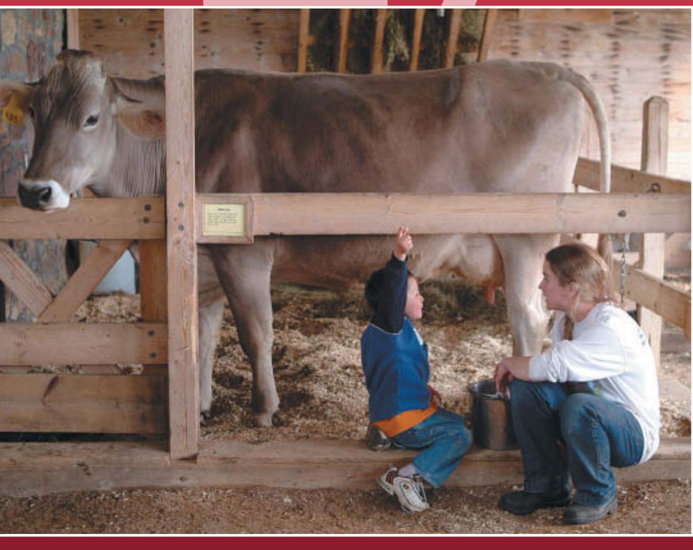
27

Adaptive Immunity: Highly Specific Host Defenses



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Got (Raw) Milk? The Role of Unprocessed Cow's Milk in Protecting against Allergy and Asthma

In recent decades, the onset of allergies and asthma during childhood has become an increasingly common complication that, as we will see in this chapter, stems from hypersensitivity reactions in the adaptive immune response. However, for nearly 20 years now, researchers have been aware of one demographic of young people that has consistently shown resistance to asthma and allergies: kids that grow up on farms. Several hypotheses have been proposed to explain this phenomenon, including early childhood exposure to livestock and their feed (see photo), but while this may contribute to a protective "farm effect," it is the consumption of unprocessed cow's milk that is emerging as the major underlying explanation for the trend.

A recent report documented the correlation between the consumption of raw, unpasteurized cow's milk and the incidence of asthma in over 1100 children from rural regions of five European countries. Researchers found that compared to shop milk purchased at a supermarket, which is pasteurized (heated to 72°C for at least 15 seconds), centrifuged, and homogenized to achieve a uniform product, regular ingestion of unprocessed farm milk was inversely related to the onset of asthma in children, with the asthma-protective effect increasing over time.

To elucidate this farm milk effect, the scientists compared the fatty acid composition of unprocessed farm milk samples to that of shop milk samples. They found that the raw milk contained substantially higher levels of omega-3 (ω-3) fatty acids than shop milk, and this was attributed to both the higher overall fat content of raw milk and its lack of pasteurization, which breaks down heat-labile components of the milk. This finding was important because ω-3 fatty acids are precursors of anti-inflammatory immune mediators that suppress hypersensitivity reactions, including those that trigger allergies and asthma.

Although the implications of this research in asthma prevention are potentially significant, public health officials strongly discourage ingestion of unpasteurized milk, mainly because of the risk of foodborne illnesses, including salmonellosis, listeriosis, Q fever, staphylococcal food poisoning, and gastroenteritis. Interestingly, changing this stance may be neither warranted nor necessary since it may be possible to restore the asthma-protective effect by supplementing industrially processed milk with ω-3 fatty acids. Only time—and more research—will tell!

Source: Brick, T., et al. 2016. ω-3 fatty acids contribute to the asthma-protective effect of unprocessed cow's milk. *J. Allergy Clin. Immunol. 137(6):* 1699–1706.e13 doi:10.1016/j.jaci.2015.10.042.

In the previous chapter we discussed the key features of innate immunity and how this system protects against infection and disease from a broad range of pathogens. Here, we build on this foundation with a focus on the powerful and highly specific immune mechanisms of adaptive immunity, mechanisms that depend on both cellular and molecular components and that complement the innate response.

I • Principles of Adaptive Immunity

Innate and adaptive immunity can be viewed as different sides of the same coin that work together to protect the host from attack by foreign substances. Whereas innate immunity is characterized by broadly specific responses triggered by *common* structural features found on and in pathogens, adaptive immunity is directed toward *specific* molecular components of the pathogen (their antigens). In adaptive immunity, pathogen-specific immune receptors are produced in large numbers only after exposure to the pathogen or its products. In this way, the individual antigenic properties of different pathogens orchestrate the adaptive immune response.

We begin this part of the chapter by considering the major characteristics of the adaptive immune response and then explore the structure of the substances that trigger this response and the different forms of adaptive immunity that we experience in our everyday lives.

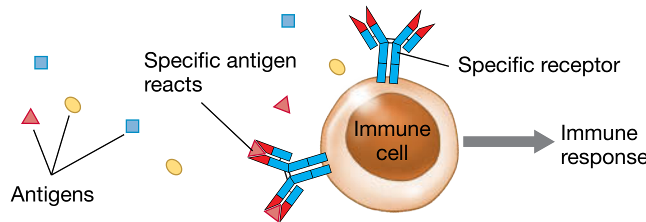
27.1 Specificity, Memory, Selection Processes, and Tolerance

Adaptive immunity is primarily a function of a special class of antigen-reactive leukocytes called *lymphocytes*. B lymphocytes (B cells) specialize in the production of antibodies that interact with and protect against extracellular antigens, thus conferring *antibody-mediated* (*humoral*) *immunity* to the host. T lymphocytes (T cells) express antigen-specific receptor proteins on their surfaces that defend against intracellular pathogens, such as viruses and certain bacteria, thus conferring *cell-mediated* (*cellular*) *immunity* to the host. The combination of antibody-mediated and cell-mediated defenses comprises adaptive immunity, a system that is characterized by three major features: *specificity*, *memory*, and *tolerance*. None of these features is characteristic of the innate immune response (Chapter 26).

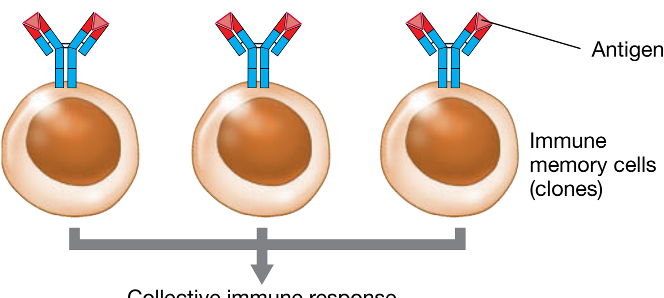
Immune Specificity and Memory

Overall, the immune response is highly specific, but the innate and the adaptive systems differ in their degree of specificity. Innate immunity is directed against features common to a broad diversity of pathogens, such as the peptidoglycan of all gram-positive bacteria or the lipopolysaccharide (LPS) of all gram-negative bacteria. By contrast, adaptive immune mechanisms are directed against pathogen-specific macromolecules, such as a specific protein associated with a single strain of a particular pathogen.

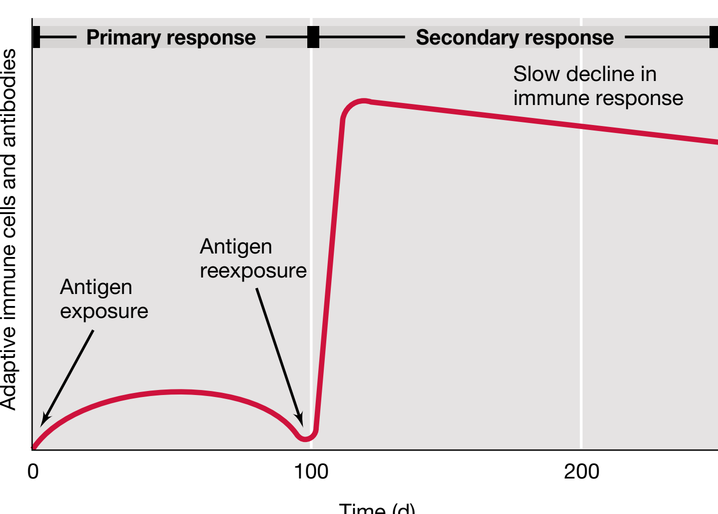
Each B cell or T cell produces a unique protein that interacts with a single type of antigen. These proteins therefore have **specificity** for that antigen. The antigen-binding proteins of B cells are membrane-bound antibodies called **B cell receptors (BCRs)**. T cells have antigen-binding **T cell receptors (TCRs)** on their surfaces. The specificity of the antigen–antibody or antigen–TCR interaction is dependent on the capacity of the lymphocyte cell receptor to interact with a particular antigen but not with other antigens (Figure 27.1*a*).



Specificity: Immune cells have surface receptors that interact with individual antigens.
(a)



Memory: The first antigen exposure induces multiplication of antigen-reactive cells, resulting in multiple copies, or *clones*. After a subsequent exposure to the same antigen, the immune response is faster and stronger due to the large number of responding cells.
(b)



Immune responses: As a result of immune memory, antigen reexposure triggers a much stronger secondary response.
(c)

Figure 27.1 Specificity and memory in the adaptive immune response. Key features of antibody-mediated and cell-mediated immunity are *(a)* specificity and *(b)* memory. *(c)* The primary response induces both immune cells and antibodies. The antigens given at day 0 and day 100 must be identical to induce a secondary response. The secondary response may generate more than 10-fold increases in immune cells and antibody concentrations.

The adaptive immune response is induced only when triggered by a unique antigen on a pathogen. For example, polysaccharide antigen from the LPS of a particular gram-negative bacterium is unique for a genus and often even for a species within the genus. Therefore, an individual lymphocyte that interacts with an LPS polysaccharide unique to the bacterium *Salmonella* will not interact with the LPS of other bacteria, even other closely related gram-negative enteric bacteria, such as *Escherichia* or *Shigella*.

In addition to specificity, the adaptive immune system exhibits **memory** (Figure 27.1*b*). Lymphocytes must encounter antigen to stimulate production of detectable and effective antigen-activated antibodies or TCRs. The first exposure to an antigen generates a **primary immune response** in which antigen contact stimulates growth and multiplication of antigen-reactive B and T cells, thereby creating large numbers of antigen-specific **clones**. Some of these clones, called **memory cells**, persist in the body for years and confer long-term specific immunity. Subsequent exposure to the same antigen activates the clones and generates a faster and stronger **secondary immune response** that peaks within a few days (Figure 27.1*c*). This capacity to respond more quickly and vigorously to subsequent exposures to the eliciting antigen provides the host with immediate resistance to previously encountered pathogens, a topic we develop in more detail in Section 27.3. Immune memory is an important facet of clinical medicine and public health, as vaccinating humans or other animals with killed or weakened pathogens (or their products) is a major means of conferring immunity to dangerous pathogens (see Section 27.2).

T Cell Selection and Tolerance

The adaptive immune system must be able to discriminate between harmless *host* antigens ("self") and potentially dangerous *foreign* antigens ("nonself") and function to destroy only the latter. T cells undergo immune selection *for* potential antigen-reactive cells and selection *against* those cells that react strongly with self antigens. Selection against self-reactive cells results in the development of another key characteristic of the adaptive immune response: **tolerance**. Tolerance is a key component of the immune response and ensures that adaptive immunity is directed against agents that pose genuine threats to the host and not against the host's own proteins. The failure to develop tolerance may result in dangerous reactions to self antigens, a condition called *autoimmunity* (Section 27.9).

T lymphocyte precursors leave the bone marrow via the bloodstream and enter the thymus, a primary lymphoid organ (**Figure 27.2**). During the process of T cell maturation in the thymus, immature T cells undergo a two-step selection process to (1) select potential antigen-reactive cells (positive selection) and (2) eliminate cells that react strongly with self antigens (negative selection). **Positive selection** requires the interaction of immature T cells in the thymus with peptide antigens that are actually of self origin. Using their TCRs, some T cells bind to *major histocompatibility complexes* (*MHCs*) (Section 27.5) that present self peptides on the thymic epithelial tissue. The T cells that do not bind MHC–peptide complexes will be of no use in the immune response and are therefore permanently eliminated via *apoptosis* (programmed cell death). By contrast, those T cells that bind thymic MHC proteins receive survival signals and therefore remain viable. Positive selection retains T cells that recognize MHC–peptide and deletes T cells that do not recognize MHC–peptide and would therefore be unable to recognize MHC–peptide outside the thymus.

The second stage of T cell maturation is **negative selection**. In this process, the positively selected T cells continue to interact with thymic MHC–peptide. Precursor T cells that react with thymic self antigens are potentially dangerous if they react very strongly with these antigens (autoimmunity). Very strongly self-reactive T cells bind tightly to thymus epithelial cells. This binding prevents the strongly self-reactive T cells from dividing and eventually causes them to die. This process, called **clonal deletion**, prevents the propagation of precursor T cells that could potentially cause autoimmune complications. Precursor T cells having TCRs that react less strongly with self MHC bound to peptide survive this selection and live (Figure 27.2).

This two-stage thymic selection process ensures the generation of self-tolerant T cells capable of reacting strongly to foreign antigens only. Precursors of T cell clones that are either useless (do not bind) or harmful (bind too tightly) are deleted in the thymus. About 95% of all immature T cells do not survive the thymic selection process. The remaining selected T cells are destined to interact very strongly with nonself antigens. They are not destroyed in the thymus because their weak binding interactions with thymic self antigens signal them to proliferate. The selected and growing T cells leave the thymus and migrate to the spleen, mucosa-associated lymphoid tissue (MALT), and lymph nodes, where they can contact foreign antigens presented by B cells and other antigen-presenting cells (APCs).

B Cell Selection and Tolerance

To respond to the nearly infinite variety of environmental antigens, the immune system must have the capacity to generate an essentially limitless variety of antigen-specific lymphocytes. To this end, and as we shall see in Section 27.4, the body produces an enormous diversity of antigen-reactive B cells, each having BCRs specific for a single antigen on its surface. B cell selection occurs when the BCRs of a particular B cell clone interact with their corresponding antigen. The antigen-stimulated B cell can then proliferate and differentiate, a process called *clonal expansion* (**Figure 27.3**). This produces a pool of cells that expresses the same antigen-specific receptors; B cells that have not interacted with antigen do not proliferate. The newly generated pool of antigen-specific B cell clones is composed of many antibody-producing *plasma cells* and comparatively fewer long-lived, antigen-specific memory cells. We explore these concepts further in Section 27.3.

