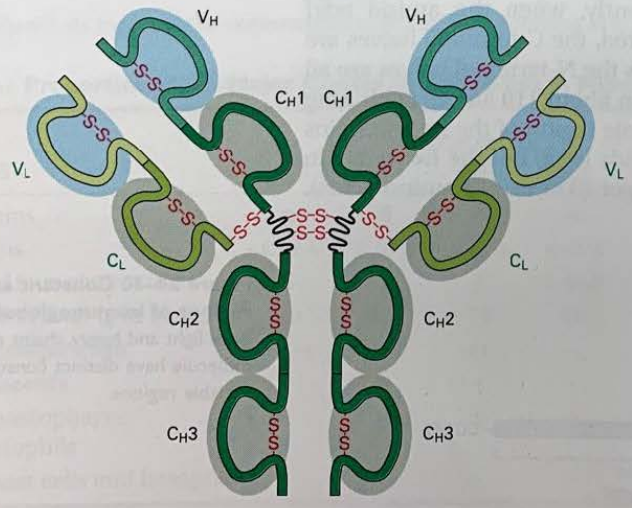


Figure 24–32 Immunoglobulin domains. The light and heavy chains in an antibody molecule are each folded into repeating domains that are similar to one another. The variable domains (shaded in *blue*) of the light and heavy chains (V_L and V_H) make up the antigen-binding sites, while the constant domains 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 have an important role in holding the chains together in the antibody molecule: V_L binds to V_H, C_L binds to C_H1, and so on (see Figure 24–34).

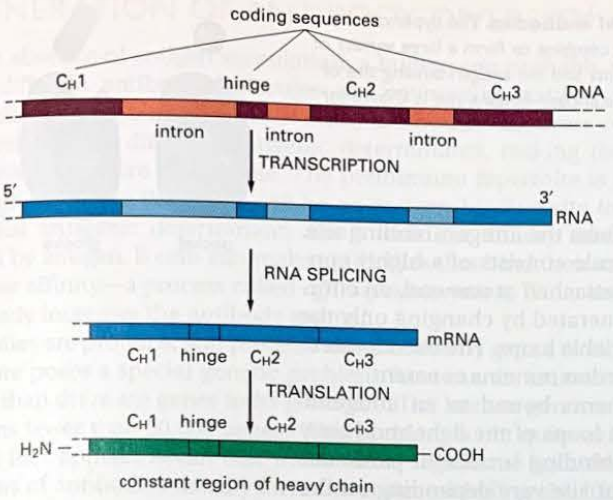


Figure 24–33 The organization of the DNA sequences that encode the constant region of an antibody heavy chain. The coding sequences (exons) for each domain and for the hinge region are separated by noncoding sequences (introns). The intron sequences are removed by splicing the primary RNA transcripts 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 7). The DNA and RNA sequences that encode the variable region of the heavy chain are not shown.

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. This hypothesis is supported by the finding that each domain of the constant region of a heavy chain is encoded by a separate coding sequence (exon) (Figure 24–33).

An Antigen-Binding Site Is Constructed From Hypervariable Loops

A number of fragments of antibodies, as well as 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 24–34, each Ig domain has a very similar three-dimensional structure based on what is called the *immunoglobulin fold,* which consists of a sandwich of two β sheets held together by a disulfide bond. We shall see later that 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 24–34). The hypervariable loops of both the light and

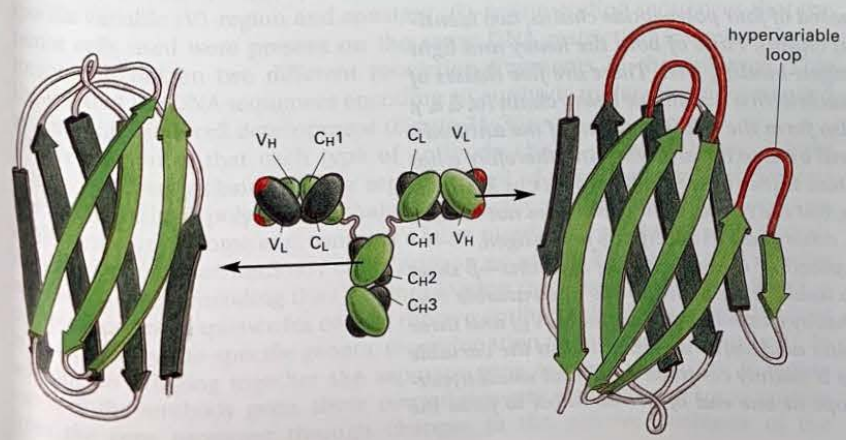


Figure 24–34 The folded structure of an IgG antibody molecule, based on x-ray crystallography studies. The structure of the whole protein is shown in the middle, while the structure of a constant domain is shown on the left and of a variable domain on the right. Both domains consist of two β sheets, 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.

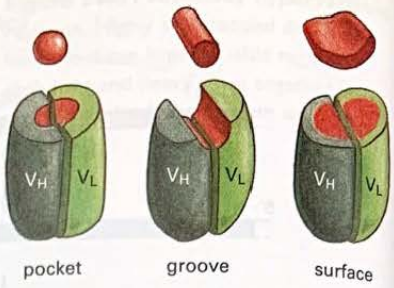


Figure 24–35 Antigen-binding sites of antibodies. The hypervariable loops of different V_L and V_H domains can combine to form a large variety of binding surfaces. The antigenic determinants and the antigen-binding site of the antibodies are shown in *red*. Only one antigen-binding site is shown for each antibody.

heavy variable domains are clustered together to form the antigen-binding site. Because the variable region of an antibody molecule consists of a highly conserved rigid framework, with hypervariable loops attached at one end, an enormous diversity of antigen-binding sites can be generated by changing only the lengths and amino acid sequences of the hypervariable loops. 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 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 24–35). 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 better fit the ligand.

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 cell to kill the invading pathogens. A typical antibody molecule is Y-shaped, with two identical antigen-binding sites at the tips of the Y and binding sites for complement components and/or 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 costimulatory 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 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, but the type of light chain does not seem to influence the properties of the antibody, other than its specificity for antigen.

Each light and heavy chain is composed of a number of Ig domains—β sheet structures containing 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 three or four constant (C_H) domains. The amino acid sequence variation in the variable domains of both light and heavy chains is mainly confined to several small hypervariable regions, which protrude as loops at one end of the domains to form the antigen-binding site.

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 antibody repertoire*. Moreover, the antigen-binding sites of many antibodies can cross-react with a variety of related but different antigenic determinants, making the antibody defense force even more formidable. The preimmune repertoire 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. After repeated stimulation by antigen, B cells can make antibodies that bind their antigen with much higher affinity—a process called *affinity maturation*. Thus, antigen stimulation greatly increases the antibody arsenal.

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 fewer than 50,000 genes.) This problem is not quite as formidable as it might first appear. Recall that the variable regions of both the light and heavy chains of antibodies usually 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, the mammalian immune system has evolved unique genetic mechanisms that enable it to generate an almost unlimited number of different light and heavy chains in a remarkably economical way, by joining separate *gene segments* together before they are transcribed. Birds and fish use very different strategies for diversifying antibodies, and even sheep and rabbits use somewhat different strategies from mice and humans. We shall confine our discussion to the mechanisms used by mice and humans.

We begin this section by discussing the mechanisms that B cells use to produce antibodies with an enormous diversity of antigen-binding sites. We then consider how a B cell can alter the tail region of the antibody it makes, while keeping the antigen-binding site unchanged. This ability allows the B cell to switch from making membrane-bound antibody to making secreted antibody, or from making one class of antibody to making another, all without changing the antigen-specificity of the antibody.

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

The first direct evidence that DNA is rearranged during B cell development came in the 1970s from experiments in which molecular biologists compared DNA from early mouse embryos, which do not make antibodies, with the DNA of a mouse B cell tumor, which makes a single species of antibody molecule. The specific variable (V)-region and constant (C)-region coding sequences that the tumor cells used were present on the same DNA restriction fragment in the tumor cells but on two different restriction fragments in the embryos. This showed that the DNA sequences encoding an antibody molecule are rearranged at some stage in B cell development (Figure 24–36).

We now know that each type of antibody chain—κ light chains, λ light chains, and heavy chains—has a separate pool of **gene segments** and exons from which a single polypeptide chain is eventually synthesized. Each pool is on a different chromosome and contains a large number of gene segments encoding the V region of an antibody chain and, as we saw in Figure 24–33, a smaller number of exons encoding the C region. During the development of a B cell, 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 and the C-region exons of the antibody gene, these rearrangements also activate transcription from the gene promoter through changes in the relative positions of the

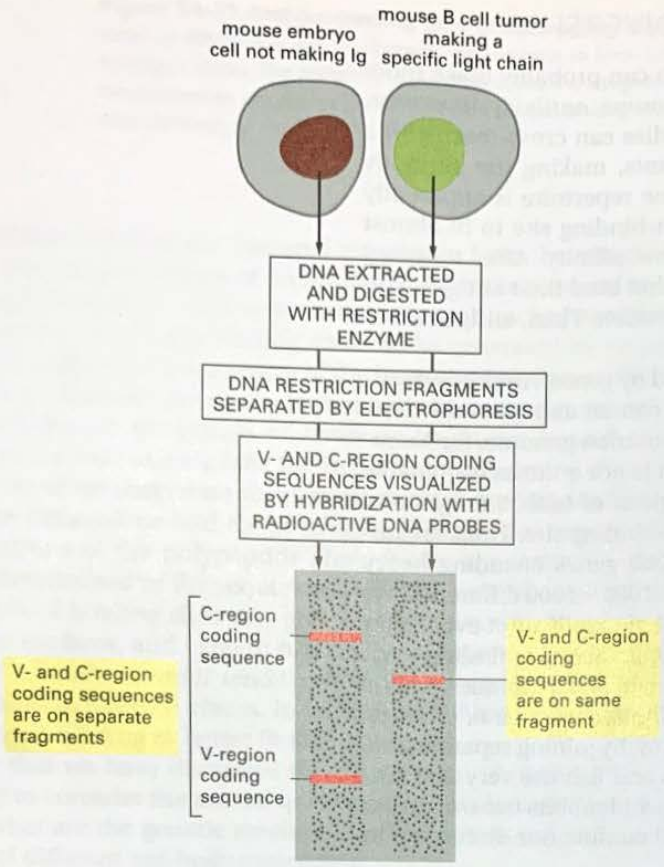


Figure 24–36 Drawing of an experiment that directly demonstrates that DNA is rearranged during B cell development. The B cell tumor arose from a single B cell and therefore makes a single species of antibody molecule. The two radioactive DNA probes used are specific for the DNA sequences encoding the C region and the V region of the light chain that the tumor cells make.

enhancers and silencers acting on the promoter. Thus, a complete antibody chain can be synthesized only after the DNA has been rearranged. As we shall see, the process of joining gene segments contributes to the diversity of antigen-binding sites in several ways.

Each Variable Region Is Encoded by More Than One Gene Segment

When genomic DNA sequences encoding V and C regions were first analyzed, it was found that a single region of DNA encodes the C region of an antibody chain (see Figure 24–33), but two or more regions of DNA have to be assembled to encode each 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 24–23), which is encoded elsewhere in the genome). Figure 24–37 illustrates the genetic mechanisms involved in producing a human κ light-chain polypeptide from a C-region exon and separate *V* and *J* gene segments.

Each heavy-chain V region is encoded by a DNA sequence assembled from three gene segments—a *V* segment, a *J* segment, and a *diversity segment*, or ***D* gene segment**. Figure 24–38 shows the number and organization of the gene segments used in making human heavy chains.

The large number of inherited *V*, *J*, and *D* gene segments available for encoding antibody chains makes a substantial contribution on its own to antibody diversity, but 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 gene-segment pool, for example, can be joined to any of the 5 *J* segments (see Figure 24–37), so that at least 200 (40 × 5) different κ-chain V regions can be encoded by this pool. Similarly, any of the 51 *V* segments in the human heavy-chain pool can be joined to any of the 6 *J* segments and any of the 27 *D* segments to encode at least 8262 (51 × 6 × 27) different heavy-chain V regions.

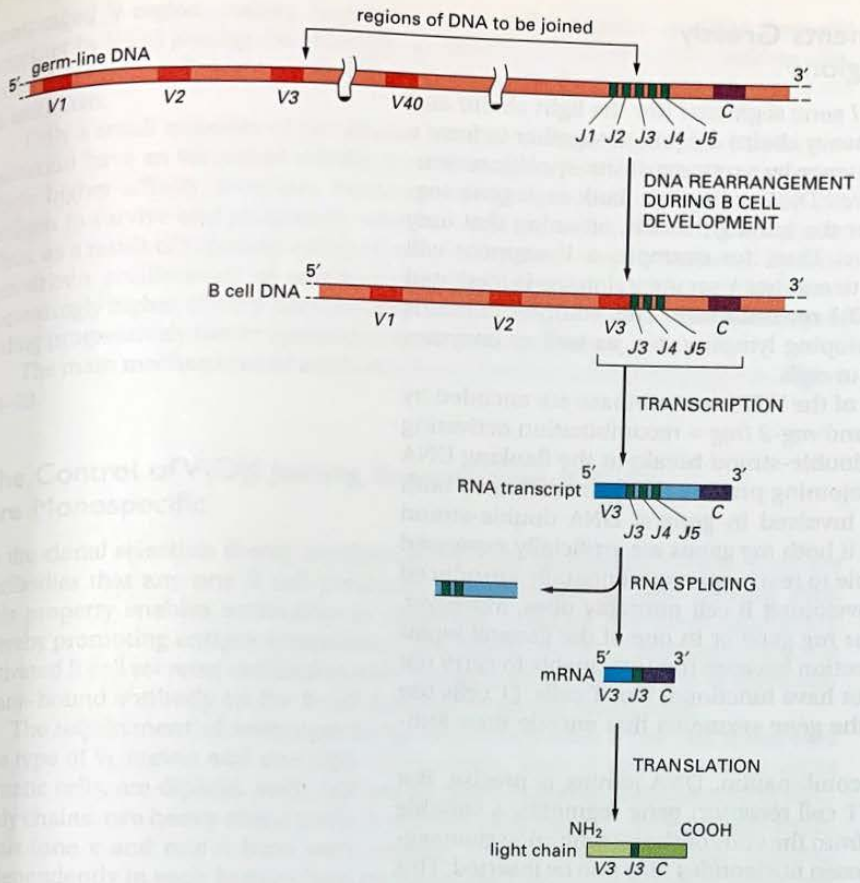


Figure 24–37 The *V–J* joining process involved in making a human κ light chain. In the "germ-line" DNA (where the antibody genes are not being expressed and are therefore not rearranged), the cluster of five *J* gene segments is separated from the *C*-region exon by a short intron and from the 40 *V* gene segments by thousands of nucleotide pairs. During the development of a B cell, the 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 exon) and then removed by RNA splicing to generate mRNA molecules in which the *V3*, *J3*, and *C* sequences are contiguous. 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 the *V–J* segment junction coincides with the third hypervariable region of the light chain, which is the most variable part of the V region.

The combinatorial diversification resulting from the assembly of different combinations of inherited *V*, *J*, and *D* gene segments just discussed is an important mechanism for diversifying the antigen-binding sites of antibodies. By this mechanism alone, a human can produce 287 different V_L regions (200 κ and 116 λ) and 8262 different V_H regions. In principle, these could then be combined to make about 2.6×10^6 (316×8262) 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 it much greater than the total number of B cells (about 10^{12}) in a human.

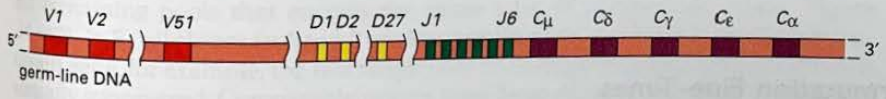


Figure 24–38 The human heavy-chain gene-segment pool. There are 51 *V* segments, 27 *D* segments, 6 *J* segments, and an ordered cluster of *C*-region exons, each cluster encoding a different class of heavy chain. The *D* segment (and part of the *J* segment) encodes amino acids in the third hypervariable region, which is the most variable part of the V region. The figure is not drawn to scale: the total length of the heavy chain locus is over 2 megabases. Moreover, many details are omitted. For instance, each *C* region is encoded by multiple exons (see Figure 24–33); there are four clusters of C_γ-region exons ($C_{\gamma 1}$, $C_{\gamma 2}$, $C_{\gamma 3}$, and $C_{\gamma 4}$), and the V_H gene segments are clustered on the chromosome in groups of homologous families. The genetic mechanisms involved in producing a heavy chain are the same as those shown in Figure 24–37 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.