As with T cells, the development of tolerance in B cells is necessary because antibodies produced by self-reactive B cells (autoantibodies) may cause autoimmunity and damage to host tissues (Section 27.9). Therefore, B cells must undergo a similar clonal deletion process. However, unlike the thymic selection process of T cells, many self-reactive B cells are eliminated during development in the bone marrow, the primary lymphoid organ responsible for B cell maturation in mammals.

In addition to clonal deletion, **clonal anergy** (clonal unresponsiveness) also plays a role in final selection of the B cell pool. Some immature B cells are reactive to self antigens but are not activated by them. This is because B cell activation requires a

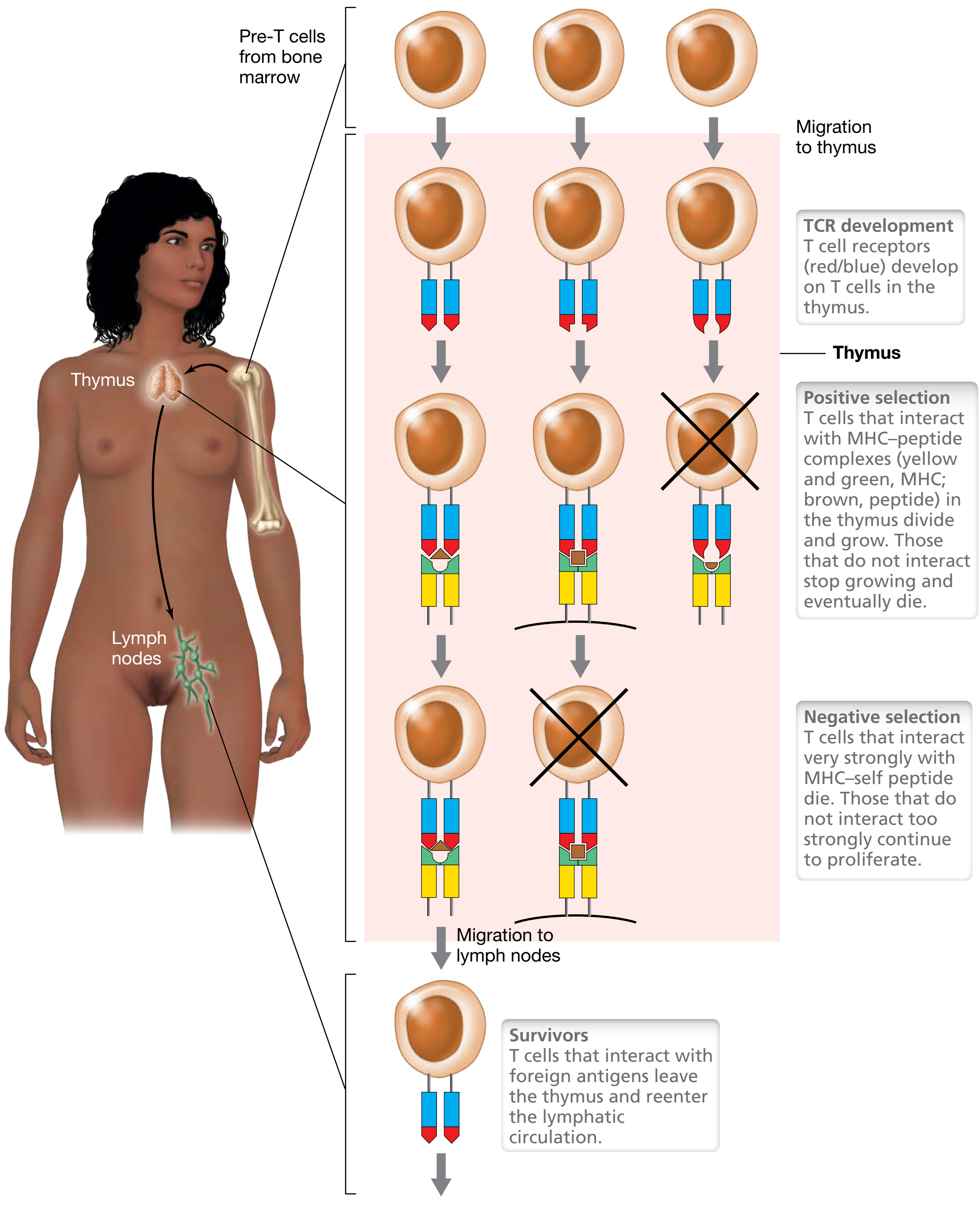


Figure 27.2 T cell selection and clonal deletion. T cells undergo selection for functionality and recognition of dangerous nonself antigens in the thymus.

second signal from a subset of T cells called *T-helper* (*Th*) *cells* (Section 27.8). The nature of this second signal is a positive interaction between proteins on the surface of B cells and Th cells that triggers the release of cytokines, especially IL-4 (*i*nter*l*eukin-4), from the Th cell that activates the B cell. If no second signal is generated because the available Th cells have been rendered tolerant to the antigen in the thymus, the B cell remains unresponsive. Using a similar mechanism, a second signal is also required to activate T cells that are interacting with antigens presented by APCs. The requirement for a second activation signal is the key to establishing and maintaining clonal anergy in potentially dangerous self-reactive B and T lymphocytes.

With their key properties of specificity, memory, and tolerance, lymphocytes stand ready to deploy the adaptive immune response. The selection and control processes just described ensure that this response will be directed only against foreign antigens. We now

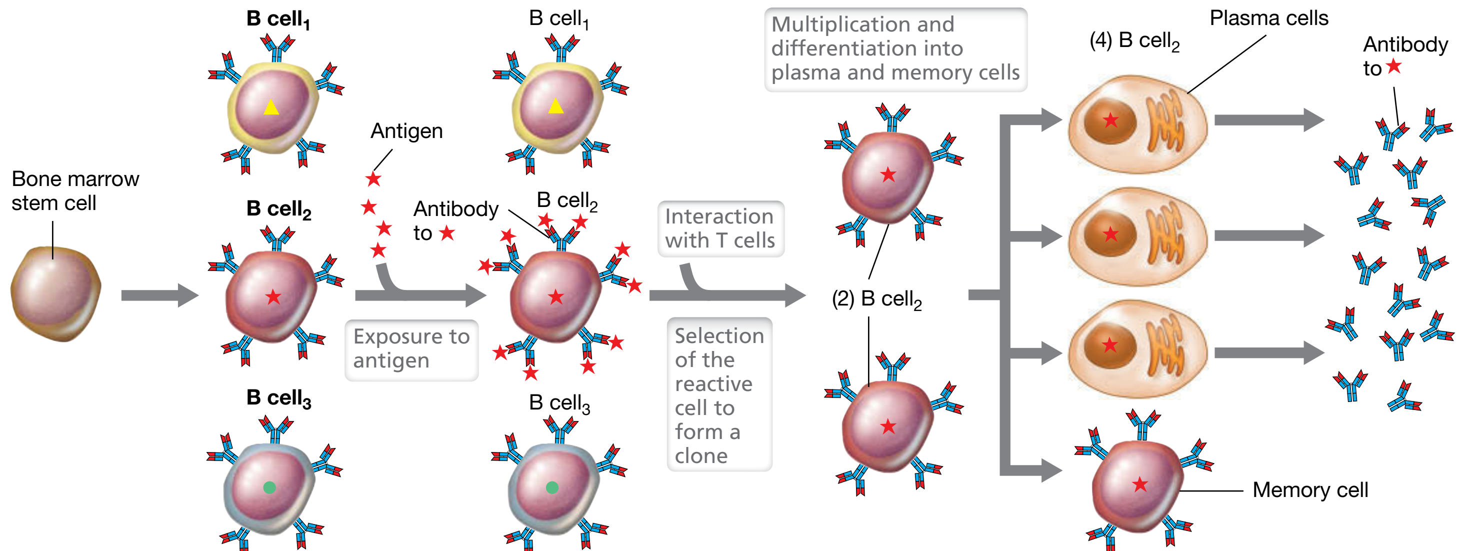


Figure 27.3 B cell clonal selection and expansion. Individual B cells, specific for a single antigen, proliferate and expand to form a clone after interaction with the specific antigen. The antigen drives selection and then proliferation of the individual antigen-specific B cell. Clonal copies of the original antigen-reactive cell have the same antigen-specific surface antibody. Continued exposure to antigen results in continued expansion of the clone.

examine the nature of antigens in more detail and consider the basic classes of adaptive immunity.

MINIQUIZ

- Distinguish between immune specificity, memory, and tolerance.
- Distinguish between positive and negative T cell selection. How do positive selection and negative selection contribute to the development of tolerance in T cells?
- Distinguish between clonal deletion and clonal anergy in B cells.

27.2 Immunogens and Classes of Immunity

The adaptive immune response recognizes a vast range of foreign macromolecules. The macromolecules are degraded and processed in host cells to produce antigens that are in turn presented to T cells. As we have discussed, **antigens** are substances that react with antibodies or TCRs. Most, but not all, antigens are **immunogens**, substances that induce an immune response. Here we examine the features of effective immunogens, define the features of antigens that promote interactions with antibodies and TCRs, and discuss the classes of immunity.

Immunogens and Antigen Binding

Immunogens share several intrinsic properties that enable them to induce an adaptive immune response. First, *molecular size* is an important property of immunogenicity; for a molecule to be immunogenic, it must be sufficiently large. Certain low-molecular-weight compounds called *haptens* do not induce immune responses themselves. However, antibodies can still bind them and they may induce an immune response if attached to a larger *carrier* molecule. Because antibodies can bind them, haptens—such as the disaccharide lactose—are considered antigens, but they are not themselves immunogens. Proteins and complex carbohydrates are effective immunogens, whereas nucleic acids, simple polysaccharides with repeating subunits, and lipids are typically not. Thus, *sufficient molecular complexity* is another key property of immunogenicity. Large, insoluble macromolecules are usually excellent immunogens because phagocytes readily engulf and process them but not soluble molecules. Thus, *appropriate physical form* is another intrinsic property of immunogenicity.

Extrinsic factors, such as the immunogen dose and the route of administration, also influence immunogenicity. The administered dose of an immunogen can be important for an effective immune response, and in general, doses of 10 μg to 1 g are effective in most mammals. Immunogens administered parenterally

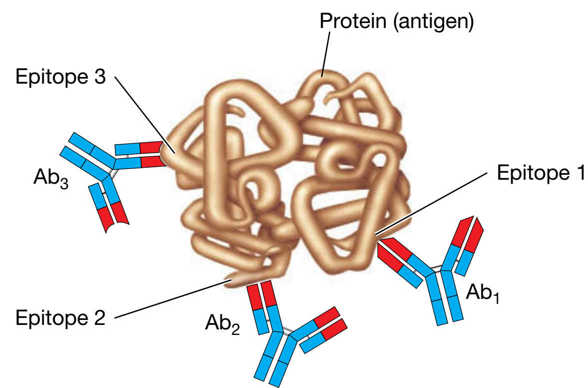


Figure 27.4 Antigens and epitopes for antibodies. Antigens may contain several different epitopes, each capable of reacting with a different antibody (Ab). The epitope 1 recognized by Ab$_1$ is a conformational epitope. Epitope 1 consists of two nonlinear parts of the folded polypeptide; the folding brings two distant portions of the polypeptide together to make a single epitope.

Natural Immunity
1
The immune system responds to an active infection such as measles.
CDC/PHIL
Active
2
Antibodies are transferred from mother to infant in utero and in breast milk.
NURSE THE BABY
YOUR PROTECTION AGAINST TROUBLE
US WPA/Library of Congress
Passive

Artificial Immunity
3
The immune system responds to antigens present in this vaccine.
CDC/PHIL/D. Jordan, M.A.
Active
4
Venom from this rattlesnake can be neutralized by antibodies in rattlesnake antivenom.
CDC/PHIL/Edward J. Wozniak, D.V.M., Ph.D
Passive

Figure 27.5 Natural and artificial immunity. Photos, left to right: (1) Childhood measles showing typical systemic measles rash. Natural active immunity requires infection with a pathogen to activate the adaptive immune response. (2) A 1934 United States government poster promoting breast-feeding. Natural passive immunity occurs when immunity is transferred from one individual to another by natural means, such as the transfer of maternal antibodies in breast milk. (3) Vaccination by nasal inhalation of antigen. Artificial active immunity occurs from exposure to particular antigens in a vaccine. (4) Timber rattlesnakes produce highly toxic venom. An antivenom consisting of purified antibodies to timber rattlesnake venom can be prepared in horses and artificial passive immunity conferred on a snakebite victim by injecting the victim with the antivenom.

(outside of the gastrointestinal tract), usually by injection, are normally more effective than those given topically or orally. When administered by oral or topical routes, antigens may be significantly degraded before contacting a phagocyte, and because of this, key immunogenic properties of the original antigen may be diminished or even lost.

Antibodies and TCRs do not interact with the immunogen as a whole but only with a distinct portion of the molecule called an **epitope** (also called an *antigenic determinant*) (Figure 27.4). Epitopes may include sugars, short peptides, and other organic molecules that are components of the larger immunogen. Antibodies interact with a sequence of four to six amino acids—the optimal size for an epitope—and can recognize conformational epitopes on proteins or polysaccharides expressed in their native conformations. By contrast, TCRs recognize epitopes only after the immunogens have been partially degraded, or *processed*, for example, by antigen-presenting cells. Antigen processing destroys the conformational structure of a macromolecule, breaking proteins into peptides shorter than about 20 amino acids long.

Antibodies and TCRs can discriminate between closely related epitopes. However, specificity is not absolute, and an individual antibody or TCR may react to some extent with several different but structurally similar epitopes. The antigen that induced the antibody or TCR is called the *homologous* antigen, and any noninducing antigens that react with the antibody are called *heterologous* antigens. An interaction between an antibody or TCR and a heterologous antigen is called a *cross-reaction*.

The engagement of antibodies and TCRs with naturally encountered immunogens induces an adaptive immune response that often leads to long-term immunity. But as we now discuss, immunity may also be conferred either by purposely introducing an eliciting antigen or through passive mechanisms, either in the absence of or in response to antigen exposure.

Classes of Adaptive Immunity: Active and Passive

Adaptive immunity may develop following a natural exposure to an infectious agent, or it may result from an intentional exposure to immunogens. In addition, an immune response can be either *active*—generated by actual exposure to the antigen—or *passive*, such as from the transfer of antibodies or immune cells from an immune individual to a nonimmune one. Active and passive immunity are illustrated in Figure 27.5 and contrasted in Table 27.1.

Natural active immunity is the outcome of exposure to antigens through infection and typically generates protective immunity from both antibodies and T cells. By contrast, *natural passive immunity* occurs when a nonimmune person acquires immune cells or antibodies through natural transfer of cells or antibodies from an immune person, such as from mothers to the fetus before birth or from mothers to newborns in breast milk (Figure 27.5).

Artificial active immunity is conferred by **vaccination (immunization)** and is a major weapon for the prevention and treatment of many infectious diseases. The introduction of antigen into the host triggers antibody production in a primary immune response but, more importantly, produces a population of immune memory cells. A second dose, often called a "booster," of the same antigen results in a secondary immune response in

TABLE 27.1 A comparison of active and passive immunity

Active immunity	*Passive immunity*
Exposure to antigen; immunity achieved by purposely administering antigen or through infection	No exposure to antigen; immunity achieved by injecting antibodies or antigen-reactive T cells
Specific immune response made by individual achieving immunity	Specific immune response made by the donor of antibodies or T cells
Immunity activated by antigen; immune memory in effect	No immune system activation; no immune memory
Immunity can be maintained via stimulation of memory cells (i.e., booster immunization)	Immunity cannot be maintained and decays rapidly
Immunity develops over a period of weeks	Immunity develops immediately

which existing memory cells are quickly activated, producing much higher levels of antibodies and a larger population of immune memory cells (Figure 27.1*c*). Active immunity may persist throughout life as a result of immune memory.

Artificial passive immunity is conferred when an individual receives antibodies from an immune individual through injection of an *antiserum*. These antibodies gradually disappear from the body, no immune memory is conferred, and a later exposure to the antigen does not elicit a secondary response. Artificial passive immunity is often used as postexposure therapy for acute infectious diseases, such as tetanus or rabies (for individuals not already protected against such diseases due to previous vaccination), and for the treatment of bites from venomous animals (antivenom, Figure 27.5). Antisera are obtained from immunized animals, such as horses, or from humans with high levels of antibody from a natural or artificial active immune response against the antigen.

MINIQUIZ

- Identify the intrinsic and extrinsic properties of an immunogen.
- Describe an epitope recognized by an antibody, and compare it to an epitope recognized by a TCR.
- Give an example for each: natural and artificial active immunity and natural and artificial passive immunity.

II • Antibodies

Antibodies provide antigen-specific immunity that protects against pathogens and dangerous soluble proteins, such as toxins. Here we look at the production, structural diversity, and antigen-binding function of antibodies. We also consider the organization and recombination of genes that encode antibodies, which underlie the nearly unlimited potential for the adaptive immune response to react to foreign molecules.

27.3 Antibody Production and Structural Diversity

An antibody, or *immunoglobulin* (*Ig*), is a soluble protein made by a B lymphocyte or a plasma cell (Figure 26.4) in response to antigen exposure. Each antibody binds to a specific antigen. Antibody-mediated immunity controls the spread of infection by recognizing and binding antigens from pathogens and their products in extracellular environments, such as blood and mucus secretions, and in so doing triggers the removal of these foreign substances from the body.

B Cells, Antibodies and Their Activities, and Memory

B cells are lymphocytes that specialize in antibody production and contain antibodies (B cell receptors, BCRs) on their surfaces; each B cell has an estimated 100,000 BCRs of identical antigen specificity. To make antibodies, a B cell must first bind antigen by way of its BCR (**Figure 27.6**). The surface antibody–antigen interaction induces the B cell to ingest the bound antigen, which is often part of an entire pathogen, by endocytosis. The B cell then digests the material and generates from it a suite of pathogen-derived antigenic peptides that are affixed to proteins of the *class II major histocompatibility complex* (*MHC II*) (Section 27.5) and displayed (*presented*) on the surface of the B cell. The B cell now functions as an APC to initiate an antibody-mediated immune response through interaction with an antigen-specific class of T lymphocyte called a *T helper* (*Th*) *cell*, more specifically, a Th2 cell. We discuss MHCs and the various T cell subsets in Section 27.8.

Th cells do not interact directly with pathogens but instead stimulate, or "help," other cells to become activated to carry out an immune response. In this case, the cell being "helped" is the antigen-presenting B cell on which the Th2 cell recognized the MHC–peptide antigen. Th2 cells produce cytokines that stimulate antigen-reactive B cells to grow and divide, which establishes a clone of the original antigen-reactive B cell. Each activated B cell clone can then proliferate and differentiate into several thousand plasma cell clones, each with the ability to produce and secrete large amounts of antibodies of identical antigenic specificity (Figure 27.6). This primary antibody response (Figure 27.1*c*) is detectable within about five days after antigen exposure, and serum levels of antibodies reach their peak within several weeks.

Some of the activated B cell clones remain in circulation in the immune system as long-lived *memory B cells* (Figure 27.6). Subsequent exposure to the same antigen, for example by reinfection with the same pathogen, stimulates the antigen-reactive memory B cells, producing a secondary antibody response characterized by a faster development of higher quantities of antibodies (Figures 27.1*c* and 27.6). Recall that the secondary response, conferred by immune memory, is the basis for vaccination (Section 27.2).

Antibodies released from plasma cells interact with antigen, which is often located directly on the pathogen. Antibody binding may have multiple effects on a pathogen, but most antibody interactions do not directly kill pathogens but instead block interactions between pathogens (or their products) and host cells. For example, antibodies present on mucous membranes may specifically interact with influenza virus antigens able to bind to host cells, thereby blocking attachment of the influenza virus to host cells on the mucosal surface. In addition, circulating antibodies in blood and lymph serum can *neutralize* toxins by binding them and preventing their attachment to host cell receptors (**Figure 27.7**). In other cases, antibodies coat pathogens by binding to their surface antigens, a process called *opsonization*, thereby marking them for destruction by phagocytosis (Section 26.9). Phagocytes have antibody receptors called *Fc receptors* (*FcR*) that bind to any antibody attached to an antigen, resulting in enhanced phagocytosis of the antibody-coated cells or viruses (Figure 26.14*a*).

Immunoglobulin G Structure and Function

There are five classes of immunoglobulins—*IgG, IgM, IgA, IgD*, and *IgE*—distinguished from one another by their different physical, chemical, and immunological properties. Based on these distinctions, each antibody class has a defined distribution and general function (**Table 27.2**). IgG is the most common circulating antibody, comprising up to 80% of serum immunoglobulins. IgG is composed of four polypeptide chains that are interconnected by disulfide (S—S) bonds (**Figure 27.8**). In each IgG molecule, two identical short chains (called *light chains*) are paired with two

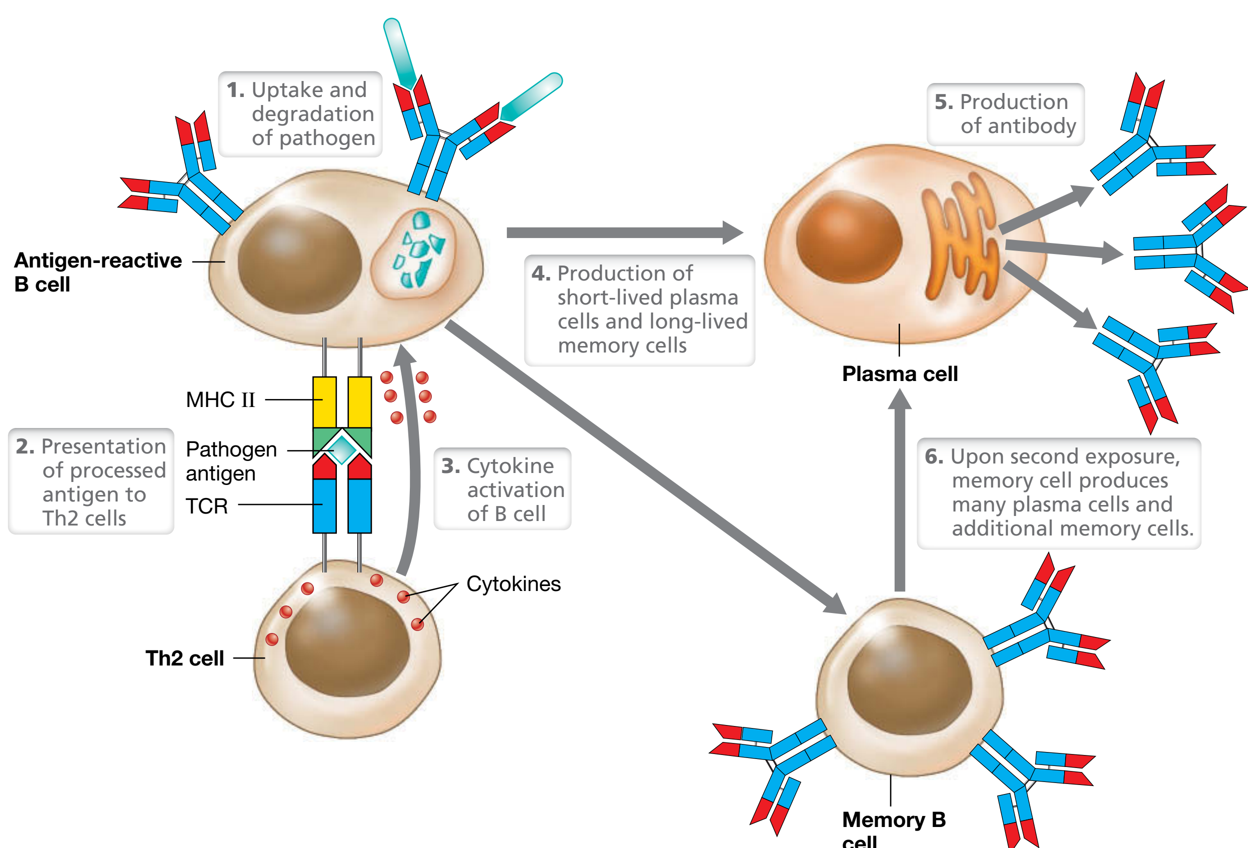


Figure 27.6 B cell–T cell interaction and antibody-mediated immunity. B cells interact with antigen and Th2 cells to produce antibodies. B cells initially function as antigen-presenting cells. First, their antigen-specific Ig receptor traps antigen. Following endocytosis, antigens are processed into peptide fragments, which are bound by MHC II and transported to the B cell surface. The MHC II–peptide complex is bound by the TCR on the Th2 cell, causing the Th2 cell to secrete IL-4 and IL-5. These cytokines activate the B cell to produce clones that differentiate into many antibody-producing plasma cells and a smaller number of memory cells. The latter are long-lived and can differentiate into antibody-producing plasma cells upon secondary exposure to the same antigen.

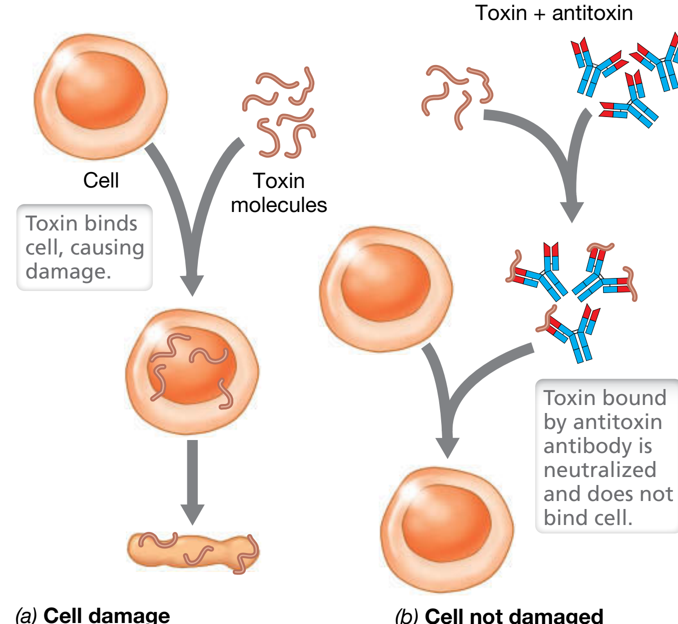


Figure 27.7 Neutralization of an exotoxin by an antitoxin antibody. *(a)* Toxin results in cell destruction. *(b)* Antitoxin prevents cell destruction. Examples of exotoxins include botulism and tetanus toxins and are discussed in Section 25.6.

identical longer chains (called *heavy chains*), yielding a Y-shaped, symmetrical molecule. Each light chain has about 220 amino acids, and each heavy chain has about 440 amino acids. Each heavy chain interacts with a light chain to form a functional *antigen-binding site*. Therefore, an IgG antibody molecule is *bivalent* because it has two identical sites that bind two identical antigenic determinants.

Heavy and light chains consist of a series of distinct protein domains, each composed of about 110 amino acids. A heavy-chain *variable domain* is connected to three heavy-chain *constant domains* (Figure 27.8*a*). The variable domain and the first constant domain (C_H1) compose part of the *Fab* (*f*ragment, *a*ntigen-*b*inding) portion of the immunoglobulin, so named because it contains the antigen-binding site. The two constant domains located most distal to the variable domain (C_H2 and C_H3) compose the *Fc* (*f*ragment, *c*rystalline) region of the antibody, named for its tendency to crystallize in solution. As previously mentioned, it is the Fc region of the antibody that binds FcR molecules on the surface of phagocytes to facilitate phagocytosis. The light chains of the antibody consist of one variable and one constant domain each and contribute to the two Fab regions only (Figure 27.8*a*).