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

During B cell development, the *V* and *J* gene segments (for the light chain) and the *V, D,* and *J* gene segments (for the heavy chain) are joined together to form a functional V_L- or V_H-region coding sequence by a process of site-specific recombination called **V(D)J joining**. Conserved DNA 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 *V* segment will always join to a *J* or *D* segment but not to another *V* segment. Joining is mediated by an enzyme complex called the **V(D)J recombinase**. This complex contains two proteins that are specific to developing lymphocytes, as well as enzymes that help repair damaged DNA in all our cells.

The lymphocyte-specific proteins of the V(D)J recombinase are encoded by two closely linked genes called *rag-1* and *rag-2 (rag = r*ecombination *a*ctivating genes). The **RAG proteins** introduce double-strand breaks at the flanking DNA sequences, and this is followed by a rejoining process that is mediated by both the RAG proteins and the enzymes involved in general DNA double-strand repair (discussed in Chapter 5). Thus, if both *rag* genes are artificially expressed in a fibroblast, the fibroblast is now able to rearrange experimentally introduced antibody gene segments just as a developing B cell normally does. Moreover, individuals who are deficient in either *rag* gene or in one of the general repair enzymes are highly susceptible to infection because they are unable to carry out *V(D)J* joining and consequently do not have functional B or T cells. (T cells use the same recombinase to assemble the gene segments that encode their antigen-specific receptors.)

In most cases of site-specific recombination, DNA joining is precise. But during the joining of antibody (and T cell receptor) gene segments, 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 recombination, specifically in the third hypervariable region. This increased diversification comes at a price, however. In many cases, it will result in a shift in the reading frame that produces 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 are stimulated to re-express the RAG proteins and undergo a second round of *V(D)J* rearrangements, thereby changing the specificity of the cell-surface antibody they make—a process referred to as **receptor editing**. Self-reactive B cells that fail to change their specificity in this way are eliminated through the process of clonal deletion (see Figure 24–13).

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 specifically in both heavy-chain and light-chain V-region coding sequences. The mutations occur long after the coding regions have been assembled, when B cells are stimulated by antigen and helper T cells to generate memory cells in a lymphoid follicle in a peripheral lymphoid organ (see Figure 24–16). They occur at the rate of about one per V-region coding sequence per cell generation. Because this is about a million times greater than the spontaneous mutation rate in other genes, the process is called **somatic hypermutation**. The molecular mechanism is still uncertain, but it is believed to involve some form of error-prone DNA repair process targeted to the

rearranged V-region coding sequence by specific regions of DNA brought together by V(D)J joining. Surprisingly, an enzyme involved in RNA editing (discussed in Chapter 7) is required, but its function in the hypermutation process is unknown.

Only a small minority of the altered antigen receptors generated by hypermutation have an increased affinity for the antigen. The few B cells expressing these higher-affinity receptors, however, are preferentially stimulated by the antigen to survive and proliferate, whereas most other B cells die by apoptosis. Thus, as a result of repeated cycles of somatic hypermutation, followed by antigen-driven proliferation of selected clones of memory B cells, antibodies of increasingly higher affinity become abundant during an immune response, providing progressively better protection against the pathogen.

The main mechanisms of antibody diversification are summarized in Figure 24–39.

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

As the clonal selection theory predicts, 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 24–19). It also means that an activated B cell secretes antibodies with the same specificity as that of the membrane-bound antibody on the B cell that was originally stimulated.

The requirement of monospecificity means that each B cell can make only one type of V_L region and one type of V_H region. Since B cells, like most other somatic cells, are diploid, each cell has six gene-segment pools encoding antibody chains: two heavy-chain pools (one from each parent) and four light-chain pools (one κ and one λ from each parent). If DNA rearrangements occurred independently in each heavy-chain pool and each light-chain pool, a single 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 gene-segment pools: one of the two heavy-chain pools and one of the four light-chain pools. Thus, each B cell must choose not only between its κ and λ light-chain pools, but also between its maternal and paternal light-chain and heavy-chain pools. This second choice is called **allelic exclusion**, and it also occurs in the expression of genes that encode T cell receptors. For most other proteins that are encoded by autosomal genes, both maternal and paternal genes 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* joining process. A functional rearrangement in one gene-segment pool suppresses rearrangements in all remaining pools that encode the same type of polypeptide chain (Figure 24–40). In B cell clones isolated from transgenic mice expressing a rearranged μ-chain gene, for example, the rearrangement of 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 are involved in the suppression of 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 pools of gene segments encoding light chain variable regions. Having two

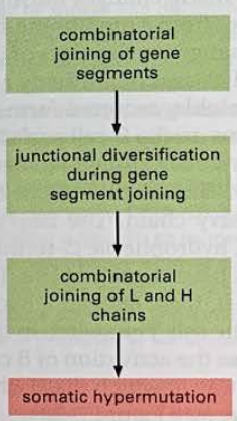


Figure 24–39 The four main mechanisms of antibody diversification. Those shaded in *green* occur during B cell development in the bone marrow (or fetal liver), while the mechanism shaded in *red* occurs when B cells are stimulated by foreign antigen and helper T cells in peripheral lymphoid organs to produce memory B cells.

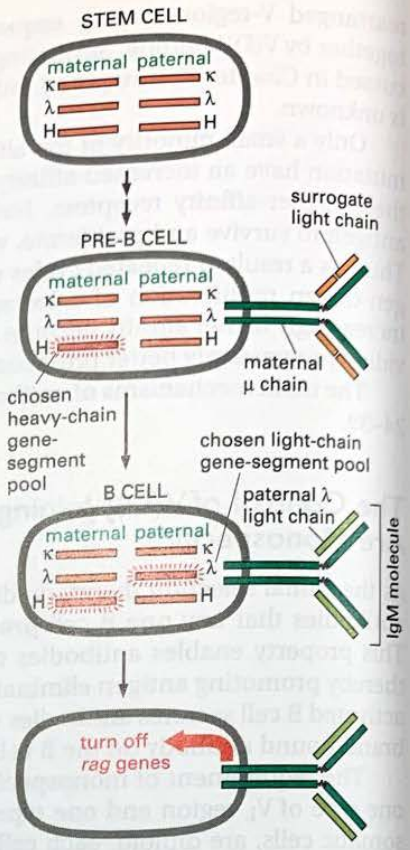


Figure 24–40 Antibody gene-pool selection in B cell development. To produce antibodies with only one type of antigen-binding site, a developing B cell must use only one L-chain gene-segment pool and one H-chain pool. Although the choice between maternal and paternal pools is thought to be random, the 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 heavy-chain pool. In this pool, *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 heavy chains made) leads to their expression on the cell surface in association with surrogate light chains. The cell now shuts down all further rearrangements of V_H-region-encoding gene segments and initiates V_L rearrangement. Although not shown, V_L rearrangement usually occurs first in a κ gene-segment pool, and only if that fails does it occur in the other κ pool or in the λ pools. 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* joining, by turning off the expression of the *rag-1* and *rag-2* genes. If a developing B cell makes a receptor that recognizes a self-antigen, it is stimulated to re-express the *rag* genes and undergo another round of *V(D)J* joining (called receptor editing), 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).

separate pools increases the chance that a pre-B cell that has successfully assembled a V_H-region coding sequence will go on to assemble successfully 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 24–22), 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.

When Activated by Antigen, a B Cell Switches From Making a Membrane-Bound Antibody to Making a Secreted Form of the Same Antibody

We now turn from the genetic mechanisms that determine the antigen-binding site of an antibody to those that determine its biological properties—that is, those that determine what form of heavy-chain constant region is synthesized. The choice of the particular gene segments that encode the antigen-binding site is a commitment for the life of a B cell and its progeny, but the type of C_H region that is made changes during B cell development. The changes are of two types: changes from a membrane-bound form to a secreted form of the same C_H region and changes in the class of the C_H region made.

All classes of antibody can be made in a membrane-bound form, as well as in a soluble, secreted form. The membrane-bound form serves as an antigen receptor on the B cell surface, while the soluble form is made only after the cell is activated by antigen to become an antibody-secreting effector cell (see Figure 24–17). The sole difference between the two forms resides in the C-terminus of the heavy chain. The heavy chains of membrane-bound antibody molecules have a 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 7–93).

B Cells Can Switch the Class of Antibody They Make

During B cell development, many B cells switch from making one class of antibody to making another—a process called **class switching**. 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 switch and make both IgM and IgD molecules as membrane-bound antigen receptors, both with the same antigen-binding sites (see Figure 24–22). On stimulation by antigen and helper T cells, some of these cells are activated to secrete IgM antibodies, which dominate the primary antibody response. Later in the immune response, the combination of antigen and the cytokines that helper T cells secrete induce many B cells to switch to making IgG, IgE, or IgA antibodies. These cells generate both memory cells that express the corresponding classes of antibody molecules on their surface and effector cells that secrete the antibodies. The IgG, IgE, and IgA molecules are collectively referred to as *secondary* classes of antibodies, both because they are produced only after antigen stimulation and because they dominate secondary antibody responses. As we saw earlier, each different class of antibody is specialized to attack microbes 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 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 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 (Figure 24–41). Switch recombination differs from *V(D)J* joining in several ways: (1) it involves noncoding sequences only and therefore leaves the coding sequence unaffected; (2) it uses different flanking recombination sequences and different enzymes; (3) it happens after antigen stimulation; and (4) it is dependent on helper T cells.

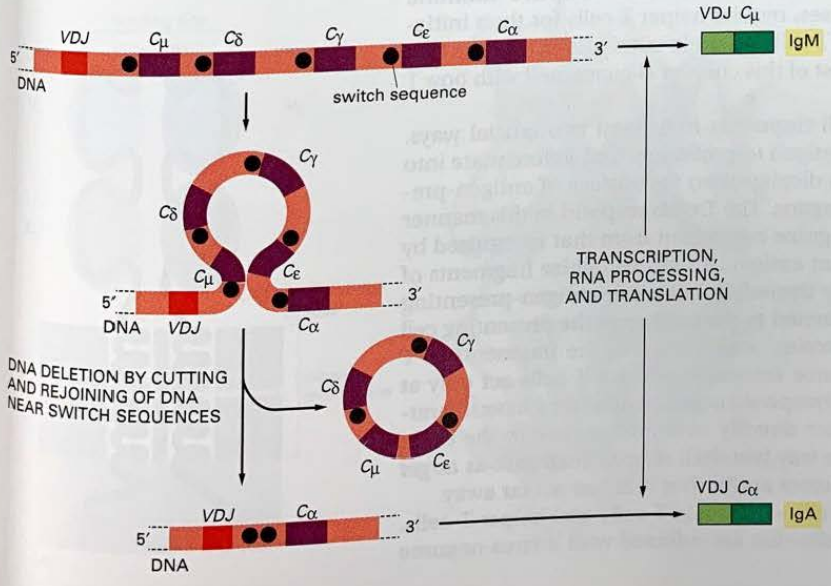


Figure 24–41 An example of the DNA rearrangement that occurs in class switch recombination. A B cell making an IgM antibody from an assembled *VDJ* DNA sequence is stimulated by antigen and the cytokines made by helper T cells to switch to making an IgA antibody. In the process, it deletes the DNA between the *VDJ* sequence and the C_α-coding sequence. Specific DNA sequences *(switch sequences)* located upstream of each C_H-coding sequence recombine with each other to delete the intervening DNA. Class switch recombination is thought to be mediated by a *switch recombinase*, which is directed to the appropriate switch sequences when these become accessible under the influence of cytokines, as we discuss later.

Summary

Antibodies are produced from three pools of gene segments and exons. One pool encodes κ light chains, one encodes λ light chains, and and one encodes heavy chains. In each pool, separate gene segments that code for different parts of the variable region of the light or heavy chains are brought together by site-specific recombination during B cell development. The light-chain pools contain one or more constant- (C-) region exons and sets of variable (V) and joining (J) gene segments. The heavy-chain pool contains sets of C-region exons and sets of V, diversity (D), and J gene segments.

To make an antibody molecule, 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 cotranscribed with the appropriate C-region sequence to produce an RNA molecule that codes for the complete polypeptide chain. Cells making functional heavy and light chains turn off the V(D)J joining process to ensure that each B cell makes only one species of antigen-binding site.

By randomly combining inherited gene segments that code for V_L and V_H regions, 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 pair to form antibodies with millions of different antigen-binding sites. This number is enormously increased by the loss and gain of nucleotides at the site of gene-segment joining, as well as by somatic mutations that occur with very high frequency in the assembled V-region coding sequences after stimulation by antigen and helper T cells.

All B cells initially make IgM antibodies, and most then make IgD as well. Later many switch and make antibodies of other classes but with the same antigen-binding site as the original IgM and IgD antibodies. Such class switching depends on antigen stimulation and helper T cells, and it allows the same antigen-binding sites to be distributed among antibodies with varied biological properties.

T CELLS AND MHC PROTEINS

The diverse responses of T cells are collectively called *cell-mediated immune reactions*. This is to distinguish them from antibody responses, which, of course, also depend on cells (B cells). Like antibody responses, T cell 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 antibody responses, require helper T cells for their initiation. Most importantly, unlike B cells, T cells can help eliminate pathogens that reside 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 in peripheral lymphoid organs. The T cells respond in this manner because the form of antigen they recognize is different from that recognized by B cells. Whereas B cells recognize intact antigen, T cells recognize fragments of protein antigens that have been partly degraded inside the antigen-presenting cell. The peptide fragments are then carried to the surface of the presenting cell on special molecules called *MHC proteins*, which present the fragments to T cells. 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. They interact directly with another cell in the body, which they either kill or signal in some way (we shall refer to such cells as *target cells*). Activated B cells, by contrast, secrete antibodies that can act far away.

There are two main classes of T cells—cytotoxic T cells and helper T cells. Effector *cytotoxic T cells* directly kill cells that are infected with a virus or some

other intracellular pathogen. Effector *helper T cells*, by contrast, help stimulate the responses of other cells—mainly macrophages, B cells, and cytotoxic T cells.

In this section, we describe these two 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 Are Antibodylike Heterodimers

Because T cell responses depend on direct contact with an antigen-presenting cell or a target cell, the antigen receptors made by T cells, unlike antibodies made by B cells, exist only in membrane-bound form and are not secreted. For this reason, T cell receptors were difficult to isolate, and it was not until the 1980s that they were first identified biochemically. On both cytotoxic and helper T cells, the receptors are similar to antibodies. They are composed of two disulfide-linked polypeptide chains (called α and β), each of which contains two Ig-like domains, one variable and one constant (Figure 24–42A). Moreover, the three-dimensional structure of the extracellular part of a T cell receptor has been determined by x-ray diffraction, and it looks very much like one arm of a Y-shaped antibody molecule (Figure 24–42B).

The pools of gene segments that encode the α and β chains are located on different chromosomes. Like antibody heavy-chain pools, the T cell receptor pools contain separate *V*, *D*, and *J* gene segments, which are brought together by site-specific recombination during T cell development in the thymus. With one exception, all the mechanisms used by B cells to generate antibody diversity are also used by T cells to generate T cell receptor diversity. Indeed, the same *V(D)J* recombinase is used, including the RAG proteins discussed earlier. The mechanism that does not operate in T cell receptor diversification is antigen-driven somatic hypermutation. Thus, the affinity of the receptors remains low (K_a ~ 10^5–10^7 liters/mole), even late in an immune response. We discuss later how various co-receptors and cell–cell adhesion mechanisms greatly strengthen the binding of a T cell to an antigen-presenting cell or a target cell, helping to compensate for the low affinity of the T cell receptors.

A small minority of T cells, instead of making α and β chains, make a different but related type of receptor heterodimer, composed of γ and δ chains. These

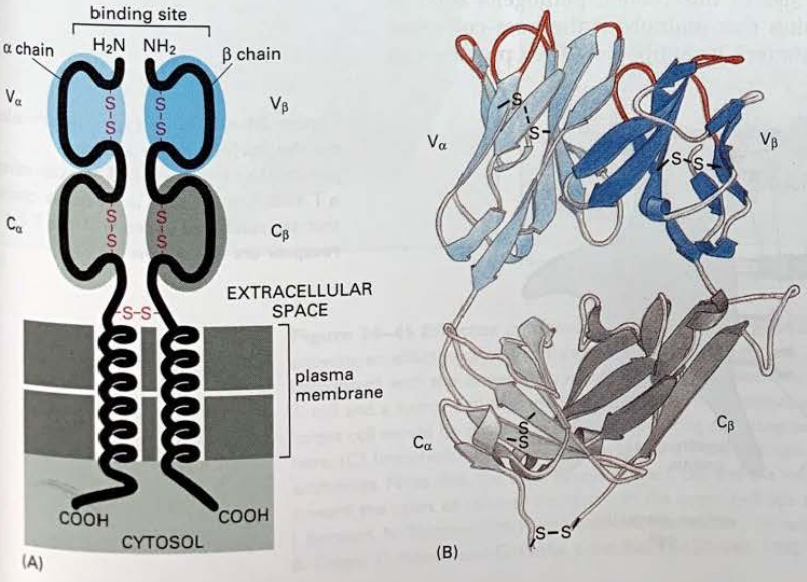


Figure 24–42 A T cell receptor heterodimer. (A) Schematic drawing showing that the receptor is composed of an α and a β polypeptide chain. Each chain is about 280 amino acids long and has a large extracellular part that is folded into two Ig-like domains—one variable (V) and one constant (C). The antigen-binding site is formed by a V_α and a V_β domain (shaded in *blue*). Unlike antibodies, which have two binding sites for antigen, T cell receptors 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 T cell receptors 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 T cell receptor. 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.)

cells arise early in development and are found mainly in epithelia (in the skin and gut, for example). Their functions are uncertain, and we shall not discuss them further.