Because the variable domains of a given antibody bind a specific antigenic determinant, their amino acid sequences differ

TABLE 27.2 Properties of human immunoglobulins

Class/H chain[a]	*Structural conformation/ Antigen-binding sites*	*Serum concentration (mg/ml)/Percent of total circulating antibody*	*Properties*	*Distribution*
IgG/γ	Monomer/2	13.5/70–80%	Major circulating antibody; four subclasses: IgG1, IgG2, IgG3, IgG4; IgG1 and IgG3 activate complement	Extracellular fluid; blood and lymph; intestine; crosses placenta
IgM/μ	Pentamer/10 Monomer/2	1.5/6–10% 0	First antibody to appear in primary response to extracellular pathogens or after immunization; pentamer especially effective in agglutinating antigens; strong complement activator	Blood and lymph; monomer is B cell surface receptor (BCR)
IgA/α	Monomer/2 Dimer/4	3.5/10–20% 0.05/0.2–0.3%	Important circulating antibody Major secretory antibody	Blood and lymph (monomer) and secretions, such as mucus, saliva, and colostrum (dimer)
IgD/δ	Monomer/2	0.03/0.2–0.3%	Minor circulating antibody; mostly associated with mature B cells	B cell surface receptor (BCR); blood and lymph (trace)
IgE/ε	Monomer/2	0.00005/0.0003%	Facilitates parasite immunity but also triggers allergic reactions	Blood and lymph; binds to mast cells and eosinophils

[a]All immunoglobulins may have either λ or κ light-chain types, but not both.

in each different antibody. The variable domain of a light chain (V_L) interacts with the variable domain of a heavy chain (V_H) to bind antigen. By contrast, the constant domains of each heavy chain are identical in amino acid sequence for all Ig molecules of a given class. Similarly, in light chains of the same type, the amino acid sequence of the constant domain is the same.

Other Immunoglobulin Classes and Their Functions

Immunoglobulins of the other classes differ from IgG in structure and function. Because the amino acid sequence of heavy-chain constant domains determines antibody class, the heavy chain called *gamma* (γ) defines the IgG class. Likewise, *alpha* (α) defines IgA; *mu* (μ) defines IgM; *delta* (δ) defines IgD; and *epsilon* (ε) defines IgE (Table 27.2). The constant-domain sequences constitute three-fourths of the heavy chains of IgG, IgA, and IgD and four-fifths of the heavy chains of IgM and IgE (**Figure 27.9*a, b***).

The structure of IgM is shown in Figure 27.9*c*. Circulating IgM forms an aggregate of five immunoglobulin molecules attached by at least one J (joining) peptide. IgM is the first class of antibody produced in a typical immune response to a bacterial infection, but IgM antibodies generally have low *affinity* (binding strength) for antigen. However, overall antigen-binding strength is enhanced because pentameric IgM molecules have ten binding sites available for interaction with antigen (Table 27.2 and Figure 27.9*c*). The ten antigen-binding sites make IgM especially effective at agglutinating (clumping) infectious particles, thereby increasing phagocytic efficiency. IgM is also a strong activator of complement through the classical pathway (Section 26.9). Whereas up to 10% of serum antibodies are pentameric IgM, IgM monomers do not circulate in the blood but rather are one of the classes of antibodies that function as B cell receptors.

Dimers of IgA are present in high numbers in secreted body fluids, including saliva, tears, breast milk colostrum, and mucosal secretions from the gastrointestinal, respiratory, and genitourinary tracts. The mucosal surfaces in an average adult, which total about 400 m^2 (skin has about 6 m^2), contain MALT and produce large amounts (about 10 g) of secretory IgA every day. By comparison, an individual produces only about 5 g of serum IgG per day. Whereas *serum* IgA typically occurs in monomeric form (Table 27.2), *secretory* IgA consists of two IgA molecules covalently linked by a J chain peptide and complexed with a protein called the *secretory component* that aids in transport of IgA across

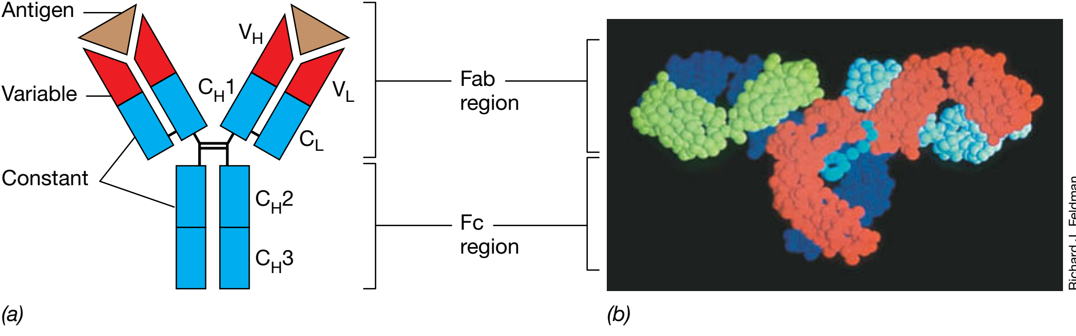


Figure 27.8 Immunoglobulin G structure. *(a)* IgG consists of two heavy chains (50,000 molecular weight) and two light chains (25,000 molecular weight), with a total molecular weight of 150,000. One heavy and one light chain interact to form an antigen-binding unit. The variable domains of the heavy and light chains (V_H and V_L) bind antigen. The constant domains (C_H1, C_H2, C_H3, C_L) are identical in all IgG proteins. The chains are covalently joined with disulfide bonds. The Fab (*f*ragment, *a*ntigen-*b*inding) region contains the antigen-binding site. The Fc (*f*ragment, *c*rystalline) stem of the antibody binds receptor molecules on phagocytes to facilitate phagocytosis of opsonized pathogens. *(b)* Space-filling model of an IgG molecule. The heavy chains are red and dark blue. The light chains are green and light blue.

membranes (Figure 27.9*d*). The high concentration of secretory IgA in breast milk colostrum likely plays a key role in preventing gastrointestinal disease in newborns.

IgE is found in very small amounts in serum; about 1 of every 3000 serum Ig molecules is IgE. Most IgE is bound to cells. For example, through its Fc region IgE binds eosinophils, arming these granulocytes to target parasitic pathogens such as schistosomes and other helminths (Section 33.7). IgE also binds to tissue mast cells, where subsequent binding of antigen to antigen-binding sites of IgE causes degranulation of the mast cells. The ensuing release of chemical mediators, such as histamine and serotonin, triggers immediate-type hypersensitivities (a type of allergic response, Section 27.9). Like IgM, IgE has a fourth heavy-chain constant domain (Figure 27.9*b*), and this allows for binding of IgE to eosinophils and mast cells, the key step for activating the protective and allergic reactions, respectively, associated with these cell types.

IgD (Figure 27.9*a*), present in serum in low concentrations, has no known unique immune protective function. However, IgD, like IgM, is abundant on the surfaces of mature B cells, especially memory B cells, where it functions as a B cell receptor.

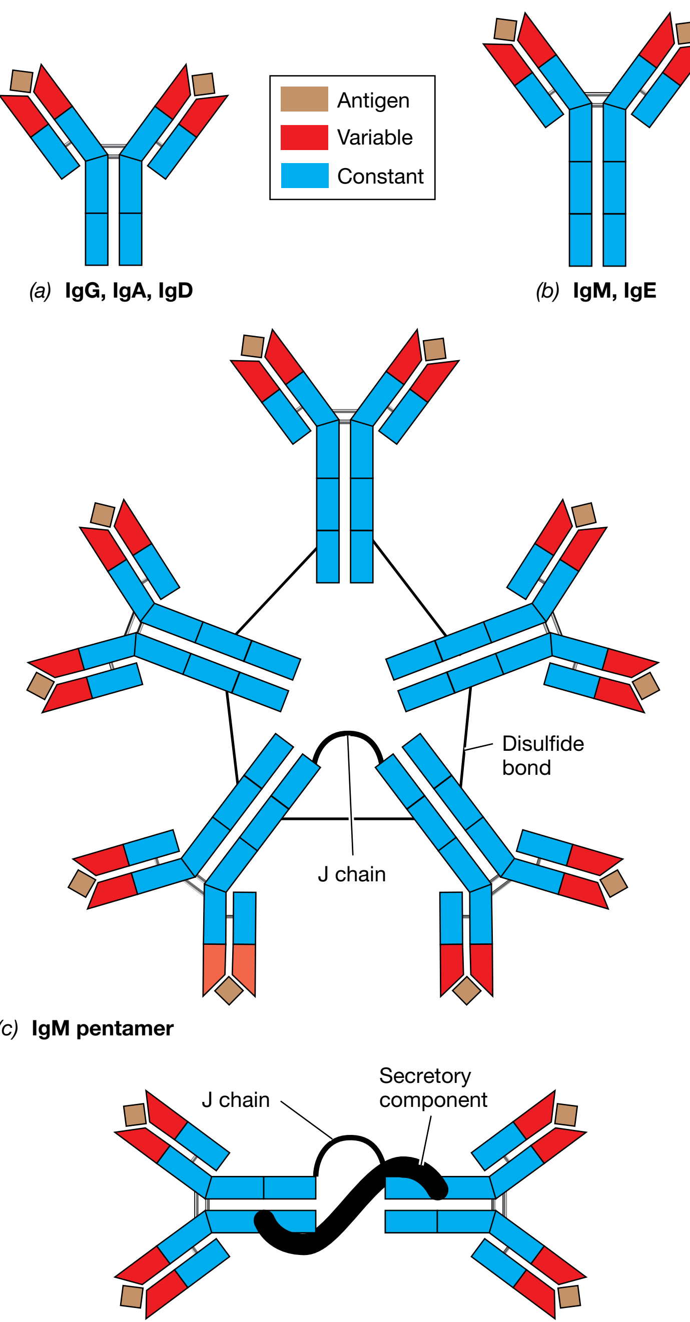


Figure 27.9 Immunoglobulin classes. All classes of Igs have V_H and V_L that bind antigen. *(a)* IgG, IgA, and IgD have three constant domains. *(b)* The heavy chains of IgM and IgE have a fourth constant domain. *(c)* IgM is found in serum as a pentameric protein consisting of five IgM proteins covalently linked to one another via disulfide bonds and a joining (J) chain protein. Because it is a pentamer, IgM can bind up to ten antigens, as shown. *(d)* Secretory IgA is often found in body secretions as a dimer consisting of two IgA proteins covalently linked to one another by a J chain protein. The secretory component aids in transport of IgA across mucosal membranes.

Antigen Exposure, Immune Memory, and the Primary and Secondary Responses

We have seen that immune memory is a major characteristic of the adaptive immune response (Figure 27.1*c*). Starting with a B cell, an antibody-mediated immune response begins with antigen exposure and culminates with the production and secretion of antigen-specific antibodies, and the route of antigen exposure influences the class of the antibodies produced. Blood and lymph, as well as the spleen and MALT (Chapter 26), are key sites for the introduction of antigens. Antigen introduced into the bloodstream by injection or natural infection travels to the spleen, where IgM, IgG, and serum IgA antibodies are formed. Antigen introduced subcutaneously, intradermally, topically, or intraperitoneally is carried through the lymphatic system to the nearest lymph nodes, again stimulating production of IgM, IgG, and serum IgA. Antigen introduced to mucosal surfaces is delivered to the nearest MALT. For example, antigen delivered orally is delivered to the MALT in the intestinal tract, preferentially stimulating production of secretory IgA in the gut.

Following initial antigen contact, each antigen-stimulated B cell multiplies and differentiates to form antibody-secreting plasma cells and memory B cells (Figure 27.6). Plasma cells in this *primary antibody response* are relatively short-lived (less than one week) and secrete large amounts of mostly IgM antibody (**Figure 27.10**). After a latent period of several days, antibody appears in the blood and a gradual increase in *antibody titer* (antibody quantity) occurs. As antigen disappears, the primary antibody response slowly diminishes.