As with antigen receptors on B cells, the T cell receptors 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. We discuss these proteins in more detail later. First, however, we need to consider how cytotoxic and helper T cells function and the special ways in which they recognize foreign antigen.

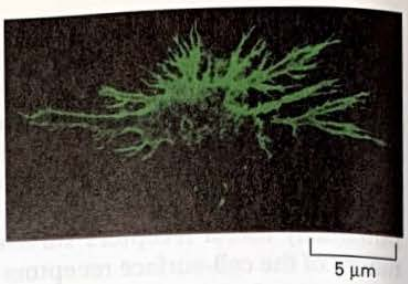


Figure 24–43 Immunofluorescence micrograph of a dendritic cell in culture. These crucial antigen-presenting cells derive their name from their long processes, or "dendrites." The cell has been labelled with a monoclonal antibody that recognizes a surface antigen on these cells. (Courtesy of David Katz.)

Antigen-Presenting Cells Activate T Cells

Before cytotoxic or helper T cells can kill or help their target cells, respectively, they must be activated to proliferate and differentiate into effector cells. This activation occurs in peripheral lymphoid organs on the surface of **antigen-presenting cells** that display foreign antigen complexed with MHC proteins on their surface.

There are three main types of antigen-presenting cells in peripheral lymphoid organs that can activate T cells—dendritic cells, macrophages, and B cells. The most potent of these are **dendritic cells** (Figure 24–43), whose only known function is to present foreign antigens to T cells. Immature dendritic cells are located in tissues throughout the body, including the skin, gut, and respiratory tract. When they encounter invading microbes at these sites, they endocytose the pathogens or their products and carry them via the lymph to local lymph nodes or gut-associated lymphoid organs. The encounter with a pathogen induces the dendritic cell to mature from an antigen-capturing cell to an antigen-presenting cell that can activate T cells (see Figure 24–5).

Antigen-presenting cells display three types of protein molecules on their surface that have a role in activating a T cell to become an effector cell: (1) *MHC proteins,* which present foreign antigen to the T cell receptor, (2) *costimulatory 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 (Figure 24–44).

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

Effector Cytotoxic T Cells Induce Infected Target Cells to Kill Themselves

Cytotoxic T cells provide protection against intracellular pathogens such as viruses and some bacteria and parasites that multiply in the host–cell cytoplasm, where they are sheltered from attack by antibodies. They provide this

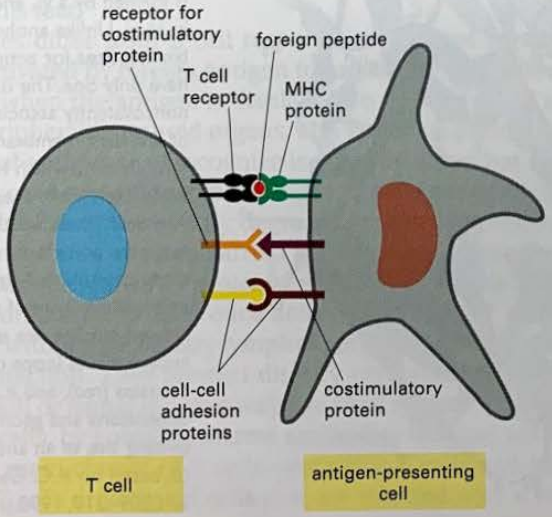


Figure 24–44 Three types of proteins on the surface of an antigen-presenting cell involved in activating a T cell. The invariant polypeptide chains that are stably associated with the T cell receptor are not shown.

protection by killing the infected cell before the microbes can proliferate and escape from the infected cell to infect neighboring cells.

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 infected with the same pathogen. When the effector T cell recognizes a microbial antigen on the surface of an infected target cell, it focuses its secretory apparatus on the target. We can observe this behavior by studying effector T cells bound to their targets: when labeled with anti-tubulin antibodies, the T cell centrosome is seen to be oriented toward the point of contact with the target cell (Figure 24–45). Moreover, antibody labeling shows that talin and other proteins that help link cell-surface receptors to cortical actin filaments are concentrated in the cortex of the T cell at the contact site. The aggregation of T cell receptors at the contact site apparently leads to a local alteration in the actin filaments in the cell cortex. A microtubule-dependent mechanism then moves the centrosome and its associated Golgi apparatus toward the contact site, focusing the killing machinery on the target cell. A similar cytoskeletal polarization is seen when an effector helper T cell interacts functionally with a target cell.

Once bound to its target cell, a cytotoxic T cell can employ at least two strategies to kill the target, both of which operate by inducing the target cell to kill itself by undergoing apoptosis (discussed in Chapter 17). 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 25–42) and polymerizes in the target cell plasma membrane to form transmembrane channels. Perforin is stored in secretory vesicles of the cytotoxic T cell and is released by local exocytosis at the point of contact with the target cell. 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

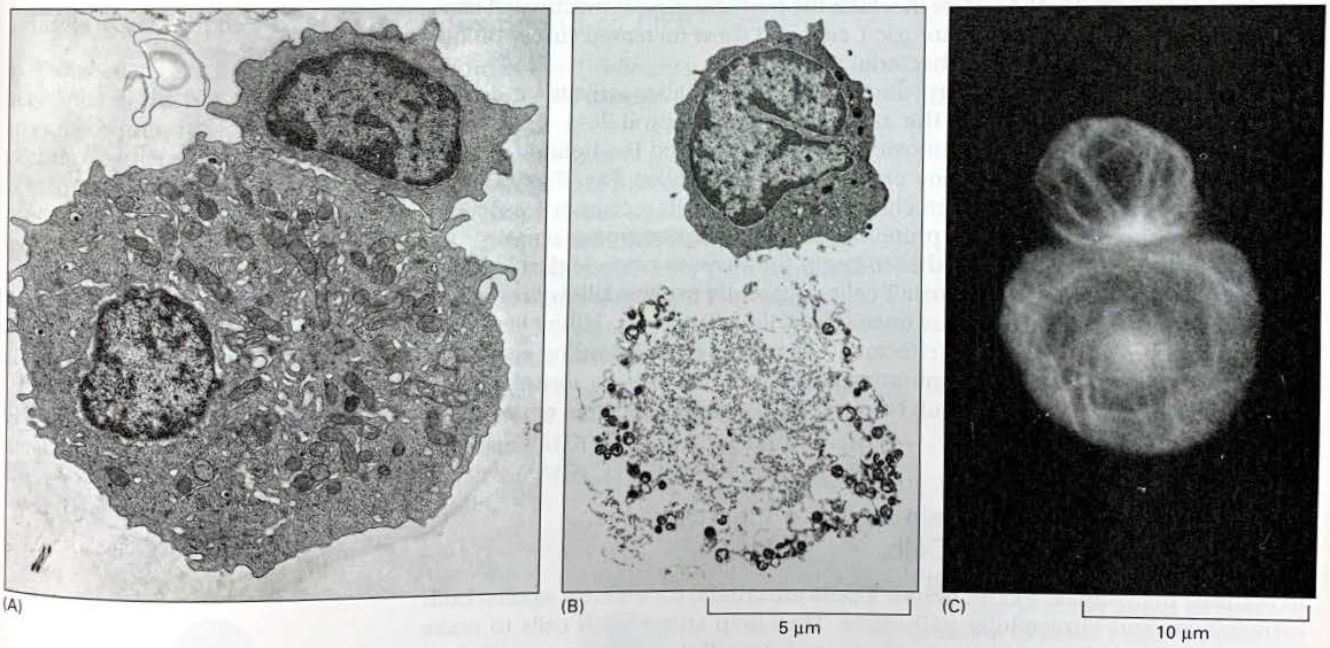


Figure 24–45 Effector cytotoxic T cells killing target cells in culture. (A) Electron micrograph showing an effector cytotoxic T cell binding to the target cell. The cytotoxic T cells were obtained from mice immunized with the target cells, which are foreign tumor cells. (B) Electron micrograph showing a cytotoxic T cell and a tumor cell that the T cell has killed. In an animal, as opposed to in a tissue culture dish, the killed target cell would be phagocytosed by neighboring cells long before it disintegrated in the way that it has here. (C) Immunofluorescence micrograph of a T cell and tumor cell after staining with anti-tubulin antibodies. Note that the centrosome in the T cell and the microtubules radiating from it are oriented toward the point of cell–cell contact with the target cell. See also Figure 16–97A. (A and B, from D. Zagury, J. Bernard, N. Thierness, M. Feldman, and G. Berke, *Eur. J. Immunol.* 5:818–822, 1975; C, reproduced from B. Geiger, D. Rosen, and G. Berke, *J. Cell Biol.* 95:137–143, 1982. © The Rockefeller University Press.)

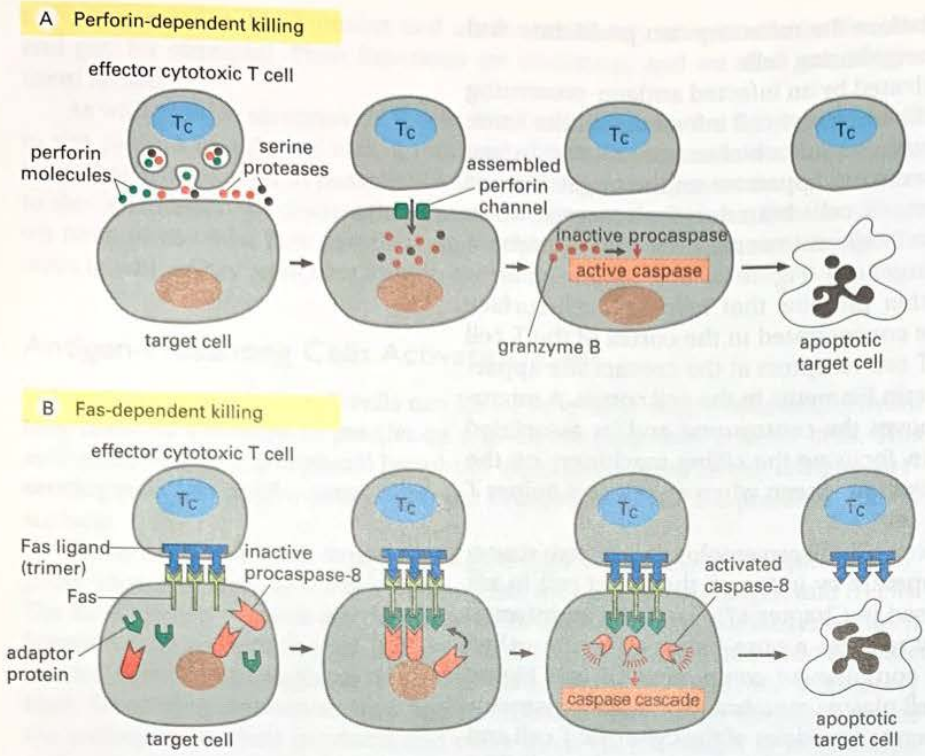


Figure 24–46 Two strategies by which effector cytotoxic T cells kill their target cells. (A) The cytotoxic T cell (T_C) releases perforin and proteolytic enzymes onto the surface of an infected target cell by localized exocytosis. The high concentration of Ca^{2+} in the extracellular fluid causes the perforin to assemble into transmembrane channels, which are thought to allow the proteolytic enzymes to enter the target cell cytosol. One of the enzymes, granzyme B, cleaves and activates specific procaspases, thereby triggering the proteolytic caspase cascade leading to apoptosis. (B) The homotrimeric Fas ligand on the cytotoxic T cell surface binds to and activates Fas receptor 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 then cleave one another to produce active caspase-8 molecules that initiate the proteolytic caspase cascade leading to apoptosis.

granzyme B, cleaves, and thereby activates, one or more members of the *caspase family* of proteases that mediate apoptosis. These caspases then activate other caspases, producing a proteolytic cascade that helps kill the cell (discussed in Chapter 17) (Figure 24–46A). Mice in which the perforin gene is inactivated cannot generate microbe-specific cytotoxic T cells and show increased susceptibility to certain viral and intracellular bacterial infections.

In the second killing strategy, the cytotoxic T cell also activates a death-inducing caspase cascade in the target cell but does it 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 the complex via an adaptor protein. The recruited procaspase-8 molecules cross-cleave and activate each other to begin the caspase cascade that leads to apoptosis (Figure 24–46B). Cytotoxic T cells apparently use this killing strategy to help contain an immune response once it is well underway, by killing excessive effector lymphocytes, especially effector T cells: if the gene encoding either Fas or Fas ligand is inactivated by mutation, effector lymphocytes accumulate in vast numbers in the spleen and lymph nodes, which become enormously enlarged.

Effector Helper T Cells Help Activate Macrophages, B Cells, and Cytotoxic T Cells

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 antibodies that help inactivate or eliminate extracellular pathogens and their toxic products. They activate macrophages to destroy any intracellular pathogen multiplying within the macrophage's phagosomes, and they help activate cytotoxic T cells to kill infected target cells.

Once a helper T cell has been activated by an antigen-presenting cell to become an effector cell, it can then help activate other cells. It does this both by secreting a variety of cytokines and by displaying costimulatory proteins on its surface. When activated by an antigen-presenting cell, a naïve helper T cell can differentiate into either of two distinct types of effector helper cell, called T_H1 and T_H2. *T_H1 cells* mainly help activate macrophages and cytotoxic T cells,

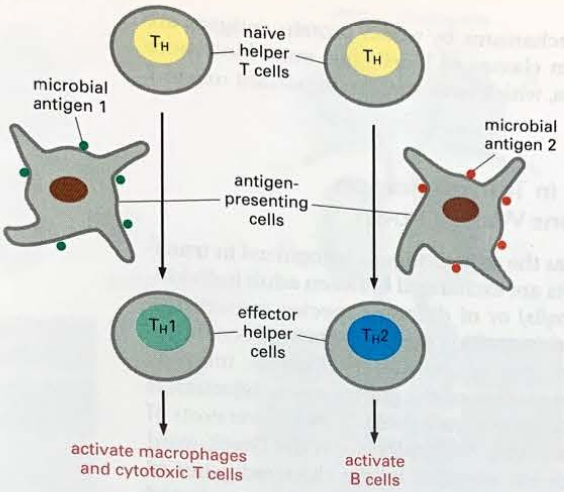


Figure 24–47 Differentiation of naïve helper T cells into either T_H1 or T_H2 effector helper cells in a peripheral lymphoid organ. The antigen-presenting cell and the characteristics of the pathogen that activated it mainly determine which type of effector helper cell develops.

whereas *T_H2 cells* mainly help activate B cells (Figure 24–47). 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.

Before discussing how helper T cells function to activate macrophages, cytotoxic T cells, or B cells, we need to consider the crucial role of MHC proteins in T cell responses.

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, usually a dendritic cell. The antigen is 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 in the antigen-presenting cell of **MHC proteins**, which bind these fragments, carry them to the cell surface, and present them there, along with a co-stimulatory signal, 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 a virus-infected cell in the case of a cytotoxic T cell.

MHC proteins are encoded by a large complex of genes called the **major histocompatibility complex (MHC)**. There are two main structurally and functionally distinct classes of MHC proteins: *class I MHC proteins*, which present foreign peptides to cytotoxic T cells, and *class II MHC proteins*, which present foreign peptides to helper cells (Figure 24–48).

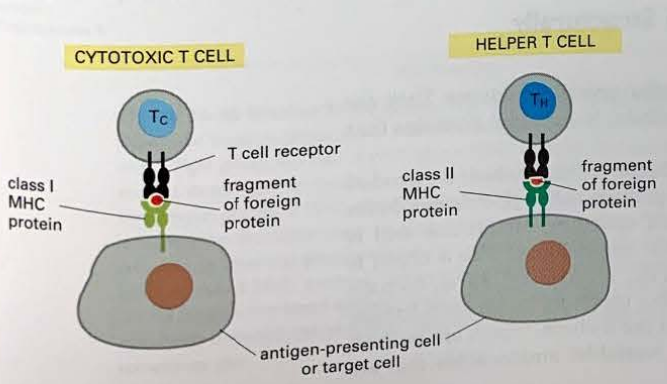


Figure 24–48 Recognition by T cells of foreign peptides bound to MHC proteins. Cytotoxic T cells recognize foreign peptides in association with class I MHC proteins, whereas helper T cells recognize foreign peptides in association with class II MHC proteins. In both cases, the peptide–MHC complexes are recognized on the surface of an antigen-presenting cell or a target cell.

Before examining the different mechanisms by which protein antigens are processed for display to the two main classes of T cells, we must look more closely at the MHC proteins themselves, which have such an important 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 *histo,* 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* 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 a long time. First, MHC proteins are overwhelmingly the preferred antigens 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 T cells respond to a typical viral antigen, more than 0.1% of them respond to a single foreign MHC antigen. Third, some of the genes that code for MHC proteins are the most *polymorphic* known in higher vertebrates. That is, within a species, there is an extraordinarily large number of *alleles* (alternative forms of the same gene) present (in some cases more than 200), without any one allele predominating. As each individual has at least 12 genes encoding MHC proteins (see later), it is very rare for two unrelated individuals to have an identical set of MHC proteins. This makes 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 solved only after it was 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 24–49A). The β_2-microglobulin does not span the membrane and is 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, 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 are

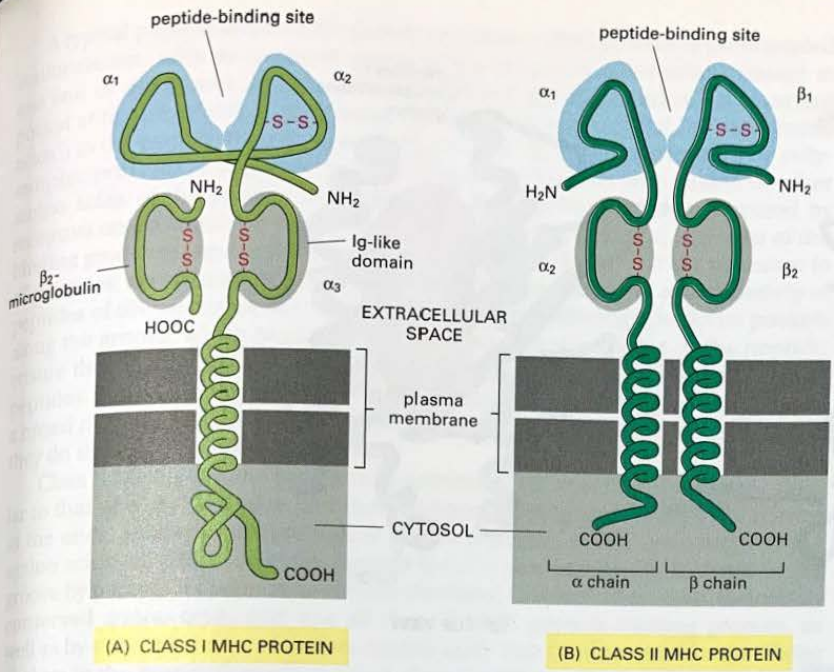


Figure 24–49 Class I and class II MHC proteins. (A) The α chain of the class I molecule has three extracellular domains, α_1, α_2 and α_3, encoded by separate exons. It is noncovalently associated with a smaller polypeptide chain, β_2-microglobulin, which is not encoded within the MHC. The α_3 domain 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*) interact to form a groove that binds peptide fragments of foreign proteins and presents them to T cells

recognized by T cells 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 24–49B). The two polymorphic domains bind a peptide and present it to helper 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 24–50, 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 *nonclassical class I MHC proteins*, which form dimers with β_2-microglobulin. These proteins are not polymorphic, but some of them present specific microbial antigens, including some lipids and glycolipids, to T cells. The functions of most of them, however, are unknown.

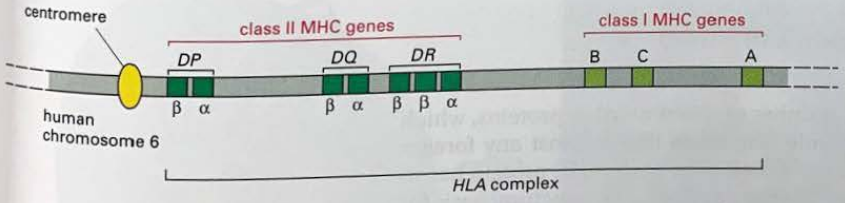
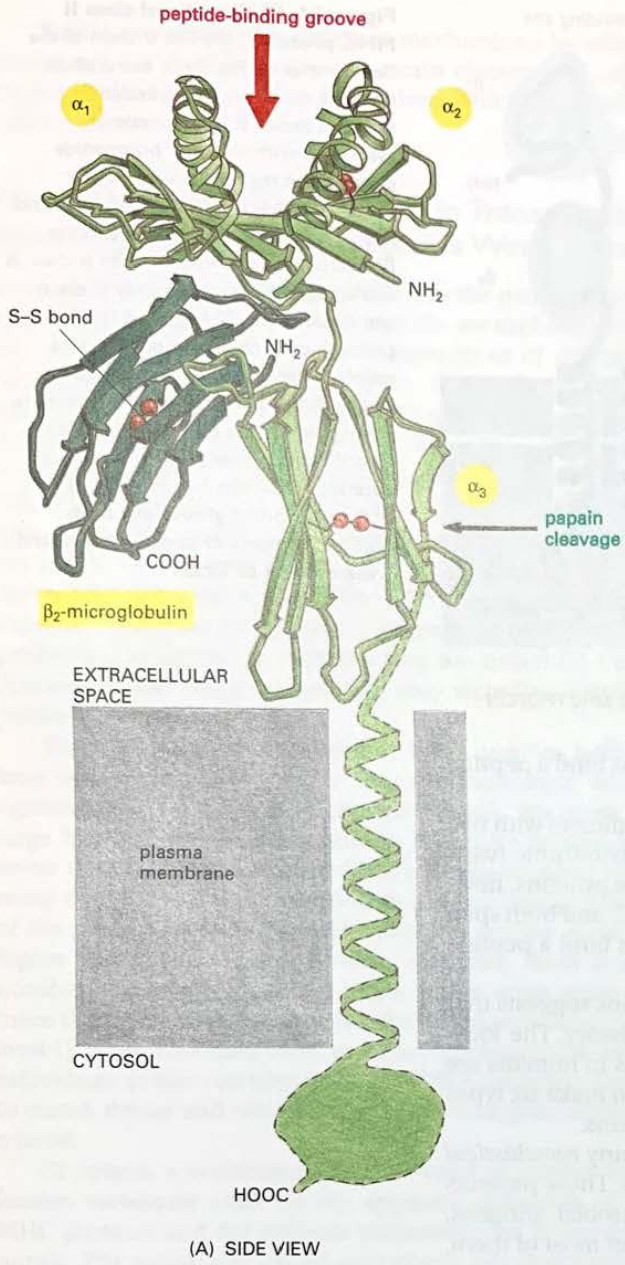


Figure 24–50 Human MHC genes. This simplified schematic drawing shows the location of the genes that encode the transmembrane subunits of class I *(light green)* and class II *(dark green)* MHC proteins. The genes shown encode three types of class I proteins (HLA-A, HLA-B, and HLA-C) and three types 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 increased because there are two DR β genes and because maternally encoded and paternally encoded polypeptide chains can sometimes pair.



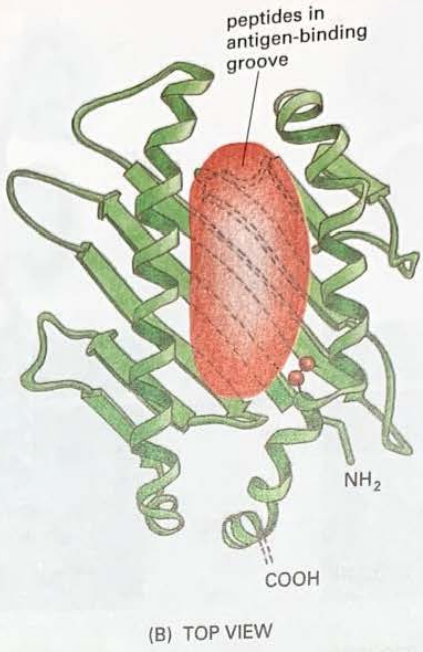


Figure 24–51 The three-dimensional structure of a human class I MHC protein as determined by x-ray diffraction analysis of crystals of the extracellular part of the molecule. The extracellular part of the protein was cleaved from the transmembrane segment by the proteolytic enzyme papain before crystallization. (A) Each of the two domains closest to the plasma membrane (α_3 and β_2-microglobulin) resembles a typical Ig domain (see Figure 24–34), 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, M.A. Saper, B. Samraoui, W.S. Bennett, J.L. Strominger, and D.C. Wiley, *Nature* 329:506–512, 1987.)

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

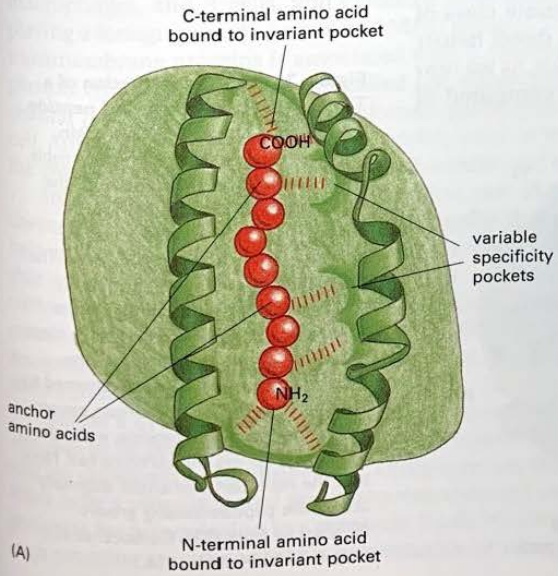
Any individual can make only a small number of different MHC proteins, which together must be able to present peptide fragments from almost any foreign protein to T cells. Thus, unlike an antibody molecule, each MHC protein has to be able to bind a very large number of different peptides. The structural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

As shown in Figure 24–51A, 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 in 1987, this groove contained bound peptides that had co-crystallized with the MHC protein (Figure 24–51B), 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 an invariant pocket at one end of the groove and its terminal carboxyl group bound to an invariant pocket at the other end of the groove. Other amino acids (called "anchor amino acids") in the peptide bind to "specificity pockets" in the groove formed by polymorphic portions of the MHC protein (Figure 24–52). The side chains of other amino acids of the peptide point outward, in a position to be recognized by receptors on cytotoxic T cells. Because the conserved pockets at the ends of the binding 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 diverse sequence. At the same time, the differing specificity pockets along the groove, which bind particular amino acid 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.

Class II MHC proteins have a three-dimensional structure that is very similar to that of class I proteins, but their antigen-binding groove does not narrow at the ends, so it can accommodate longer peptides, which are usually 13–17 amino acids long. Moreover, the peptide is not bound at its ends. It is held in the groove by parts of its peptide backbone that bind to invariant pockets formed by conserved amino acids that line all class II MHC peptide-binding grooves, as well as by the side chains of anchor amino acids that bind to variable specificity pockets in the groove (Figure 24–53). A class II MHC binding 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 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.

The way in which the T cell receptor recognizes a peptide fragment bound to an MHC protein is revealed by x-ray crystallographic analyses of complexes formed between a soluble receptor and a soluble MHC protein with peptide in its binding groove. (The soluble proteins for these experiments are produced by recombinant DNA technology.) In each case studied, the T cell receptor 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 24–54). Soluble MHC–peptide complexes are now widely used to detect T cells with a particular specificity; they are usually cross-linked into tetramers to increase their avidity for T cell receptors.



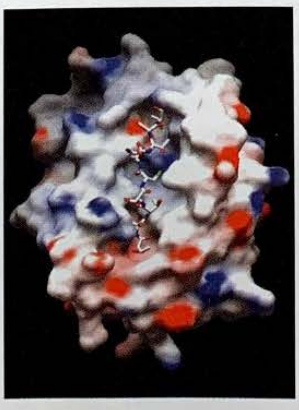

(B)

Figure 24–52 A peptide bound in the groove of a class I MHC protein. (A) Schematic drawing of a top view of the groove. The peptide backbone is shown as a string of *red* balls, each of which represents one of the nine amino acids of the peptide. The terminal amino and carboxyl groups of the peptide backbone bind to invariant pockets at the ends of the groove, while the side chains of several anchor amino acids of the peptide bind to variable specificity pockets in the groove. (B) The three-dimensional structure of a peptide bound in the groove of a class I MHC protein, as determined by x-ray diffraction. (B, courtesy of Paul Travers.)

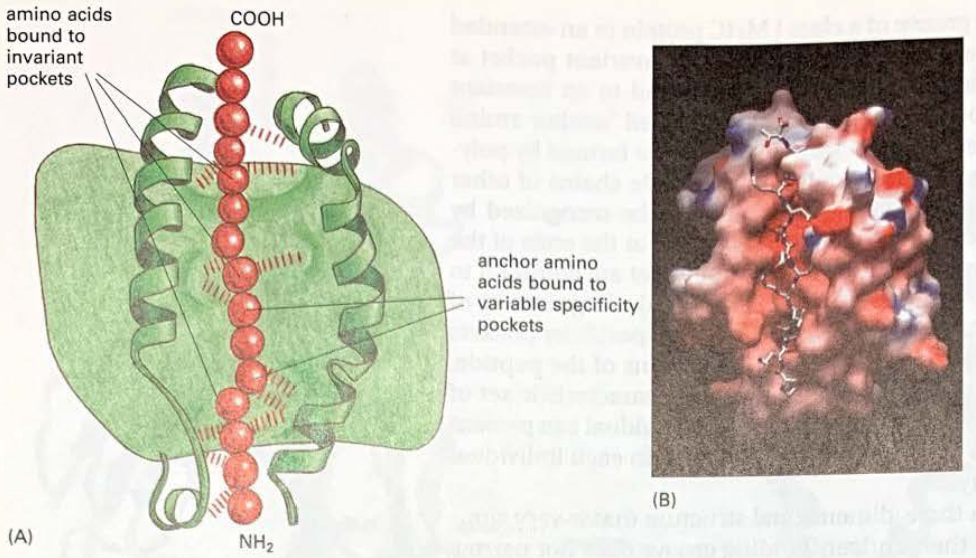


Figure 24–53 A peptide bound in the groove of a class II MHC protein. (A) Schematic drawing similar to that shown in Figure 24–52A. Note that the ends of the peptide are not tightly bound and extend beyond the cleft. The peptide is held in the groove by interactions between parts of its backbone and invariant pockets in the groove and between the side chains of several anchor amino acids of the peptide and variable specificity pockets in the groove. (B) The three-dimensional structure of a peptide bound in the groove of a class II MHC protein, as determined by x-ray diffraction. (B, courtesy of Paul Travers.)

MHC Proteins Help Direct T Cells to Their Appropriate Targets

Class I MHC proteins are expressed on virtually all nucleated cells. This is presumably because effector cytotoxic T cells must be able to focus on and kill any cell in the body that happens to become 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 include dendritic cells, which initially activate helper T cells, as well as the targets of effector helper T cells, such as macrophages and B cells. Because dendritic cells express both class I and class II MHC proteins, they can activate both cytotoxic and helper T cells.

It is important that effector cytotoxic T cells focus their attack 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 the latter type is essential for the body's immune defenses, it is vitally important that T cells never 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 two 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 T cells and cytotoxic T cells, respectively, to their appropriate targets, as we now discuss. The properies of class I and class II MHC proteins are compared in Table 24–2.