Memory B cells generated by the initial exposure to antigen may circulate in the host for years. A second exposure to the same antigen stimulates memory B cells to rapidly differentiate into plasma cells and produce antibody; memory B cells need no T cell help. The second and each subsequent exposure to antigen causes the antibody titer to rise rapidly to a level often 10–100 times greater than the titer following the first exposure (Figure 27.10). This rise in antibody titer is called the *secondary antibody response*. The secondary response is a function of immune memory and typically results in a switch from the production of IgM to another antibody class, a transition called *class switching* (Figure 27.10). This switch is induced by a cytokine signal produced in response to the specific type of

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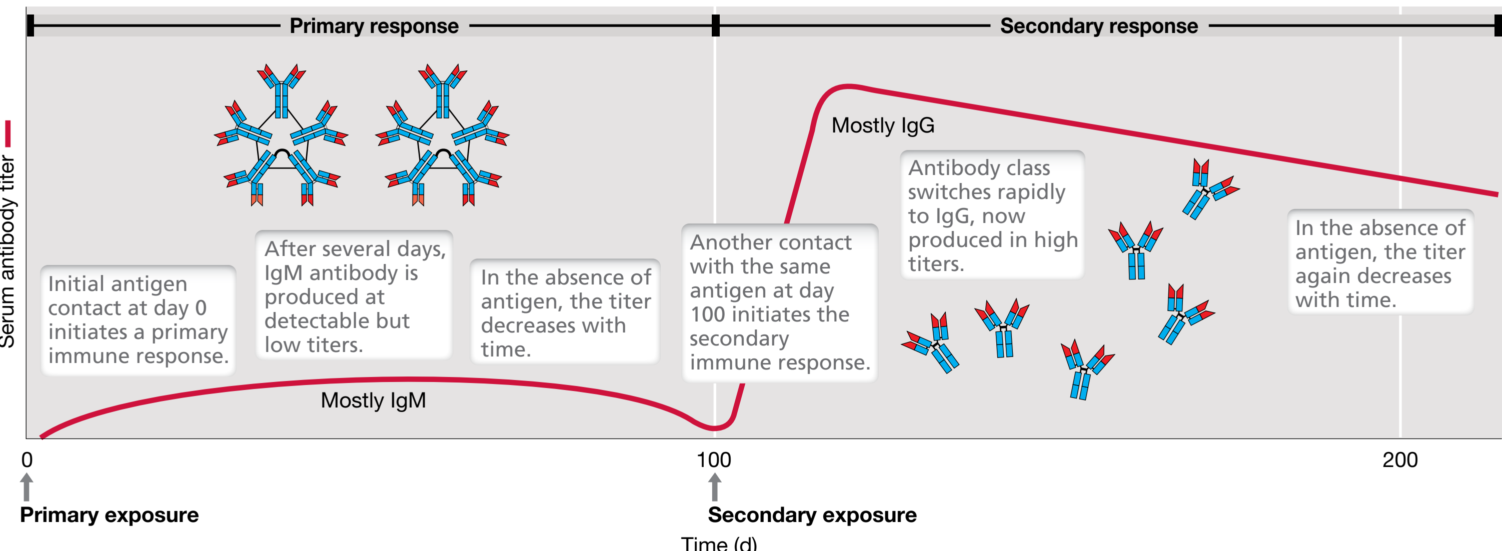

Figure 27.10 Primary and secondary antibody responses in serum. The antigen exposures at day 0 and day 100 must be to identical antigen to induce a secondary response. The secondary response, also called a booster response, may be more than 10-fold greater than the primary response. Note the class switch from IgM production in the primary response to IgG production in the secondary response.

pathogen invasion. The signal prompts a recombination event that switches the heavy-chain constant region, resulting in the production of the antibody class that can most efficiently address the threat. For example, in serum, the most common antibody class switch is from IgM to IgG, whereas in mucosal tissues, switches to IgA predominate. It is important to note that class switching does not cause a change in antigen specificity; the new class of antibody generated remains specific to the original antigen.

Serum antibody titer slowly decreases over time, but subsequent exposure(s) to the same antigen can trigger another secondary response. This rapid and strong memory response is the basis for the immunization procedure known as a "booster shot" (for example, the rabies shot given every 1–3 years to domestic animals). Periodic reimmunization maintains high levels of memory B cells and circulating antibody specific for a particular antigen, providing long-term artificial active immunity against individual infectious diseases.

MINIQUIZ

- Summarize antibody production starting with pathogen interaction with a B cell.
- Differentiate among antibody classes using structural characteristics, distribution patterns, and functional roles.
- Explain the rationale for periodic revaccination in children and adults.

27.4 Antigen Binding and the Genetics of Antibody Diversity

We have seen that antibodies consist of four polypeptides, two heavy (H) chains and two light (L) chains (Figure 27.8), and each chain is further divided into constant (C) and variable (V) domains. The V domains of one H and one L chain interact to form the antigen-binding site. Here we examine the structural features of the V domains that define the antigen-binding site and then explore the genetics behind the enormous diversity of antibodies that are possible.

Variable Domains and Antigen–Antibody Interaction

The V domains of an H and an L chain interact to form a receptor that binds antigen strongly but not covalently; the measurable strength of binding is the antibody's *binding affinity*. The vertebrate immune system can recognize, or bind, countless antigens, and each antigen binds to an antibody at a unique antigen-binding site (**Figure 27.11**). To accommodate all possible antigens that a host might encounter, the synthesis of billions of different antibodies, each with its own unique antigen-binding site, is necessary. The V domains define these unique antigen-binding sites.

Amino acid sequences differ in the V domains of Igs that bind different antigens. Amino acid variability is especially apparent in several **complementarity-determining regions (CDRs)**. The three CDRs in each of the V domains provide most of the molecular contacts with antigen (Figure 27.11). The amino acid sequences of CDR1 and CDR2 differ in minor ways between different Igs, while CDR3s differ in sequence dramatically from one another. Three distinct gene segments encode CDR3 of the H chain: the carboxy-terminal portion of the V domain, followed by a short "diversity" (D) segment of about three amino acids, and a longer "joining" (J) segment about 13–15 amino acids long. The light-chain CDR3 is similar except that it lacks the D segment. The heavy- and light-chain CDRs, six in total, confer highly specific antigen binding on the antibody molecule (Figure 27.11).

The Ig three-dimensional structure was shown in Figure 27.8*b*. Each antigen–antibody interaction requires the specific binding of an antigenic *epitope* with the CDR domains of the H and

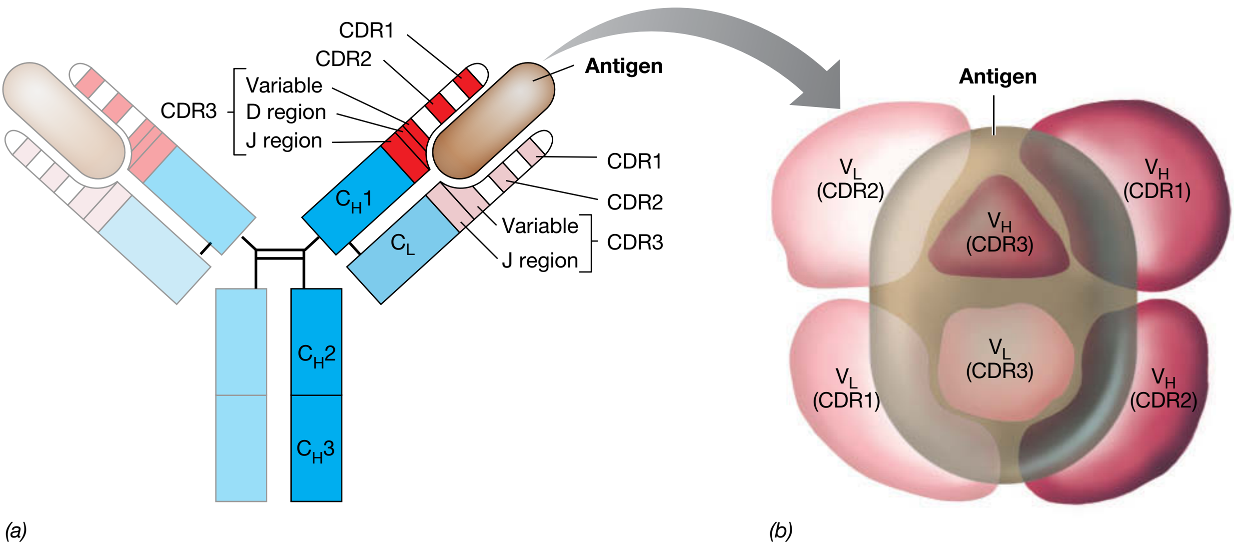

Figure 27.11 Antigen binding by immunoglobulin light and heavy chains. *(a)* An Ig is shown schematically, with bound antigen (light brown). The V domains on the H and L chains are shown in red (heavy chain) and pink (light chain), with the antigen-binding CDR1, CDR2, and CDR3. C_H1, C_H2, and C_H3 (dark blue) are constant domains in the H chain, and C_L (light blue) is the constant domain in the L chain. *(b)* Complementarity-determining regions (CDRs) from both H (red) and L (pink) chains, shown from above, are conformed to make a single antigen-binding site on the Ig. The highly variable CDR3s from both H and L chains cooperate at the center of the site. Antigen (light brown) may contact all CDRs. The shape of the site may be a shallow groove or a deep pocket, depending on the antibody–antigen pair involved.

L chains. A single antigen-binding site of an antibody molecule measures about 2×3 nm, creating a space large enough to accommodate an epitope of 10 to 15 amino acids. Antigen binding is ultimately a function of the Ig folding pattern of the H and L polypeptide chains. The Ig folds of the V region bring all six CDRs (CDR1, 2, and 3 from both heavy and light chains) together, resulting in the formation of a unique and specific antigen-binding site (Figure 27.11).

Because an individual can recognize and bind an immense variety of antigens, the immune system must be capable of virtually unlimited antibody variation. In order to accomplish this from the relatively small genomic investment evolution has made in immunoglobulin genes, several unusual genetic mechanisms come into play. **Table 27.3** summarizes these diversity-generating mechanisms, and we explore each below.

Genetic Organization of the Immunoglobulin Molecule

The gene encoding each immunoglobulin H or L chain is constructed from several gene segments. In each B cell, Ig gene segments undergo a series of random, somatic rearrangements characterized by genetic recombination followed by deletion of intervening sequences. These events produce a single, functional antibody gene derived from a relatively small pool of Ig gene segments. As a B cell matures, V, D, and J gene segments are enzymatically recombined to form a single Ig heavy-chain gene (**Figure 27.12*a***). The single V gene segment encodes CDR1 and CDR2, whereas CDR3 is encoded by a mosaic of the 3′ end of the V region, followed by the D and J gene segments.

In each B cell, only one protein-producing rearrangement occurs in the heavy- and light-chain genes. Called *allelic exclusion*, this mechanism causes each B cell to express Ig genes from only one of the two inherited parental alleles, thus ensuring that all Igs from a given clone of B cells have identical antigen specificity. Finally, separate C gene segments encode the class-defining constant domains of Igs. Therefore, four different gene segments—V, D, J, and C—recombine to form one functional heavy-chain gene (Figure 27.12*a*). Light chains, which lack D segments, are encoded in a similar way by recombination of light-chain V, J, and C segments (Figure 27.12*b*). The gene segments required for all Igs exist in all nucleated cells of the body but undergo recombination only in developing B lymphocytes.

V, D, J, and C gene segments are separated by noncoding sequences (introns) typical of gene arrangements in eukaryotes. Genetic recombination occurs in each B cell during its development. One each of the V, D, and J segments is recombined at random to form a functional heavy-chain gene, while randomly recombined V and J segments form a complete light-chain gene.

TABLE 27.3 Generation of antigen-binding receptor diversity in B cells and T cells

Diversity-generating mechanism	*B cell Ig receptors, heavy and light chains*	*T cell receptors, α and β chains*
Somatic recombination of tandem genes	Yes	Yes
Random reassortment	Yes	Yes
Imprecise V-D-J or V-J joining	Yes	Yes
Nucleotide additions at V-D-J or V-J junctions	Yes	Yes
D gene segments read in all 3 frames	No	Yes
Somatic hypermutation	Yes	No

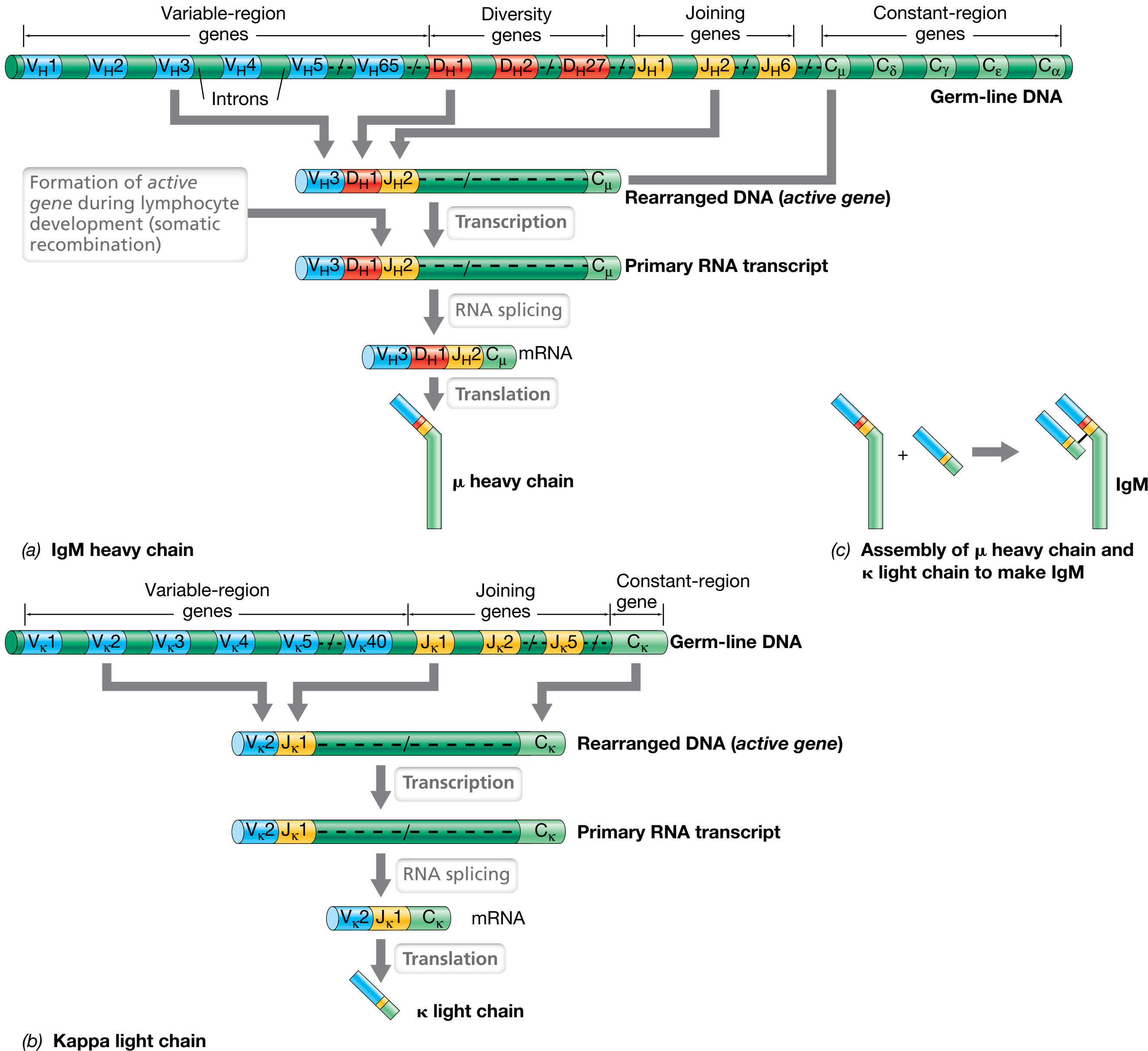

Figure 27.12 Immunoglobulin gene rearrangement in human B cells. Ig genes are arranged in tandem on three different chromosomes. *(a)* The H chain gene complex on human chromosome 14. The filled boxes represent Ig coding genes. The broken lines indicate intervening sequences and are not shown to scale. *(b)* The κ light-chain complex on human chromosome 2. The λ light-chain genes are in a similar complex on human chromosome 22. *(c)* Assembly of one-half of an antibody molecule.

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The active gene, still containing an intervening sequence between the VDJ or VJ gene segments and the C gene segments, is transcribed, and the resulting primary RNA transcript is spliced to yield the final messenger RNA (mRNA). The mRNA is then translated to make the heavy and light chains of the Ig molecule.

Reassortment, VDJ Joining, and Hypermutation

If one considers all of the random reassortments possible in genes encoding immunoglobulin heavy and light chains and from these calculates the number of unique molecules that could be encoded, the total diversity is astonishing. In humans, for example, based on the numbers of kappa (κ) light-chain gene segments, there are about 40 V × 5 J possible rearrangements, or 200 possible κ light chains. For the alternative lambda (λ) light chain, the type produced instead of κ by some B cells in every individual, there are about 30 V × 4 J = 120 possible combinations. About 10,500 possible heavy chains can be formed by the rearrangement of about 65 V × 27 D × 6 J genes. Assuming that each heavy chain and light chain has an equal chance to be expressed, there are 10,500 × 200 = 2,100,000 possible immunoglobulins with κ light chains and 10,500 × 120 = 1,260,000 possible immunoglobulins with λ chains. Therefore, at least 3,360,000 possible antibodies can be expressed based on random reassortment alone!

On top of this impressive diversity, additional diversity is generated in the CDR3 regions of both heavy and light chains by several unique mechanisms. First, the DNA-joining mechanism that constructs V-D or D-J gene segments in the heavy chain or the V-J gene segments in the light chain is an imprecise process. The final nucleotide sequence at these regions occasionally varies by a few nucleotides from the original genomic sequence, and when this occurs, it will alter the amino acid sequence in this region. Even more diversity is generated at V-D and D-J coding joints in the heavy-chain genes and at V-J coding joints in light-chain genes by either random (N) or template-specific (P) nucleotide additions. Because these coding joints are contained within the sequences that encode CDR3 on both heavy and light chains, N and P diversity at V-domain coding joints changes or adds amino acids in the CDR3 of both heavy and light chains.

Immunoglobulin diversity is further expanded by the process of **somatic hypermutation**, the mutation of B cell Ig genes at much higher rates than those observed in other genes. Somatic hypermutation of Ig genes is typically evident after a second exposure to an immunizing antigen and occurs only in the V regions of rearranged heavy- and light-chain genes, creating B cells with slightly altered Ig cell surface receptors. These mutated B cells compete for available antigen, and B cells whose receptors have a higher affinity for antigen than the original B cell receptor are selected. This *affinity maturation* process is one of the factors responsible for a dramatically stronger secondary immune response (Figures 27.1*c* and 27.10). Affinity maturation also adds more diversity to the pool of antibody specificity in the adaptive immune response, thus making the potential antibody repertoire essentially infinite.

MINIQUIZ

- Draw a complete Ig molecule and identify antigen-binding sites on the antibody.
- Describe antigen binding to the CDR1, 2, and 3 regions of the heavy-chain and light-chain variable domains.
- Describe the recombination events that produce a mature heavy-chain gene and other somatic events that further enhance antibody diversity.

III • The Major Histocompatibility Complex (MHC)

The *major histocompatibility complex* (*MHC*) is a series of genes found in all vertebrates that encodes a group of proteins important in antigen presentation. The MHC proteins in humans are called **human leukocyte antigens (HLAs)** and were first identified as the major antigens responsible for immune-mediated tissue transplant rejection. We now know that MHC proteins function primarily as antigen-presenting molecules, binding pathogen-derived peptides and displaying these peptides for interaction with T cell receptors.

27.5 MHC Proteins and Their Functions

The MHC proteins consist of two protein classes encoded by about 4 megabase pairs (Mbp) of DNA (Figure 27.13*a*). **MHC class I proteins** are found on the surfaces of all nucleated cells and function to present peptide antigens to *T-cytotoxic* (*Tc*) *cells*. If a peptide antigen presented by MHC class I is recognized by the TCR of a Tc cell, the antigen-containing cell is quickly destroyed (Section 27.8). **MHC class II proteins** are found only on the surface of B lymphocytes, macrophages, and dendritic cells—the antigen-presenting cells (APCs) (Section 26.4). Through their class II proteins, APCs present antigens to *T-helper* (*Th*) *cells*, stimulating cytokine production that leads to antibody-mediated immunity or inflammatory responses (Section 27.8).

Class I and Class II MHC Proteins

Class I MHC proteins consist of two polypeptides (Figure 27.13*b, d*, and *e*). The first of these is the membrane-embedded *alpha* (α) *chain*, encoded by a gene located in the MHC gene region. The second is *beta-2 microglobulin* ($\beta_2 m$), a smaller, noncovalently associated protein encoded by a gene not included in the MHC gene cluster. The class I α chain folds to form a peptide-binding groove that accommodates peptides of 8 to 11 amino acids. The peptides in infected cells are derived from *endogenous* (intracellular) foreign antigens (Figure 27.13*d*), for example, from viral proteins produced inside the cell. Following translocation to the cell surface, the MHC–virus-peptide complexes, which to the immune system look much like the variant MHC proteins associated with a tissue transplant, are recognized as foreign by TCRs on Tc cells and are destroyed.

Class II MHC proteins consist of two membrane-integrated polypeptides, α and β, found only on APCs. One α and one β polypeptide, expressed together, form a functional heterodimer (Figure 27.13*c, f*). The α1 and β1 domains of the class II protein interact to form a binding site for TCR–peptide similar to the class I binding site for TCR–peptide. However, the peptide-binding groove in the class II MHC is able to bind and display peptides that may be significantly longer than 8–11 amino acids. Peptides presented by class II MHCs are typically 10 to 20 amino acids long and are proteolytic fragments derived from *exogenous* (extracellular) pathogens that have been internalized and processed by APCs. The APCs use the class II–peptide complex to interact with TCRs on Th cells, leading to Th activation and an adaptive immune response (Section 27.8).

Antigen Processing and Presentation to T Cells

MHC proteins are expressed on cell surfaces only when they are complexed with a peptide, either a self peptide or a foreign peptide. In essence, then, *MHC–peptide complexes reveal to the immune system the protein composition of the cell*, and in this capacity, function to alert the immune system when a cell contains foreign antigens. T cells, through their TCRs, conduct surveillance of cell surfaces to identify any cells expressing foreign antigens. When the latter are encountered, the TCR interacts with the foreign antigen presented on the MHC protein, and this interaction targets the cell for destruction. T cells do not react with MHC complexes containing only self peptides because self-reactive T cells are eliminated from the host during the development of immune tolerance (Section 27.1).

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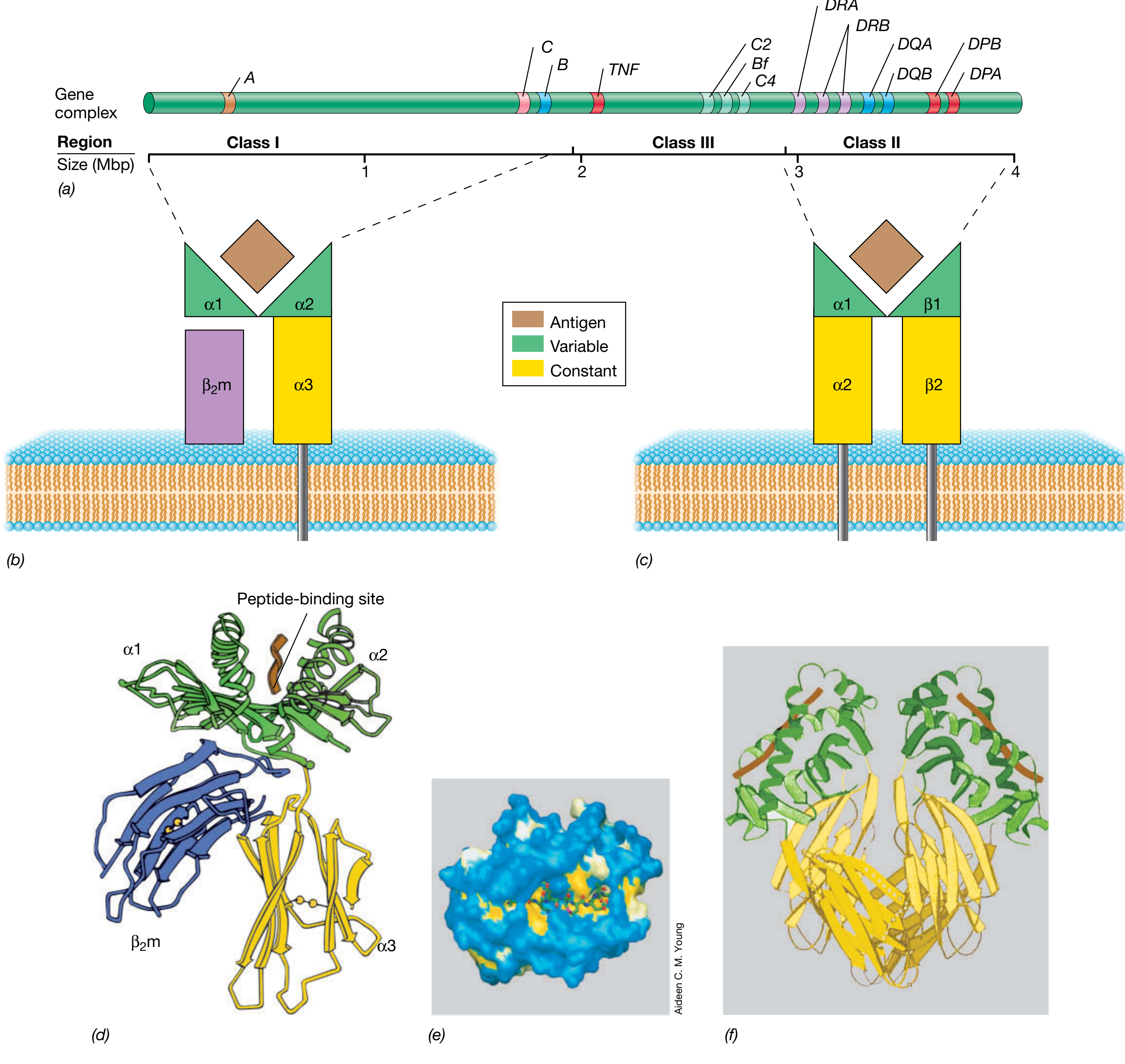
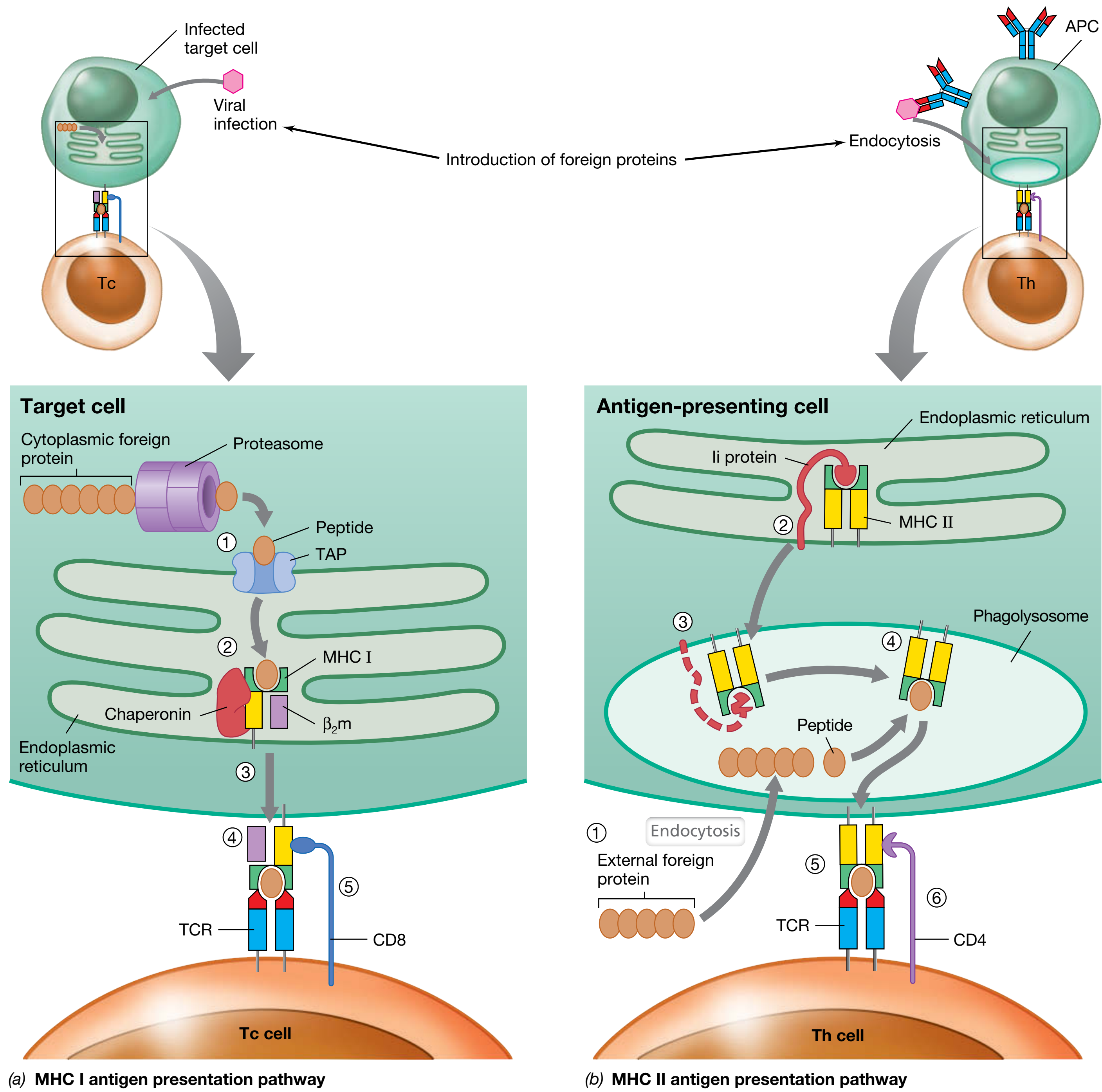