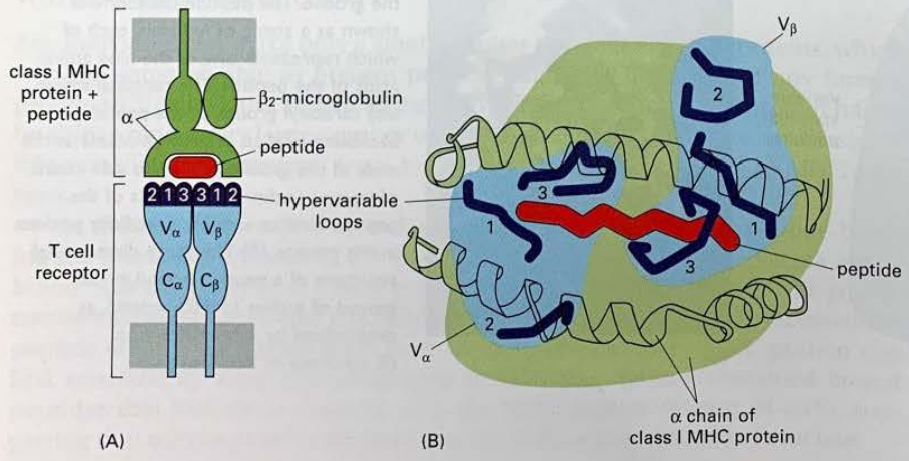


Figure 24–54 The interaction of a T cell receptor with a viral peptide bound to a class I MHC protein. (A) Schematic view of the hypervariable loops of the V_α and V_β domains of the T cell receptor interacting with the peptide and the walls of the peptide-binding groove of the MHC protein. The precise contacts are not illustrated. (B) Drawing of the "footprint" of the V domains *(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.)

TABLE 24–2 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	antigen-presenting cells (including B cells), thymus epithelial cells, some others
Involved in presenting antigen to	cytotoxic T cells	helper T cells
Source of peptide fragments	proteins made in cytoplasm	endocytosed plasma membrane and extracellular proteins
Polymorphic domains	$\alpha_1 + \alpha_2$	$\alpha_1 + \beta_1$
Recognition by co-receptor	CD8	CD4

CD4 and CD8 Co-receptors Bind to Nonvariable Parts of MHC Proteins

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

When accessory receptors also 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 T cell receptors, they recognize MHC proteins, but, unlike T cell receptors, they bind to nonvariable parts of the protein, far away from the peptide-binding groove. **CD4** is expressed on helper 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 24–55). Thus, CD4 and CD8 contribute to T cell recognition by helping to focus the cell on particular MHC proteins, and thus on particular types of cells—helper T cells on dendritic cells, macrophages, and B cells, and cytotoxic cells on any nucleated host cell displaying a foreign peptide on a class I MHC protein. The cytoplasmic tail of these transmembrane proteins is associated with a member of the Src family of cytoplasmic tyrosine protein kinases called *Lck*, which phosphorylates various intracellular proteins on tyrosines and thereby participates in the activation of the T cell. Antibodies to CD4 and CD8 are widely used as tools to distinguish between the two main classes of T cells, in both humans and experimental animals.

Ironically, the AIDS virus (HIV) makes use of CD4 molecules (as well as chemokine receptors) to enter helper T cells. It is the eventual depletion of helper T cells that renders AIDS patients susceptible to infection by microbes that are not normally dangerous. 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 cytotoxic or helper 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 first 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.

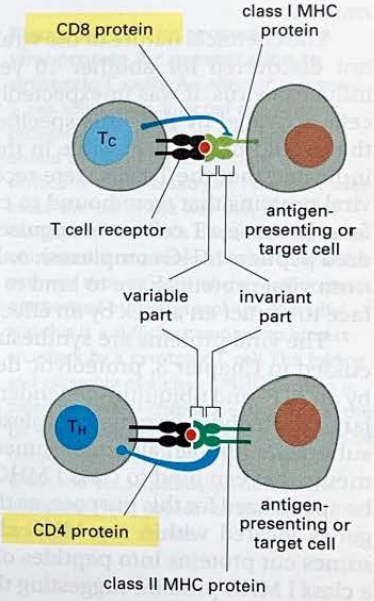


Figure 24–55 CD4 and CD8 co-receptors on the surface of T cells. Cytotoxic T cells (T_C) express CD8, which recognizes class I MHC proteins, whereas helper T cells (T_H) express CD4, which recognizes class II MHC proteins. Note that the co-receptors bind to the same MHC protein that the T cell receptor has engaged, so that they are brought together with T cell receptors during the antigen recognition process. Whereas the T cell receptor binds to the variable (polymorphic) parts of the MHC protein that form the peptide-binding groove, the co-receptor binds to the invariant part, far away from the groove.

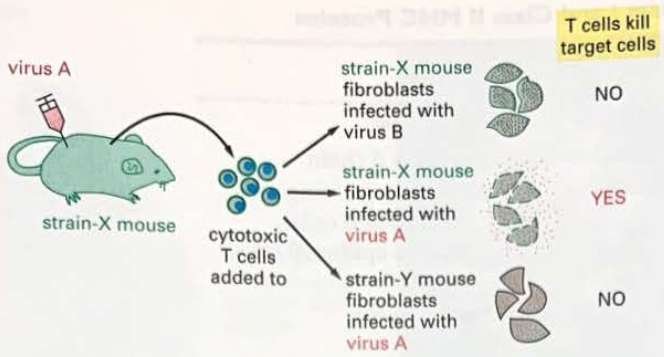


Figure 24–56 The classic experiment showing that an effector cytotoxic T cell recognizes some aspect of the surface of the host target cell in addition to a viral antigen. Mice of strain X are infected with virus A. Seven days later, the spleens of these mice contain effector cytotoxic T cells able to 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 suggested that class I MHC proteins are necessary to present cell-surface-bound viral antigens to effector cytotoxic T cells.

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

One of the first, and most dramatic, demonstrations that MHC proteins present foreign antigens to T cells came from an experiment performed in the 1970s. It was 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 (Figure 24–56). This experiment demonstrated that the T cells of any individual that recognize a specific antigen do so only when that antigen is associated with the allelic forms of MHC proteins expressed by that individual, a phenomenon known as *MHC restriction*.

The chemical nature of the viral antigens recognized by cytotoxic T cells was not discovered for another 10 years. In experiments on cells infected with influenza virus, it was unexpectedly found that some of the effector cytotoxic T cells activated by the virus specifically recognize internal proteins of the virus that would not be accessible in the intact virus particle. Subsequent evidence indicated that the T cells were recognizing degraded fragments of the internal viral proteins that were bound to class I MHC proteins on the infected cell surface. Because a T cell can recognize tiny amounts of antigen (as few as one hundred peptide–MHC complexes), only a small fraction of the fragments generated from viral proteins have to bind to class I MHC proteins and get to the cell surface to attract an attack by an effector cytotoxic T cell.

The 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. Although all proteasomes are probably able to generate peptide fragments that can bind to class I MHC proteins, some proteasomes are thought to be specialized for this purpose, as they contain two subunits that are encoded by genes located within the MHC chromosomal region. Even bacterial proteasomes cut proteins into peptides of about the length that fits into the groove of a class I MHC protein, suggesting that the MHC groove evolved to fit this length of peptide.

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 24–57)? The answer was discovered through observations on 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 protein 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 in the MHC chromosomal region, and, if either gene is inactivated by mutation, cells are unable to supply peptides to class I MHC proteins. The class I MHC proteins in such mutant cells are degraded in the cell 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 (Figure 24–58).

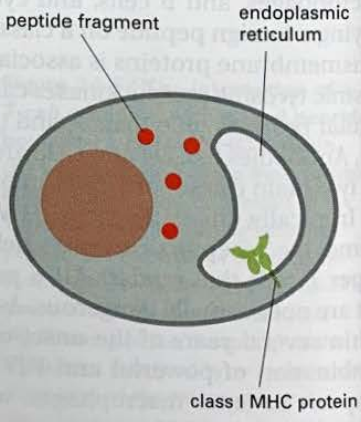


Figure 24–57 The peptide-transport problem. How do peptide fragments get from the cytosol, where they are produced, into the ER lumen, where the peptide-binding grooves of class I MHC proteins are located? A special transport process is required.

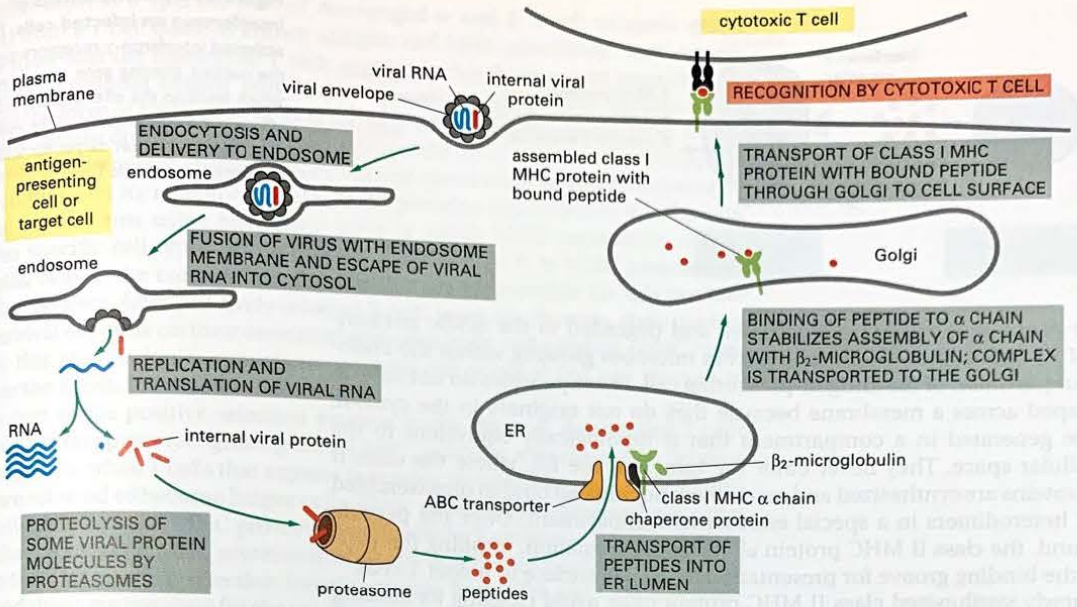


Figure 24–58 The processing of a viral protein for presentation to cytotoxic T cells. An effector cytotoxic T cell kills a virus-infected cell when it recognizes fragments of viral protein bound to class I MHC proteins on the surface of the infected cell. Not all viruses enter the cell in the way that this enveloped RNA virus does, but fragments of internal viral proteins always follow the pathway shown. Some of the viral proteins synthesized in the cytosol are degraded, and this is a sufficient amount to attract an attack by a cytotoxic T cell. The folding and assembly of a class I MHC protein is aided by several chaperone proteins in the ER lumen, only one of which is shown. The chaperones bind to the class I MHCα chain and act sequentially. The last one binds the MHC protein to the ABC transporter, as shown.

In cells that are not infected, 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 pumped into the ER and are carried to the cell surface by class I MHC proteins. They are not antigenic because the cytotoxic T cells that could recognize them have been eliminated or inactivated during T cell development, as we discuss later.

When cytotoxic T cells and some helper T cells are activated by antigen to become effector cells, they secrete the cytokine **interferon-γ (IFN-γ)**, which greatly enhances anti-viral responses. The IFN-γ acts on infected 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 (and class II) MHC proteins, the two specialized proteasome subunits, and the two subunits of the peptide transporter located in the ER (Figure 24–59). Thus, all of the machinery required for presenting viral antigens to cytotoxic T cells is coordinately called into action by IFN-γ, creating a positive feedback that amplifies the immune response and culminates in the death of the infected cells.

Helper T Cells Recognize 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 so as to eliminate microbes. 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 antigen-presenting 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 are different from those of peptide fragments presented by class I MHC proteins to cytotoxic T cells.

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

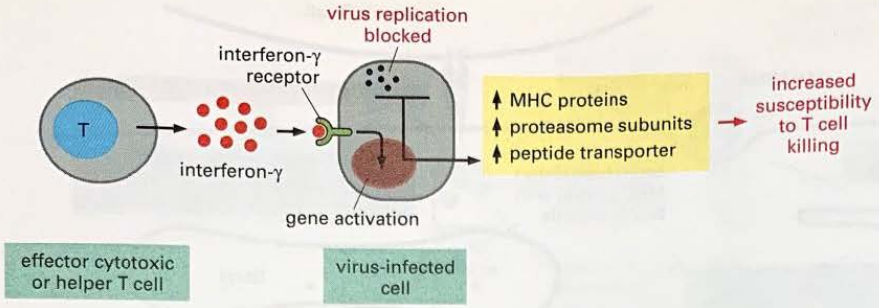


Figure 24–59 Some effects of interferon-γ on infected cells. The activated interferon-γ receptors signal to the nucleus, altering gene transcription, which leads to the effects indicated. The effects shaded in *yellow* tend to make the infected cell a better target for killing by an effector cytotoxic T cell.

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. These peptides do not have to be pumped across a membrane because they do not originate in the cytosol; they are generated in a compartment that is topologically equivalent to the extracellular space. They never enter the lumen of the ER, where the class II MHC proteins are synthesized and assembled, but instead bind to preassembled class II heterodimers in a special endosomal compartment. Once the peptide has bound, the class II MHC protein alters its conformation, trapping the peptide in the binding groove for presentation at the cell surface to helper T cells.

A newly synthesized class II MHC protein must avoid clogging its binding groove prematurely in the ER lumen with peptides derived from endogenously synthesized proteins. 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 other 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, the invariant chain is cleaved by proteases, leaving only a short fragment bound in the peptide-binding groove of the MHC protein. This fragment is then released (catalyzed by a class II-MHC-like protein called HLA-DM), freeing the MHC protein to bind peptides derived from endocytosed proteins (Figure 24–60). In this way, the functional differences between class I and class II MHC proteins are ensured—the former presenting molecules that come from the cytosol, the latter presenting molecules that come from the endocytic compartment.

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 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 have foreign peptides bound to them. This is sufficient, however, because only a hundred or so of such molecules are required to stimulate a helper T cell, just as in the case of peptide–class-I-MHC complexes stimulating a cytotoxic T cell.

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 24–56): 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 that will be capable of recognizing foreign peptides presented by self MHC proteins are selected to survive, while 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 T cell receptor 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 T cell receptor. 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 T cell depend on a match between its receptor and the MHC proteins expressed in the thymus. 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 process. After positively selected T cells leave the thymus, their continued survival depends on their continual stimulation by self-peptide–MHC complexes; this stimulation is enough to promote cell survival but not enough to activate the T cells to become effector cells.

As part of the positive selection process in the thymus, developing T cells that express receptors recognizing class I MHC proteins are selected to become cytotoxic cells, while T cells that express receptors recognizing class II MHC proteins are selected to become helper cells. Thus, genetically engineered mice that lack cell-surface class I MHC proteins specifically lack cytotoxic T cells, whereas mice that lack class II MHC proteins specifically lack helper T cells. 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 T cells fail to develop, and without CD8, cytotoxic T cells fail to develop.

Positive selection still leaves a large problem to be solved. If developing 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.

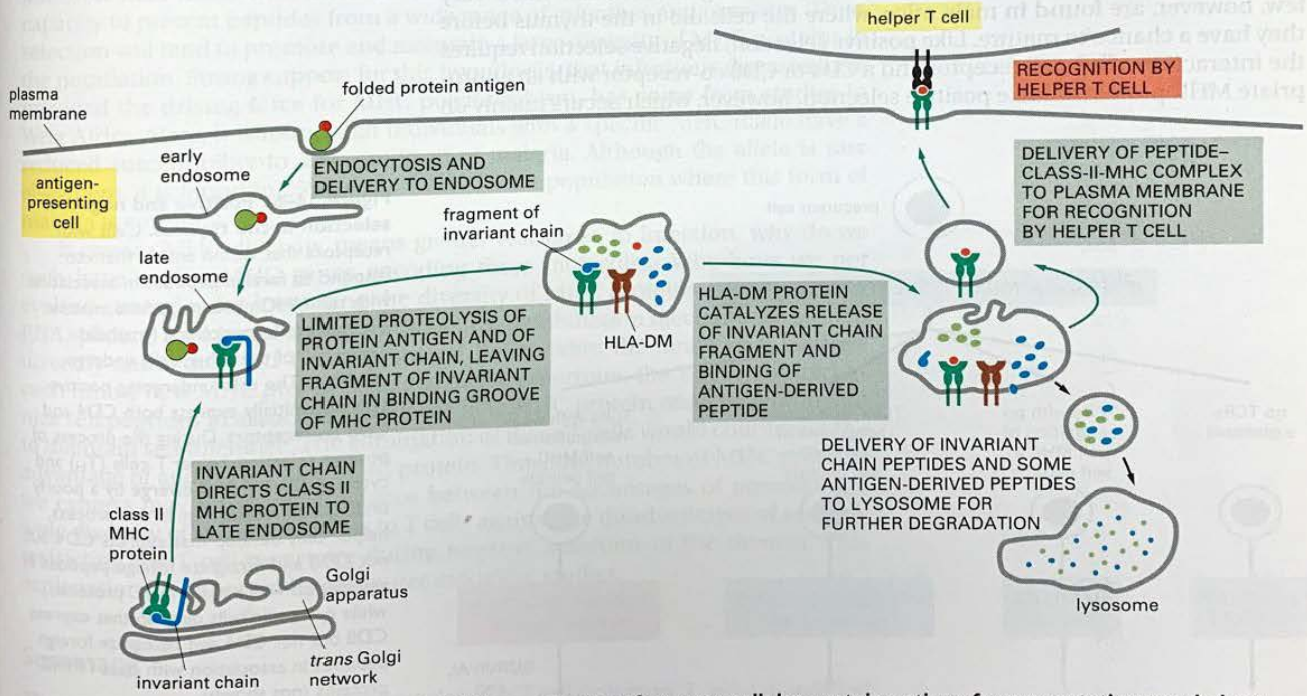


Figure 24–60 The processing of an extracellular protein antigen for presentation to a helper T cell. The drawing shows a simplified view of how peptide–class-II-MHC complexes are formed in endosomes and delivered to the cell surface. Note that the release of the invariant-chain fragment from the binding 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. They are made in the ER, are transported to the plasma membrane, and can then enter endosomes after endocytosis.

Many Developing T Cells That Could Be Activated by Self Peptides Are Eliminated in the Thymus

As discussed previously, a fundamental feature of the adaptive immune system is that it can 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 T cells—that is, T cells whose receptors 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 self-reactive B cells that escape B cell tolerance induction are harmless.

It is not enough, therefore, for the thymus to select *for* T cells that recognize self MHC proteins; it must also select *against* T cells that could be activated by self MHC proteins complexed with self peptides. In other words, it must pick out for survival just those T cells that will be capable of responding to self MHC proteins complexed with foreign peptides, even though these peptides are not present in the developing thymus. It is thought that these T cells bind weakly in the thymus to self MHC proteins that are carrying self peptides mismatched to the T cell receptors. Thus, the required goal can be achieved by (1) ensuring the death of 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 is the positive selection we have just discussed. Process 1 is called **negative selection**. In both death processes, the cells that die undergo apoptosis (Figure 24–61).

The most convincing evidence for negative selection derives once again from experiments with transgenic mice. After the introduction of T cell receptor transgenes encoding a receptor that recognizes a male-specific peptide antigen, for example, large numbers of mature T cells expressing the transgenic receptor are found in the thymus and peripheral lymphoid organs of female mice. Very few, however, are found in male mice, where the cells die in the thymus before they have a chance to mature. Like positive selection, negative selection requires the interaction of a T cell receptor and a CD4 or CD8 co-receptor with an appropriate MHC protein. Unlike positive selection, however, which occurs mainly on

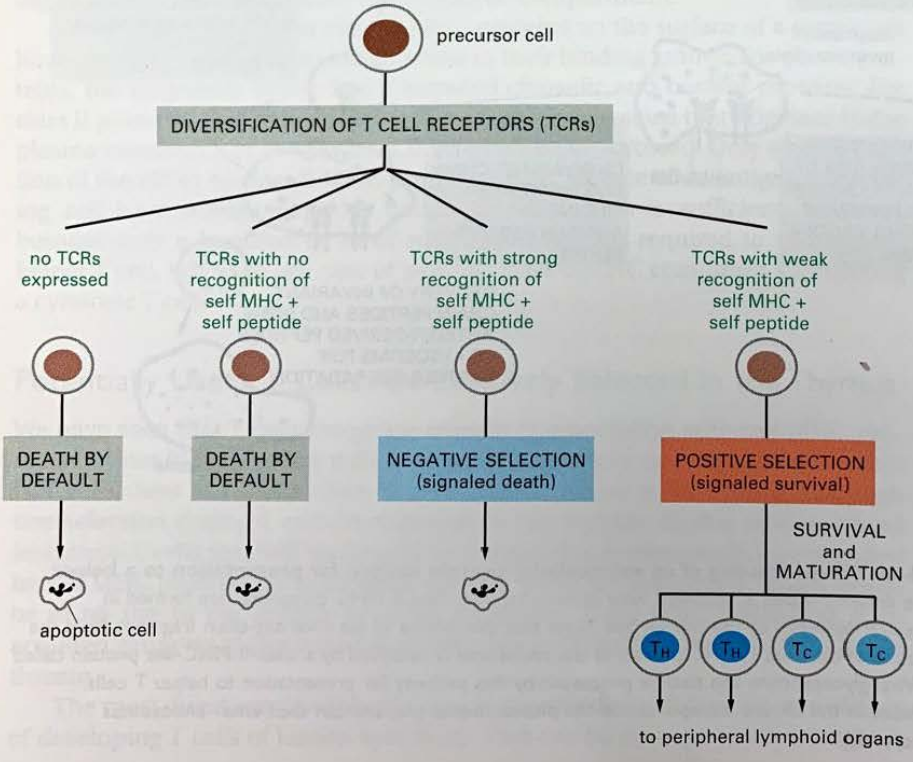


Figure 24–61 Positive and negative selection in the thymus. Cells with receptors that would enable them to respond to foreign peptides in association with self MHC proteins survive, mature, and migrate to peripheral lymphoid organs. All of the other cells undergo apoptosis. The cells undergoing positive selection initially express both CD4 and CD8 co-receptors. During the process of positive selection, helper T cells (T_H) and cytotoxic T cells (T_C) diverge by a poorly understood mechanism. In this process, helper cells develop that express CD4 but not CD8 and recognize foreign peptides in association with class II MHC proteins, while cytotoxic cells develop that express CD8 but not CD4 and recognize foreign peptides in association with class I MHC proteins (not shown).

the surface of thymus epithelial cells, negative selection occurs on the surface of thymus dendritic cells and macrophages, which, as we have seen, function as antigen-presenting cells in peripheral lymphoid organs.

The deletion of self-reactive T cells in the thymus cannot eliminate all potentially self-reactive T cells, as some self molecules are not present in the thymus. Thus, some potentially self-reactive T cells are deleted or functionally inactivated after they leave the thymus, presumably because they recognize self peptides bound to MHC proteins on the surface of dendritic cells that have not been activated by microbes and therefore do not provide a costimulatory signal. As we discuss later, antigen recognition without costimulatory signals can delete or inactivate a T or B cell.

Some potentially self-reactive T cells, however, are not deleted or inactivated. Instead, special *regulatory* (or *suppressor) T cells* are thought to keep them from responding to their self antigens by secreting inhibitory cytokines such as TGF-β (discussed in Chapter 15). These self-reactive T cells may sometimes escape from this suppression and cause autoimmune diseases.

The Function of MHC Proteins Explains Their Polymorphism

The role of MHC proteins in binding foreign peptides and presenting them to T cells provides an explanation for the extensive polymorphism of these proteins. In the evolutionary war between pathogenic microbes and the adaptive immune system, microbes tend to change their antigens to avoid associating with MHC proteins. When a microbe 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 microbe 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 microbes and parasites. Thus, selection will tend to promote and maintain a large diversity of MHC proteins in the population. Strong support for this hypothesis, that infectious diseases have provided the driving force for MHC polymorphism, has come from studies in West Africa. Here, it is found that 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 T cell receptors? Presumably, the limits exist because each time a new MHC protein is added to the repertoire, the T cells that recognize self peptides in association with 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 may 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. This explanation is supported by computer modeling studies.

Summary

There are two main functionally distinct classes of T cells: cytotoxic T cells kill infected cells directly by inducing them to undergo apoptosis, while helper T cells help activate B cells to make antibody responses and macrophages to destroy microorganisms that either invaded the macrophage or were ingested by it. Helper T cells also help activate cytotoxic T cells. Both classes of T cells express cell-surface, antibodylike receptors, which are encoded by genes that are assembled from multiple gene segments during T cell development in the thymus. These receptors recognize fragments

of foreign proteins that are displayed on the surface of host cells in association with MHC proteins. Both cytotoxic and helper T cells are activated in peripheral lymphoid organs by antigen-presenting cells, which express peptide–MHC complexes, costimulatory proteins, and various cell-cell adhesion molecules on their cell surface.

Class I and class II MHC proteins have crucial roles in presenting foreign protein antigens to cytotoxic and helper T cells, respectively. 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 and characteristic set of peptides, which are produced intracellularly by protein degradation: class I MHC proteins generally bind fragments produced in the cytosol, while class II MHC proteins bind fragments produced in the endocytic compartment. 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 T cell receptors, which interact with both the peptide and the walls of the peptide-binding groove. T cells also express CD4 or CD8 co-receptors, which recognize nonpolymorphic regions of MHC proteins on the target cell: helper cells express CD4, which recognizes class II MHC proteins, while cytotoxic T cells express CD8, which recognizes class I MHC proteins.

The T cell receptor repertoire is shaped mainly by a combination of positive and negative selection processes that operate during T cell development in the thymus. These processes help to ensure that only T cells with potentially useful receptors survive and mature, while the others die by apoptosis. T cells that will be able to respond to foreign peptides complexed with self MHC proteins are positively selected, while many T cells that could react strongly with self peptides complexed with self MHC proteins are eliminated. T cells with receptors that could react strongly with self antigens not present in the thymus are eliminated, functionally inactivated, or actively kept suppressed after they leave the thymus.

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 not only help activate B cells to secrete antibodies and macrophages to destroy ingested microbes, but they also help activate cytotoxic T cells to kill infected target cells. As dramatically demonstrated in AIDS patients, without helper T cells we cannot defend ourselves even against many microbes that are normally harmless.

Helper T cells themselves, however, can only function when activated to become effector cells. They are activated on the surface of antigen-presenting cells, which mature during the innate immune responses triggered by an infection. The innate responses also dictate what kind of effector cell a helper T cell will develop into and thereby determine the nature of the adaptive immune response 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 either T_H1 or T_H2 effector cells.

Costimulatory Proteins on Antigen-Presenting Cells Help Activate T Cells

To activate a cytotoxic or helper T cell to proliferate and differentiate into an effector cell, an antigen-presenting cell provides two kinds of signals. *Signal 1* is provided by a foreign peptide bound to an MHC protein on the surface of the presenting cell. This peptide–MHC complex signals through the T cell receptor

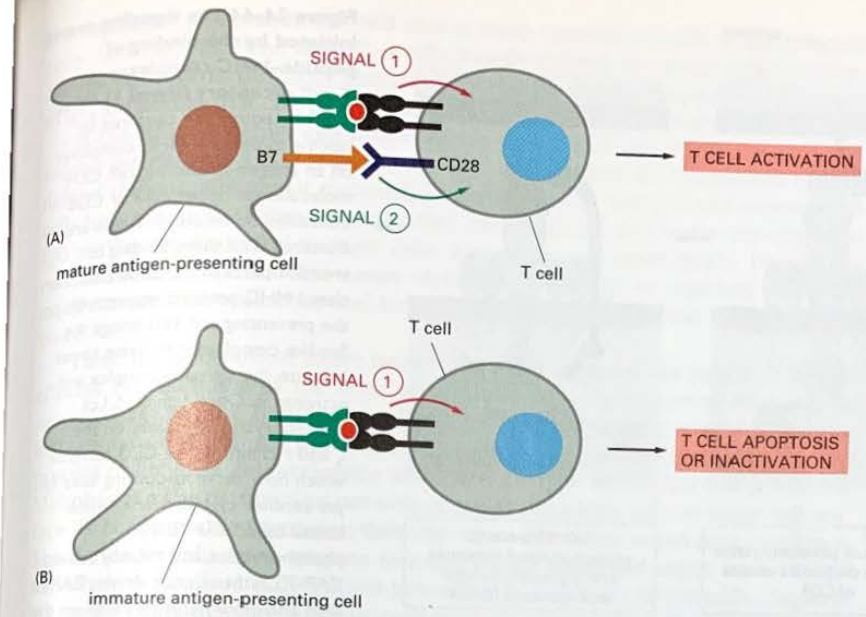


Figure 24–62 The two signals that activate a helper T cell. (A) A mature antigen-presenting cell can deliver both signal 1 and 2 and thereby activate the T cell. (B) An immature antigen-presenting cell delivers signal 1 without signal 2, which can kill or inactivate the T cell; this is one mechanism for immunological tolerance to self antigens. One model for the role of signal 2 is that it induces the active transport of signaling proteins in the T cell plasma membrane to the site of contact between the T cell and the antigen-presenting cell. The accumulation of signaling proteins around the T cell receptor is thought to greatly enhance the intensity and duration of the signaling process activated by signal 1. In this way, "immunological synapses" form in the contact zone, with the T cell receptors (and their associated proteins—see Figure 24–63) and co-receptors in the center and cell–cell adhesion proteins forming a peripheral ring (not shown).

and its associated proteins. *Signal 2* is provided by costimulatory proteins, especially the **B7 proteins** (CD80 and CD86), which are recognized by the co-receptor protein **CD28** on the surface of the T cell. The expression of B7 proteins on an antigen-presenting cell is induced by pathogens during the innate response to an infection. Effector T cells act back to promote the expression of B7 proteins on antigen-presenting cells, creating a positive feedback loop that amplifies the T cell response.

Signal 2 is thought to amplify the intracellular signaling process triggered by signal 1. If a T cell receives signal 1 without signal 2, it may undergo apoptosis or become altered so that it can no longer be activated, even if it later receives both signals (Figure 24–62). This is one mechanism by which a T cell can become *tolerant* to self antigens.

The T cell receptor does not act on its own to transmit signal 1 into the cell. It is associated with a complex of invariant transmembrane proteins called **CD3**, which transduces the binding of the peptide–MHC complex into intracellular signals (Figure 24–63). In addition, the CD4 and CD8 co-receptors play important parts in the signaling process, as illustrated in Figure 24–64.

The combined actions of signal 1 and signal 2 stimulate the T cell to proliferate and begin to differentiate into an effector cell by a curiously indirect mechanism. In culture, they cause the T cells to stimulate their own proliferation and differentiation by inducing the cells to secrete a cytokine called **interleukin-2 (IL-2)** and simultaneously to synthesize high affinity cell-surface receptors that bind it. The binding of IL-2 to the IL-2 receptors activates intracellular signaling pathways that turn on genes that help the T cells to proliferate and differentiate into effector cells (Figure 24–65). As discussed in Chapter 15, there are advantages to such an autocrine mechanism. It helps ensure that T cells differentiate into effector cells only when substantial numbers of them respond to antigen simultaneously in the same location, such as in a lymph node during an infection. Only then do IL-2 levels rise high enough to be effective.

Once bound to the surface of an antigen-presenting cell, a T cell increases the strength of the binding by activating an integrin adhesion protein called *lymphocyte-function-associated protein 1 (LFA-1)*. Activated LFA-1 now binds more strongly to its Ig-like ligand, *intracellular adhesion molecule 1 (ICAM-1)*, on the surface of the presenting cell. This increased adhesion enables the T cell to remain bound to the antigen-presenting cell long enough for the T cell to become activated.

The activation of a T cell is controlled by negative feedback. During the activation process, the cell starts to express another cell-surface protein called

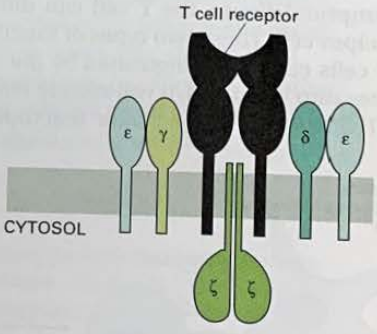


Figure 24–63 The T cell receptor and its associated CD3 complex. All of the CD3 polypeptide chains (shown in *green*), except for the ζ (zeta) chains, have extracellular Ig-like domains and are therefore members of the Ig superfamily.