Figure 27.13 Human leukocyte antigen (HLA) genes and MHC proteins. *(a)* The HLA complex, located on human chromosome 6, contains more than 4 megabase pairs (Mbp). Class II genes *DPA* and *DPB* encode class II proteins DPα and DPβ; *DQA* and *DQB* encode DQα and DQβ; *DRA* and two *DRB* loci encode DRα and DRβ proteins. The class I MHC proteins HLA-A, HLA-C, and HLA-B are encoded by genes *A, C*, and *B*. The class II and class I loci are highly polymorphic and encode peptide-binding proteins. Class III MHC genes encode proteins associated with immune-related functions, such as complement proteins C4 and C2 and the cytokine TNF (tumor necrosis factor). *(b)* Schematic of MHC class I protein. The α1 and α2 domains interact to form the peptide antigen–binding site. *(c)* Schematic of MHC class II protein. The α1 and β1 domains combine to form the peptide antigen–binding site. *(d)* MHC class I protein structure. Beta-2 microglobulin (β_2m) binds noncovalently to the α chain. The antigen peptide (brown) is bound cooperatively by the α1 and α2 domains. *(e)* An MHC I protein with a bound peptide, as seen from above. A peptide of nine amino acids is shown as a carbon backbone structure, embedded in a space-filling model of a mouse MHC I protein. *(f)* Structure of MHC class II protein dimer. The peptides (brown) are shown in their positions in the binding sites of the MHC II proteins.

Two distinct antigen-processing schemes are at work to initiate adaptive immune responses: one for MHC I antigen presentation and another for MHC II antigen presentation. MHC I proteins present peptide from viruses or other intracellular pathogens; such infected cells are called *target cells* (Figure 27.14*a*). Proteins derived from infecting viruses, for example, are taken up and digested in the cytoplasm in a structure called the *proteasome*. Peptides about ten amino acids long are transported into the endoplasmic reticulum (ER)



(a) **MHC I antigen presentation pathway**

(b) **MHC II antigen presentation pathway**

Figure 27.14 Antigen presentation by MHC I and MHC II proteins. *(a)* ① Protein antigens, such as virus components manufactured within the cell, are degraded by the proteasome in the cytoplasm. The peptide fragments are transported into the endoplasmic reticulum (ER) through a pore formed by the TAP proteins. ② MHC I proteins in the ER are stabilized by chaperonins until peptide fragments are bound. ③ When peptide fragments are bound by MHC I, the complex is transported to the cell surface. ④ The MHC I–peptide complex interacts with T cell receptors (TCRs) on the surface of Tc cells. ⑤ The CD8 coreceptor on the Tc cell engages MHC I, resulting in a stronger complex. The Tc cell is activated by the binding events, causing it to release cytokines and cytolytic toxins and kill the target cell. *(b)* ① External foreign proteins are imported into the cell and digested into peptide fragments in phagolysosomes. ② MHC II proteins in the ER are assembled with Ii, a blocking protein that prevents MHC II from binding with peptides in the ER. ③ The MHC II–Ii assembly is transported to the lysosome, where it remains until the lysosome fuses with the phagosome, forming a phagolysosome where Ii is degraded, ④ freeing the MHC II protein to bind the foreign peptide fragments. ⑤ The MHC II–peptide complex is transported to the cell surface, where it interacts with TCRs and ⑥ the CD4 coreceptor on Th cells. The Th cells then release cytokines that interact with other cells to promote an immune response. Note that the APC in part (b) may be either a B cell, which ingests antigen by endocytosis (shown), or a macrophage or dendritic cell, which engulf antigens through phagocytosis.

through a pore called the *transporter associated with antigen processing* (*TAP*). Once the foreign peptides have entered the ER they are bound by MHC I, and the MHC I–peptide complex moves to the cell surface where it integrates into the cell membrane. When the TCR on the surface of a Tc cell interacts with both the foreign peptide (recognized as "nonself") and the MHC I protein (recognized as "self") on the surface of the target cell, the Tc cell releases *perforin* and *granzymes*, cytotoxic proteins that kill the virus-infected target cell.

MHC II proteins are the antigen-presenting proteins in a second pathway (Figure 27.14*b*). MHC II proteins are expressed exclusively on APCs, where they function to present peptide antigens from engulfed pathogens. Similar to MHC I proteins, MHC class II proteins are assembled in the endoplasmic reticulum. However, unlike events with MHC I proteins (Figure 27.14*a*), a protein called Ii binds MHC II, and this blocks peptide loading and labels the entire complex for transport from the ER to lysosomes. After ingestion of a pathogen or pathogen product by an APC, the phagosome containing the foreign antigen fuses with a lysosome to form a phagolysosome (Section 26.7). Within the phagolysosome, enzymes digest both the foreign antigens and the Ii peptide but not MHC II. The foreign peptides then bind to the newly opened MHC II antigen-binding site and the complex is inserted on the cell surface for presentation to T-helper cells. The latter, through their TCRs, recognize the MHC II–peptide complex and secrete cytokines that stimulate antibody production by B cells or induce inflammation.

In addition to the TCR, each T cell expresses a unique cell surface protein that functions as a *coreceptor*. Th cells express the **CD4 coreceptor**, and Tc cells express the **CD8 coreceptor** (Figure 27.14). When the TCR binds to the peptide–MHC complex, the coreceptor on the T cell also binds to the MHC protein on the antigen-presenting cell, strengthening the molecular interactions between the cells and enhancing activation of the T cell. CD4 binds only to MHC II, strengthening Th cell interaction with APCs that express MHC II protein. Likewise, CD8 binds only to MHC I, enhancing the binding of Tc cells to MHC I–bearing target cells. In clinical medicine, the CD4 and CD8 proteins are used as T cell markers to differentiate Th (CD4) cells from Tc (CD8) cells in diagnostic tests, for example, in assessing the course of disease in an AIDS patient (Section 30.15 and Figure 30.44).

MINIQUIZ

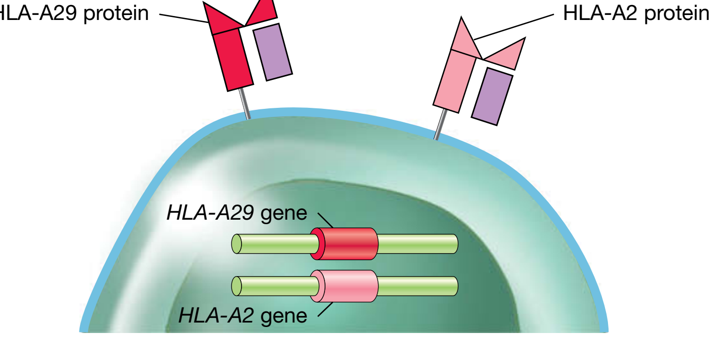
- Identify the cells that display MHC class I and MHC class II proteins on their surfaces.
- Compare the MHC I and MHC II protein structures and peptide-binding sites. How do they differ? How are they similar?
- Define the sequence of events for processing and presenting antigens from both intracellular (endogenous) and extracellular (exogenous) pathogens.

27.6 MHC Polymorphism, Polygeny, and Peptide Binding

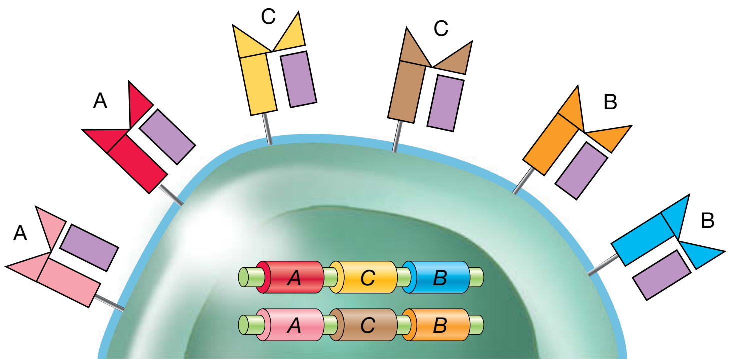
Although MHC class I and class II proteins theoretically can bind all possible antigen peptides for presentation to T cells, MHC proteins in different individuals of the same species are not identical. Different individuals typically have subtle differences in the amino acid sequence of homologous MHC proteins. These genetically encoded MHC variants, called *polymorphisms*, are the major immunological barriers for successful tissue transplantation from one individual to another.

Polymorphism, Polygeny, and the Immune Barrier to Tissue Transplantation

Polymorphism is the occurrence, within a population, of multiple alleles (alternate forms of a gene) at a specific locus (the location of the gene on the chromosome). For example, the MHC class I locus *HLA-A* (Figure 27.13*a*) has over 2000 known alleles, each of which encodes a distinct HLA-A protein that occurs within the human population. The genome of each person, however, contains only two of the *HLA-A* alleles; one allele is of paternal origin and one is of maternal origin. The two allelic protein products are expressed equally (**Figure 27.15*a***).



(*a*) **Polymorphism**



(*b*) **Polygeny**

Figure 27.15 Polymorphism and polygeny in MHC genes and proteins. *(a)* Polymorphism in *HLA-A* loci results in equal expression of proteins encoded by both alleles. There are over 2000 HLA-A alleles in the human population, but only two (one at each locus) are found in each individual. *HLA-B* and *HLA-C* exhibit similar levels of polymorphism. *(b)* Polygeny in MHC results in duplicated polymorphic *HLA-A, HLA-B*, and *HLA-C* genes that potentially encode three pairs of different MHC proteins. The colors represent alternate alleles of each gene and their respective protein.

Additional MHC class I protein diversity is a result of **polygeny**, in which evolutionary gene duplication events have led to the occurrence of two additional genetically, structurally, and functionally related loci, *HLA-B* and *HLA-C*. These gene loci are also polymorphic, having more than 2600 and 1500 allelic variants, respectively. Thus, an individual typically displays six structurally distinct proteins derived from the three polymorphic class I loci (three products derived from maternal origin and three products derived from paternal origin) (Figure 27.15*b*). Likewise, highly polymorphic and equally expressed alleles encode MHC class II proteins at the *HLA-DR, HLA-DP*, and *HLA-DQ* alpha- and beta-chain loci (Figure 27.13*a*).

As a result of polymorphism and polygeny, most individuals have unique MHC profiles. Only closely related family members are likely to have all of the same MHC genes and proteins, and this property can be used to prove (or disprove) paternity or to trace ancestral lineages (**Figure 27.16**). These highly polymorphic variations in MHC proteins are major barriers to successful tissue transplants because the MHC proteins on the donor tissue (graft) are recognized as foreign by the recipient's immune system. An immune response directed against the graft MHC proteins thus causes rejection and death of the graft tissue. However, matching MHC alleles between donors and recipients minimizes tissue graft rejection. Control of tissue rejection may also be accomplished by administering drugs that suppress the immune system.

Figure 27.16 Polymorphic *HLA* genes and tracing ancestral lineages. By analyzing the distribution of specific *HLA* alleles within different populations, researchers are increasingly able to reconstruct migration and interbreeding patterns of ancient humans and other hominids, including the Neanderthals. The intermixing of *HLA* genes between disparate ancient populations increased genetic diversity and likely enhanced survival by helping to strengthen and shape the modern immune response.

Peptide Antigen Binding

Most of the allelic variations in MHC proteins occur as amino acid changes concentrated in the antigen-binding groove (Figure 27.13), and each polymorphic variation of the MHC protein binds a different set of peptide antigens. The peptides bound by a single MHC protein share a common structural pattern—a peptide **motif**—and each different MHC protein binds a different motif. For example, for a certain class I protein, the bound peptides contain eight amino acids with a phenylalanine (F) at position 5 and a leucine (L) at position 8 (Figure 4.28 for the structures of amino acids). All other positions in the peptide can be occupied by any amino acid (X). Thus, all peptides sharing the sequence X-X-X-X-**F**-X-X-**L** would bind that MHC protein. Another MHC class I protein encoded by a different MHC allele binds a peptide motif of nine amino acids with a tyrosine (Y) at position 2 and an isoleucine (I) at position 9 (X-**Y**-X-X-X-X-X-X-**I**).

The invariant amino acids in each motif are called *anchor residues*—they bind directly and specifically within an individual MHC–peptide binding groove. Thus, an individual MHC protein can bind and present many different peptide antigens if the peptides contain the same anchor residues. Because each MHC protein binds a different motif with different anchor residues, the six possible MHC I proteins encoded in an individual's genome bind six different motifs. In this way, each individual can present a large number of different peptide antigens using the limited number of MHC I molecules available. MHC II proteins bind peptides in a similar manner.

Because of polymorphisms and polygeny within the human species, at least a few peptide antigens from virtually any pathogen will display a motif that can be bound and presented by the MHC proteins. This system of generating antigen-binding diversity is therefore quite different from the genetic mechanisms used to synthesize Igs (Section 27.4) and TCRs (Section 27.7), in which each receptor interacts specifically with only a *single* antigen.

MINIQUIZ

- Define polymorphism and polygeny as they apply to MHC genes.
- How does a single MHC protein present many different peptides to T cells?