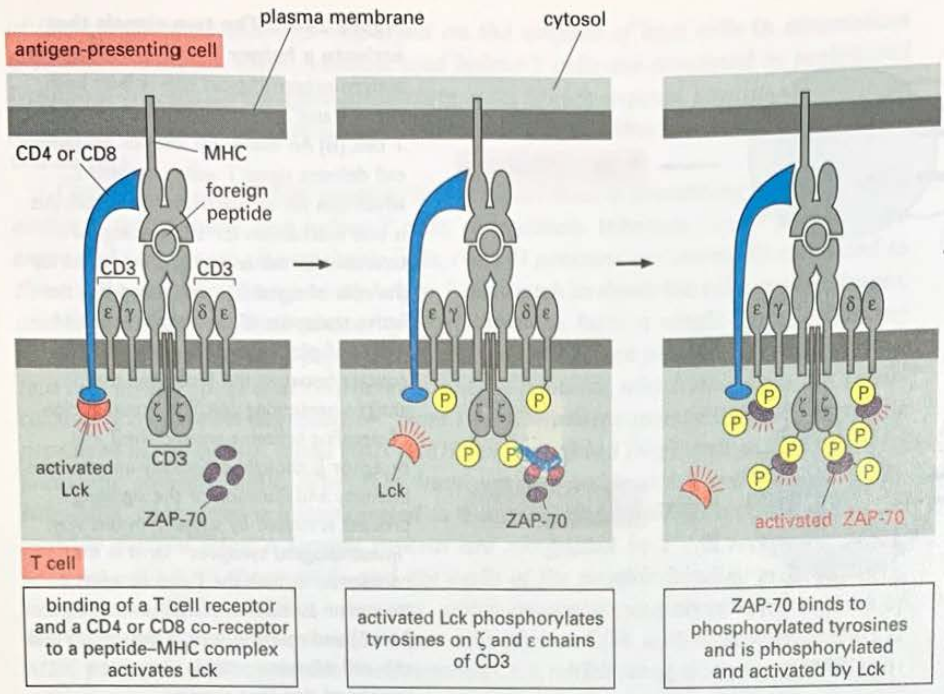


Figure 24–64 The signaling events initiated by the binding of peptide–MHC complexes to T cell receptors (signal 1). When T cell receptors are clustered by binding to peptide–MHC complexes on an antigen-presenting cell, CD4 molecules on helper cells or CD8 molecules on cytotoxic T cells are clustered with them, binding to invariant parts of the same class II or class I MHC proteins, respectively, on the presenting cell. This brings the Src-like cytoplasmic tyrosine kinase Lck into the signaling complex and activates it. Once activated, Lck phosphorylates tyrosines on the ζ and ε chains of the CD3 complex, which now serve as docking sites for yet another cytoplasmic tyrosine kinase called *ZAP-70*. Lck phosphorylates, and thereby activates, ZAP-70. Although not shown, ZAP-70 then phosphorylates tyrosines on the tail of another transmembrane protein, 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).

CTLA-4, which acts to inhibit intracellular signaling. It resembles CD28, but it binds to B7 proteins on the surface of the antigen-presenting cell with much higher affinity than does CD28, and, when it does, it holds the activation process in check. Mice with a disrupted *CTLA-4* gene die from a massive accumulation of activated T cells.

Most of the T (and B) effector cells produced during an immune response must be eliminated after they have done their job. As antigen levels fall and the response subsides, effector cells are deprived of the antigen and cytokine stimulation that they need to survive, and the majority die by apoptosis. Only memory cells and some long-lived effector cells survive.

Table 24–3 summarizes some of the co-receptors and other accessory proteins found on the surface of T cells.

Before considering how effector helper T cells help activate macrophages and B cells, we need to discuss the two 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 a an antigen-presenting cell activates a naïve helper T cell in a peripheral lymphoid tissue, the T cell can differentiate into either a T_H1 or T_H2 effector helper cell. These two types of functionally distinct subclasses of effector helper T cells can be distinguished by the cytokines they secrete. If the cell differentiates into a **T_H1 cell**, it will secrete *interferon-γ (IFN-γ)* and *tumor necrosis factor-α (TNF-α)* and will activate macrophages to kill microbes located within the

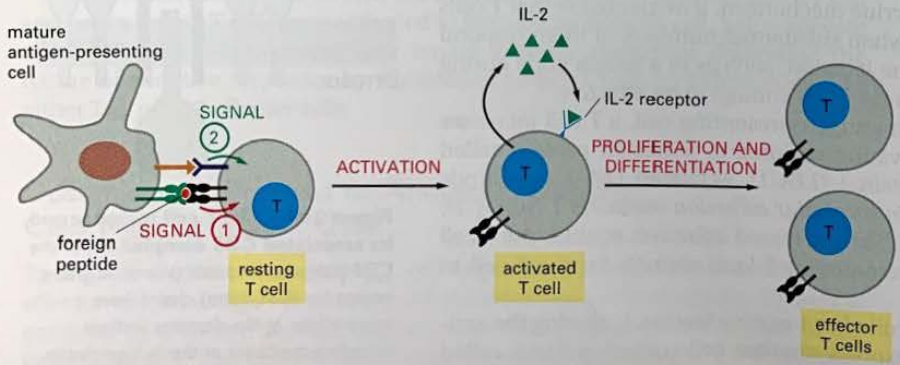


Figure 24–65 The stimulation of T cells by IL-2 in culture. Signals 1 and 2 activate T cells to make high affinity IL-2 receptors and to secrete IL-2. The binding of IL-2 to its receptors helps stimulate the cell to proliferate and differentiate into effector cells. Although some T cells do not make IL-2, as long as they have been activated by their antigen and therefore express IL-2 receptors, they can be helped to proliferate and differentiate by IL-2 made by neighboring T cells (not shown).

macrophages' phagosomes. It will also activate cytotoxic T cells to kill infected cells. Although, in these ways, T_H1 cells mainly defend an animal against intracellular pathogens, they may also stimulate B cells to secrete specific subclasses of IgG antibodies that can coat extracellular microbes and activate complement.

If the naïve T helper cell differentiates into a **T_H2** cell, by contrast, it will secrete *interleukins 4, 5, 10,* and *13* (*IL-4, IL-5, IL-10,* and *IL-13*) and will mainly defend the animal against extracellular pathogens. A T_H2 cell can stimulate B cells to make most classes of antibodies, including IgE and some subclasses of IgG antibodies that bind to mast cells, basophils, and eosinophils. These cells release local mediators that cause sneezing, coughing, or diarrhea and 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 will be mounted against the pathogen—whether it will be dominated by macrophage activation or by antibody production. The specific cytokines present during the process of helper T cell activation influence the type of effector cell produced. Microbes at a site of infection not only stimulate dendritic cells to make cell-surface B7 costimulatory proteins; they also stimulate them to produce cytokines. The dendritic cells then migrate to a peripheral lymphoid organ and activate naïve helper T cells to differentiate into either T_H1 or T_H2 effector cells, depending on the cytokines the dendritic cells produce. Some intracellular bacteria, for example, stimulate dendritic cells to produce *IL-12,* which encourages T_H1 development, and thereby macrophage activation. As expected, mice that are deficient in either IL-12 or its receptor are much more susceptible to these bacterial infections than are normal mice. Many parasitic protozoa and worms, by contrast, stimulate the production of cytokines that encourage T_H2 development, and thereby antibody production and eosinophil activation, leading to parasite expulsion (Figure 24–66).

Once a T_H1 or T_H2 effector cell develops, it inhibits the differentiation of the other type of helper T cell. IFN-γ produced by T_H1 cells inhibits the development of T_H2 cells, while IL-4 and IL-10 produced by T_H2 cells inhibit the development of T_H1 cells. Thus, the initial choice of response is reinforced as the response proceeds.

TABLE 24–3 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 T cell receptors; helps transport T cell receptors to cell surface
CD4	Ig	helper T cells	class II MHC	promotes adhesion to antigen-presenting cells and to target cells; signals T cell
CD8	Ig	cytotoxic T cells	class I MHC	promotes adhesion to antigen-presenting cells and infected target cells; signals T cell
CD28	Ig	most T cells	B7 proteins (CD80 and CD86)	provides signal 2 to some T cells
CTLA	Ig	activated T cells	B7 proteins (CD80 and CD86)	inhibits T cell activation
CD40 ligand	Fas ligand family	effector helper T cells	CD40	costimulatory protein that helps activate macrophages and B cells
LFA-1	integrin	most white blood cells, including all T cells	ICAM-1	promotes cell–cell adhesion

* 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 150 CD proteins have been identified.

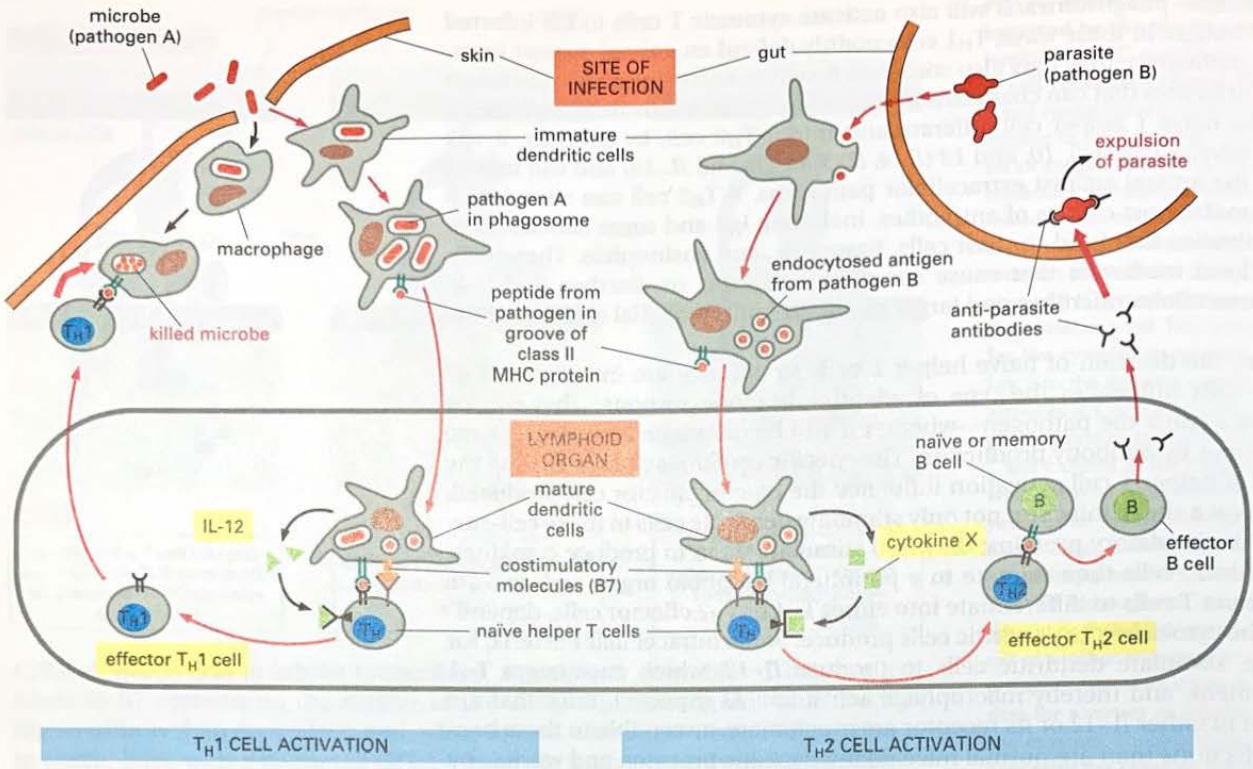


Figure 24–66 The activation of T_H1 and T_H2 cells. The differentiation of helper T cells into either T_H1 or T_H2 effector cells determines the nature of the subsequent adaptive immune responses that the effector cells activate. Whether a naïve helper T cell becomes a T_H1 or T_H2 cell depends mainly on the cytokines present when the helper T cell is activated by a mature dendritic cell in a peripheral lymphoid organ. The types of cytokines produced depend on the local environment and the nature of the microbe or parasite that activated the immature dendritic cell at the site of infection. IL-12 produced by mature dendritic cells promotes T_H1 cell development. The cytokine(s) produced by dendritic cells that promotes T_H2 cell development (cytokine X) is not known, although IL-4 produced by T cells can serve this function. 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. The antibodies arm mast cells, basophils, and eosinophils (not shown), which then can help expel the parasite from the gut.

The importance of the T_H1/T_H2 decision is illustrated by individuals infected with *Mycobacterium leprae*, the bacterium that causes leprosy. The 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. 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 disease that progresses slowly but does not kill the host. In other patients, by contrast, the *lepromatous* form of the disease occurs. 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.

T_H1 Cells Help Activate Macrophages at Sites of Infection

T_H1 cells are preferentially induced by antigen-presenting cells that harbor microbes in intracellular vesicles. The bacteria that cause tuberculosis for example, replicate mainly in phagosomes inside macrophages, where they are protected from antibodies. They are also not readily attacked by cytotoxic T cells, which mainly recognize foreign antigens that are produced in the cytosol (see Figure 24–58). The bacteria can survive in phagosomes because they inhibit both the fusion of the phagosomes with lysosomes and the acidification of the phagosomes that is necessary to activate lysosomal hydrolases. Infected dendritic cells recruit helper T cells to assist in the killing of such microbes. The dendritic cells migrate to peripheral lymphoid organs, where they stimulate the production of T_H1 cells, which then migrate to sites of infection to help activate infected macrophages to kill the microbes harboring in their phagosomes (see Figure 24–66).

T_H1 effector cells use two signals to activate a macrophage. They secrete IFN-γ, which binds to IFN-γ receptors on the macrophage surface, and they display the costimulatory protein **CD40 ligand**, which binds to **CD40** on the macrophage (Figure 24–67). (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

it contains: 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 25). 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, B7 costimulatory proteins, and various cytokines, especially IL-12. This makes them more effective at stimulating 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 stimulate an inflammatory response 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.

T_H1 cells can also help activate cytotoxic T cells in peripheral lymphoid organs by stimulating dendritic cells to produce more costimulatory proteins. In addition, they can help effector cytotoxic T cells kill virus-infected target cells, by secreting IFN-γ, which increases the efficiency with which target cells process viral antigens for presentation to cytotoxic T cells (see Figure 24–59). 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 Figure 24–46B).

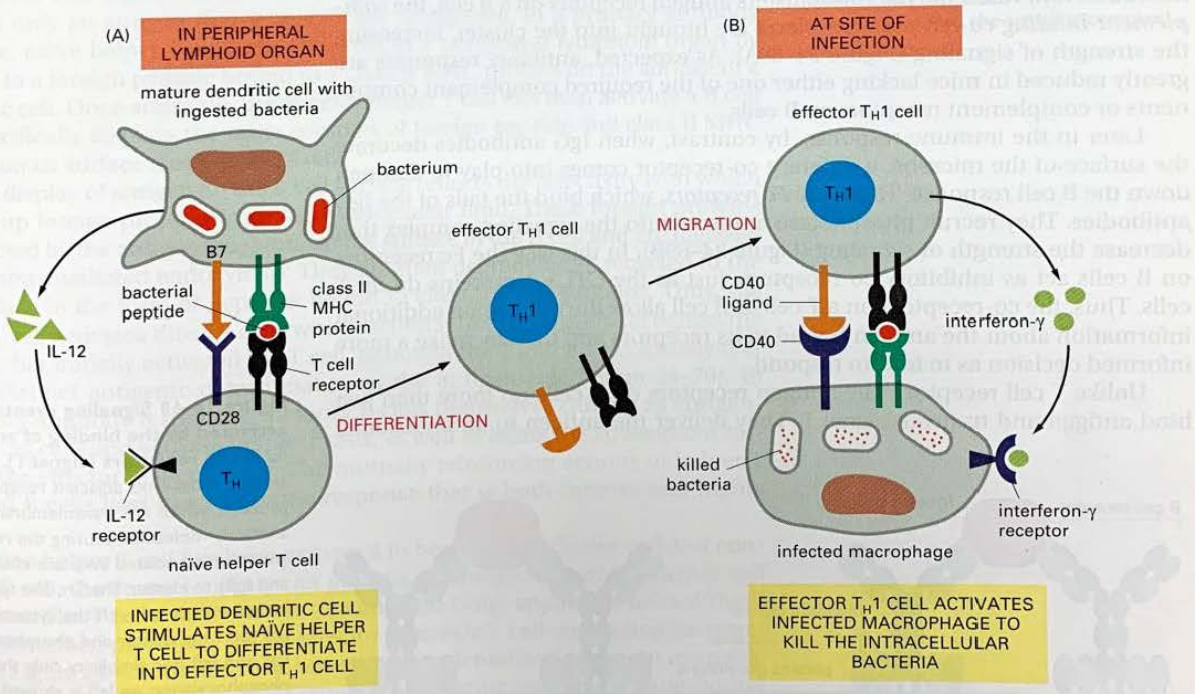


Figure 24–67 The differentiation of T_H1 cells and their activation of macrophages. (A) An infected dendritic cell that has migrated from a site of infection to a peripheral lymphoid organ activates a naïve helper T cell to differentiate into a T_H1 effector cell, using both cell-surface B7 and secreted IL-12. (B) A T_H1 effector cell that has migrated from the peripheral lymphoid organ to an infected site helps activate macrophages to kill the bacteria harboring within the macrophages' phagosomes. The T cell activates the macrophage by means of CD40 ligand on its surface and secreted interferon-γ.

Both T_H1 and T_H2 cells can help stimulate B cells to proliferate and differentiate into either antibody-secreting effector cells or memory cells. They can also stimulate B cells to switch the class of antibody they make, from IgM (and IgD) to 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 Provides Signal 1 to B Cells

Like T cells, B cells require two types of extracellular signals to become activated. Signal 1 is provided by antigen binding to the antigen receptor, which is a membrane-bound antibody molecule. Signal 2 is usually provided by a helper T cell. Like a T cell, if a B cell receives the first signal only, it is usually eliminated or functionally inactivated, which is one way in which B cells become tolerant to self antigens.

Signaling through the B cell antigen receptor works in much the same way as signaling through the T cell receptor (see Figure 24–64). The receptor is associated with two invariant protein chains, Igα and Igβ, which help convert antigen binding to the receptor into intracellular signals. When antigen cross-links its receptors on the surface of a B cell, it causes the receptors and its 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 phosphorylation cascade (Figure 24–68).

Just as the CD4 and CD8 co-receptors on T cells enhance the efficiency of signaling through the T cell receptor, so a co-receptor complex that binds complement proteins greatly enhances the efficiency of signaling through the B cell antigen receptor and its associated invariant chains. If a microbe activates the complement system (discussed in Chapter 25), complement proteins are often deposited on the microbe surface, greatly increasing the B cell response to the microbe. Now, when the microbe clusters antigen receptors on a B cell, the *complement-binding co-receptor complexes* are brought into the cluster, increasing the strength of signaling (Figure 24–69A). As expected, antibody responses are greatly reduced in mice lacking either one of the required complement components or complement receptors on B cells.

Later in the immune response, by contrast, when IgG antibodies decorate the surface of the microbe, a different co-receptor comes into play to dampen down the B cell response. These are *Fc receptors,* which bind the tails of the IgG antibodies. They recruit phosphatase enzymes into the signaling complex that decrease the strength of signaling (Figure 24–69B). In this way the Fc receptors on B cells act as inhibitory co-receptors, just as the CTLA-4 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.

Unlike T cell receptors, the antigen receptors on B cells do more than just bind antigen and transmit signal 1. They deliver the antigen to an endosomal

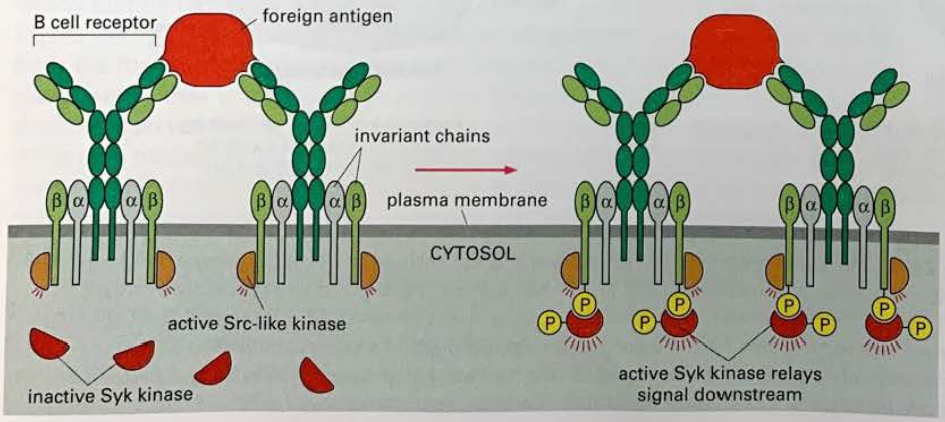


Figure 24–68 Signaling events activated by the binding of antigen to B cell receptors (signal 1). The antigen cross-links adjacent receptor proteins, which are transmembrane antibody molecules, causing the receptors and their associated invariant chains (Igα and Igβ) to cluster. The Src-like tyrosine kinase associated with the cytosolic tail of Igβ joins the cluster and phosphorylates Igα and Igβ (for simplicity, only the phosphorylation on Igβ is shown). The resulting phosphotyrosines on Igα and Igβ serve as docking sites for another Src-like tyrosine kinase called *Syk*, which is homologous to ZAP-70 in T cells (see Figure 24–64). Like ZAP-70, Syk becomes phosphorylated and relays the signal downstream.

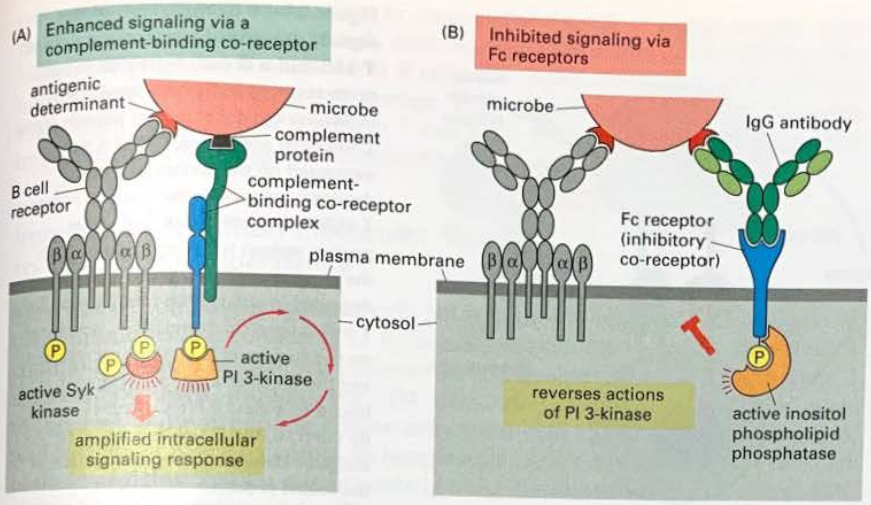


Figure 24–69 The influence of B cell co-receptors on the effectiveness of signal 1. (A) The binding of microbe–complement complexes to a B cell cross-links the antigen receptors to complement-binding, co-receptor complexes. The cytosolic tail of one component of the co-receptor complex becomes phosphorylated on tyrosines, which then serve as docking sites for PI 3-kinase. As discussed in Chapter 15, PI 3-kinase is activated to generate inositol phospholipid docking sites in the plasma membrane, which 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 become bound 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, which then serve as docking sites for an inositol phospholipid phosphatase. The phosphatase dephosphorylates the inositol phopholipid docking sites in the plamsa membrane generated by PI 3-kinase, thereby reversing the activating effects of PI 3-kinase. The Fc receptors also inhibit signaling by recruiting protein tyrosine phosphatases into the signaling complex (not shown).

compartment where the antigen is degraded to peptides, which are returned to the B cell surface bound to class II MHC proteins (see Figure 24–60). The peptide–class-II-MHC complexes are then recognized by effector helper T cells, which can now deliver signal 2. Signal 1 prepares the B cell for its interaction with a helper T cell by increasing the expression of both class II MHC proteins and receptors for signal 2.

Helper T Cells Provide Signal 2 to B Cells

Whereas antigen-presenting cells such as dendritic cells and macrophages are omnivorous and ingest and present antigens nonspecifically, a B cell generally presents only an antigen that it specifically recognizes. In a primary antibody response, 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 a dendritic cell. Once activated, the effector helper T cell can then activate a B cell that specifically displays the same complex of foreign peptide and class II MHC protein on its surface (see Figure 24–66).

The display of antigen on the B cell surface reflects the selectivity with which it takes up foreign proteins from the extracellular fluid. These foreign proteins are selected by the antigen receptors on the surface of the B cell and are ingested by receptor-mediated endocytosis. They are then degraded and recycled to the cell surface in the form of peptides bound to class II MHC proteins. Thus, the helper T cell activates those B cells with receptors that specifically recognize the antigen that initially activated the T cell, although the T and B cells usually recognize distinct antigenic determinants on the antigen (see Figure 24–70). 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 immune 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 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 24–45). In this case, however, the orientation is thought to enable the effector helper T cell to provide signal 2 by directing both membrane-bound and secreted signal molecules onto the B cell surface. The membrane-bound signal molecule is the transmembrane protein CD40 ligand, which we encountered earlier and is expressed on the surface of effector helper T cell, but not on nonactivated naïve or memory helper T cells. It is recognized by the CD40 protein on the B cell surface. The interaction between CD40 ligand and CD40 is required for helper T cells to activate B cells to proliferate and differentiate into

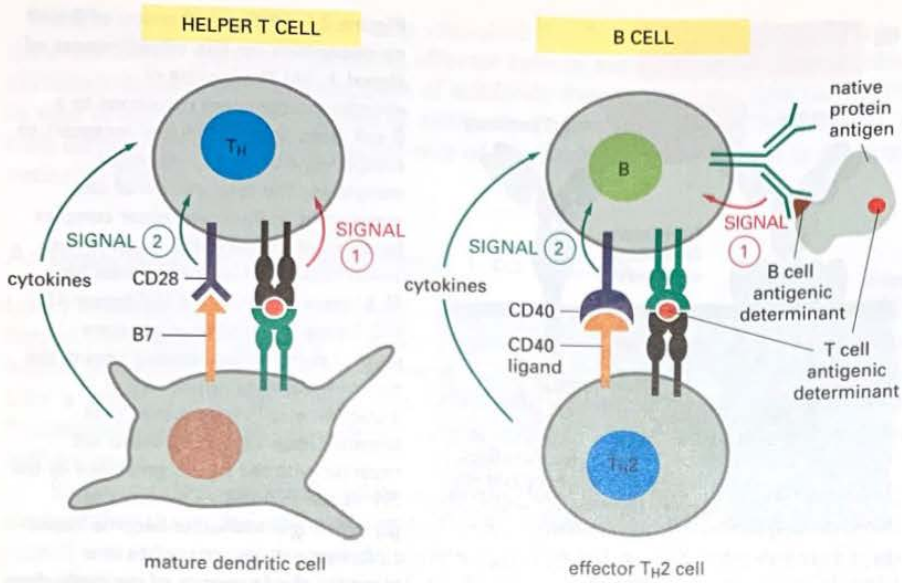


Figure 24–70 Comparison of the signals required to activate a helper T cell and a B cell. Note that in both cases secreted and membrane-bound molecules can cooperate to provide signal 2. Although not shown, CD40 is also expressed on the surface of mature dendritic cells and helps maintain helper T cells in an active state. The native protein antigen is endocytosed by both the dendritic cell and the B cell and is degraded in endosomes (not shown). The T cell antigenic determinant is presented on the surface of both the dendritic cell and the B cell as a peptide fragment bound to a class II MHC protein. By contrast, the B cell recognizes an antigenic determinant on the surface of the folded protein.

memory or antibody-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.

Secreted signals from helper T cells also help B cells to proliferate and differentiate and, in some cases, to switch the class of antibody they produce. *Interleukin-4 (IL-4)* is one such signal. Produced by T_H2 cells, it collaborates with CD40 ligand in stimulating B cell proliferation and differentiation, and it promotes switching to IgE antibody production. Mice deficient in IL-4 production are severely impaired in their ability to make IgE.

The signals required for T and B cell activation are compared in Figure 24–70, and some of the cytokines discussed in this chapter are listed in Table 24–4.

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 signal 1 and signal 2. Others are large polymers with repeating, identical antigenic determinants (see Figure 24–29B); their multipoint binding to B cell antigen receptors can generate a strong enough signal 1 to activate the B cell directly, without signal 2. Because T-cell-independent antigens do not activate helper T cells, they fail to induce B

TABLE 24–4 Properties of Some Interleukins

CYTOKINE	SOME SOURCES	SOME TARGETS	SOME ACTIONS
IL-2	all helper T cells; some cytotoxic T cells; activated mast cells	all activated T cells and B cells	stimulates proliferation and differentiation
IL-4	T_H2 cells and mast cells	B cells and T_H cells	stimulates B cell proliferation, maturation, and class switching to IgE and IgG1; inhibits T_H1 cell development
IL-5	T_H2 cells and mast cells	B cells, eosinophils	promotes proliferation and maturation
IL-10	T_H2 cells, macrophages, and dendritic cells	macrophages and T_H1 cells	inhibits macrophages and T_H1 cell development
IL-12	B cells, macrophages, and dendritic cells	naïve T cells	induces T_H2 cell development and inhibits T_H1 cell development
IFN-γ	T_H1 cells	B cells, macrophages, endothelial cells	activates various MHC genes and macrophages; increases MHC expression in many cell types
TNF-α	T_H1 cells and macrophages	endothelial cells	activates

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 an Ancient 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, T cell receptors, MHC proteins, the CD4, CD8, and CD28 co-receptors, and most of the invariant polypeptide chains associated with B and T cell receptors, 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 40% of the 150 or so polypeptides 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 24–71).

The amino acids in each Ig-like domain are usually encoded by a separate exon. 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 Figure 24–50A) or the Thy-1 protein (see Figure 24–71)—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.

The multiple gene segments that encode antibodies and T cell receptors may have arisen when a transposable element, or transposon (discussed in

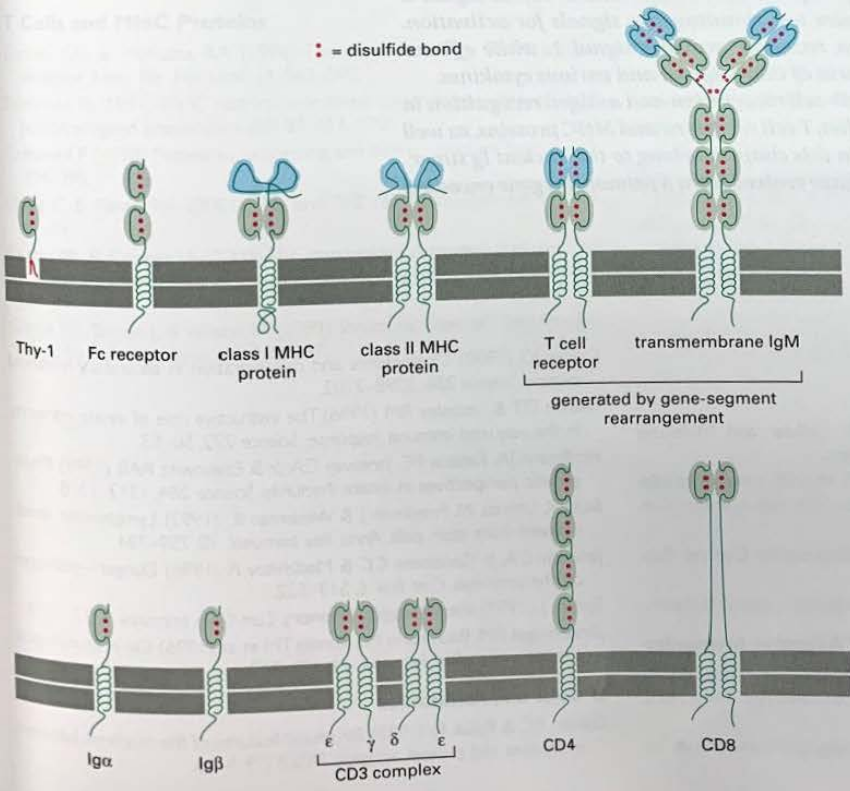


Figure 24–71 Some of the membrane proteins belonging to the Ig superfamily. The Ig and Ig-like domains are shaded in *gray*, except for the antigen-binding domains (not all of which are Ig domains), which are shaded in *blue*. The function of Thy-1 is unknown, but it is widely used to idenitfy T cells 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 (N-CAM) discussed in Chapter 19 and the receptors for various protein growth factors discussed in Chapters 15 and 17 (not shown). There are about 765 members of the Ig superfamily in humans.

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* joining; 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 the *V* and *J* gene segments of an Ig light chain gene are assembled (see Figure 24–37). 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 have generated the arrangements of gene segments that characterize the adaptive immune systems of present-day vertebrates.

Adaptive immune systems evolved to defend vertebrates against infection by pathogens. Pathogens, however, evolve more quickly, and they have acquired remarkably sophisticated strategies to counter these defenses, as we discuss in Chapter 25.

Summary

Naïve T cells require at least two signals for activation. Both are provided by an antigen-presenting cell, which is usually a dendritic cell: signal 1 is provided by MHC–peptide complexes binding to T cell receptors, while signal 2 is mainly provided by B7 costimulatory proteins binding to CD28 on the T cell surface. If the T cell receives only signal 1, it is usually deleted or inactivated. When helper T cells are initially activated on a dendritic cell, they can differentiate into either T_H1 or T_H2 effector cells, depending on the cytokines in their environment: T_H1 cells activate macrophages, cytotoxic T cells, and B cells, while T_H2 cells mainly activate 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 signal proteins. The membrane-bound signal is CD40 ligand. Like T cells, B cells require two simultaneous signals for activation. Antigen binding to the B cell antigen receptors provides signal 1, while effector helper T cells provide signal 2 in the form of CD40 ligand and various cytokines.

Most of the proteins involved in cell–cell recognition and antigen recognition in the immune system, including antibodies, T cell receptors, 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.

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