IV • T Cells and Their Receptors

Adaptive immunity is ultimately initiated by interactions of T lymphocytes with peptide antigens on infected cells. The infected cells that are first recognized by T cells may include the same phagocytes that participate in the innate immune response (Chapter 26). Antigen presentation activates precursor T lymphocytes to differentiate into T cells that carry out antigen-specific, cell-mediated immunity. In the absence of antigen-activated T cells, there is little antigen-specific immunity and no immune memory.

27.7 T Cell Receptors: Proteins, Genes, and Diversity

Antigen-presenting cells ingest bacteria, viruses, and other antigenic material by phagocytosis (in macrophages and dendritic cells) or through internalization of a molecular antigen bound to

a BCR. Ingested antigens are then digested, complexed with MHC proteins, and moved to the cell surface for antigen presentation to T cells. The TCRs of T cells can recognize (bind) antigens only when the peptides are complexed with MHC proteins on host cell surfaces (Section 27.6). For example, a phagocyte infected with a virus will display MHC I and MHC II proteins embedded with viral peptides (**Figure 27.17**). These viral peptide–MHC complexes are the targets for T cells.

Each T cell expresses a TCR that is specific for a single peptide–MHC complex. Antigen-specific T cells are found closely associated with APCs in the spleen, lymph nodes, and MALT. T cells constantly sample surrounding APCs for peptide–MHC complexes. Peptide–MHC complexes that interact with the TCR signal the T cell to grow and divide, producing antigen-reactive clones that coordinate cell-mediated killing, induce inflammation, and activate antibody-producing B cells. Here we examine how the TCR interacts with antigens presented on an APC or an infected target cell.

TCR Structure and Diversity

The TCR is a membrane-spanning protein that extends from the T cell surface into the extracellular environment. Similar to B cells with their BCRs, each T cell has thousands of copies of its specific TCR on its surface. A functional TCR consists of two polypeptides, an α chain and a β chain. Similar to Igs, each TCR chain has a variable (V) domain and a constant (C) domain (**Figure 27.18**), and the V_α and V_β domains interact cooperatively to form an antigen-binding site that contains CDR1, CDR2, and CDR3 segments. However, unlike Igs, which can bind antigens of any composition, TCRs recognize only MHC–*peptide*; other antigens, such as complex polysaccharides, are not recognized by TCRs.

The three-dimensional structure of the TCR bound to MHC I–peptide is shown in Figure 27.18*a*. Both TCR and MHC proteins bind directly to peptide antigen. The MHC protein binds one face of the peptide—the MHC motif—and the TCR binds the other peptide face—the T cell epitope (Figure 27.18*b*). The CDR regions of the TCR bind directly to the MHC–peptide complex, and each CDR has a specific binding function. The CDR3 regions of the TCR α chain and β chain bind the antigen epitope; the CDR1 and CDR2 regions of the TCR α and β chains bind mainly to the MHC proteins.

The adaptive immune response can generate TCR diversity sufficient to bind nearly every possible peptide antigen. T cells generate receptor diversity in ways similar to the generation of Ig diversity in B cells, and Table 27.3 compares the receptor diversity–generating mechanisms for each cell type. Analogous to the H and L chains of Igs, the TCR α and β chains are encoded by distinct constant- and variable-domain gene segments. TCR V-region genes are arranged as a series of tandem segments (**Figure 27.19**). The α chain has about 80 V and 61 J gene segments, whereas the β chain has 52 V, 2 D, and 13 J gene segments. As we have seen for Igs (Section 27.4), antigen-binding diversity in TCRs is generated by somatic recombination, imprecise V-D-J (β-chain) or V-J (α-chain) joining, and random reassortment. Additional TCR diversity is generated because the D region of the β chain can be transcribed in all three reading frames (Section 4.9 and Figure 4.35), leading to production of three separate transcripts from each D-region gene.

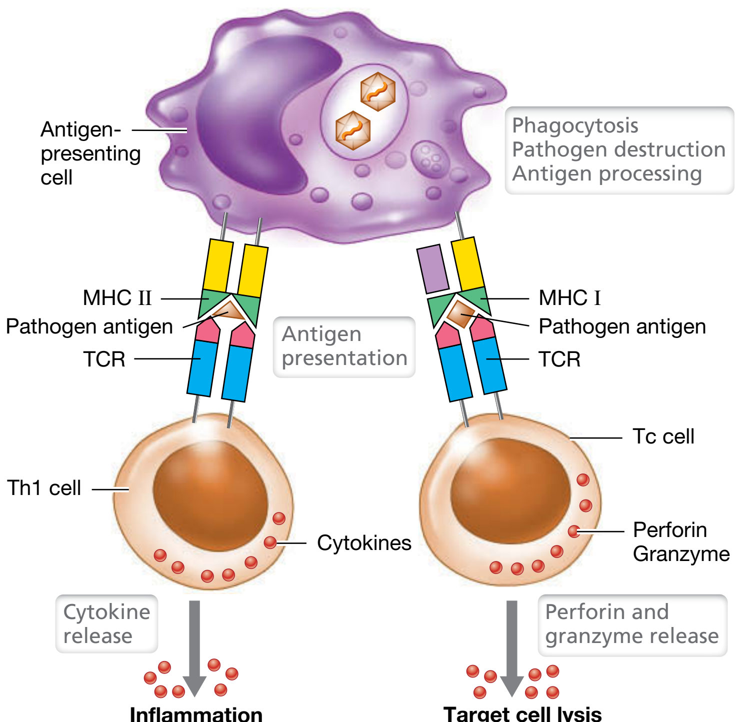


Figure 27.17 T cell immunity. Antigen-presenting cells, such as the phagocytes in innate immunity, ingest, degrade, and process antigens. They then present antigens to T cells that secrete protein cytokines that activate the adaptive immune response. T-helper 1 (Th1) cells produce cytokines that activate other cells and induce inflammation. T-cytotoxic (Tc) cells produce perforin and granzymes, proteins that destroy nearby target cells.

Similar to the assembly of Ig H and L chains, individual α and β chains are produced by each T cell at random and assembled to form a complete α:β heterodimer. The somatic hypermutation mechanisms responsible for increased receptor diversity in Ig genes do not operate in T cells, and thus additional TCR diversity from these events is not possible. However, potential TCR diversity is still enormous, as an estimated 10^{15} different TCRs can be generated.

Structural Similarities of Antigen-Binding Proteins

As we have seen, antigen-binding proteins of the adaptive immune response have common structural features. Ig, TCR, and MHC protein complexes all consist of two nonidentical polypeptides: MHC and TCR proteins are composed of α and β polypeptide chains, and Igs have a separate heavy and light chain (Section 27.4). Therefore, these protein complexes are believed to have arisen from duplication and selection of genes encoding primordial antigen receptors. Because of their shared structural, evolutionary, and functional features, genes encoding Ig, TCR, and MHC proteins are part of an extended gene family called the **immunoglobulin gene superfamily**. A comparison of Ig superfamily proteins is shown in **Figure 27.20**, with several discrete homologous domains highlighted.

The constant (C) domain of each protein in the superfamily has a highly conserved amino acid sequence consisting of about 100 amino acids with an intrachain disulfide bond spanning

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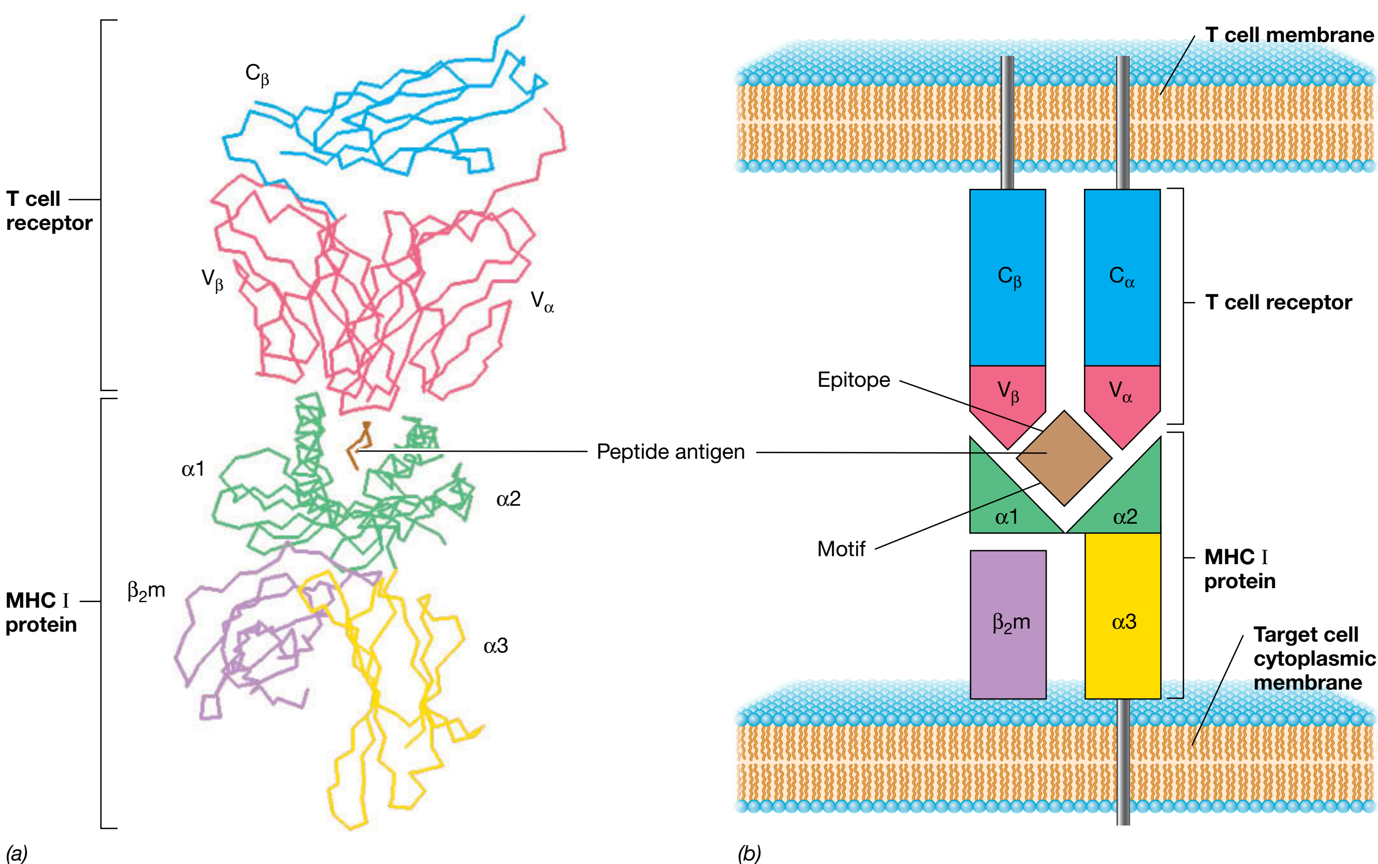


Figure 27.18 The TCR:MHC I–peptide complex. *(a)* A three-dimensional structure showing the orientation of TCR, peptide (brown), and MHC. This structure was derived from data deposited in the Protein Data Bank. *(b)* A diagram of the TCR:MHC–peptide structure. Note that the peptide is bound by both MHC and TCR proteins and has a distinct surface structure that interacts with each (see page 852 for discussion of this).

50–70 amino acids. C domains provide structural integrity for the antigen-binding molecules, anchor the antigen-binding V domains to the cytoplasmic membrane, and give each protein its characteristic shape. C domains can also provide recognition sites for accessory molecules. For example, C domains of most IgG and all IgM proteins are bound by the C1q component of complement, a critical first step in initiating the classical complement activation sequence (Section 26.9). Likewise, MHC I C

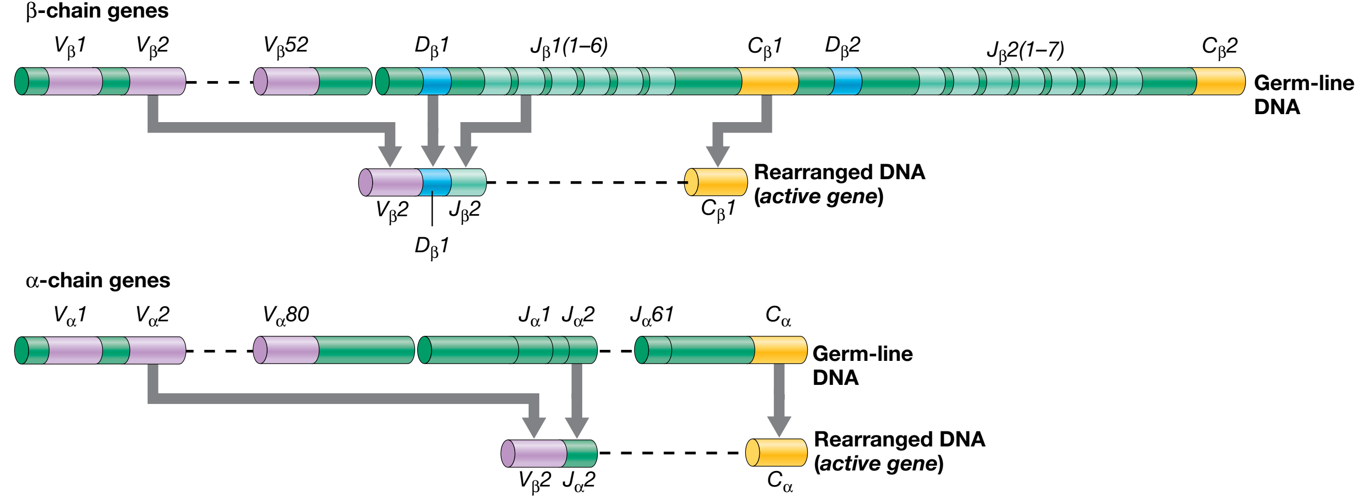


Figure 27.19 Organization of the human TCR α- and β-chain genes. The α-chain genes are located on chromosome 14 and the β-chain genes are on chromosome 6. Compare this figure with Figure 27.12.

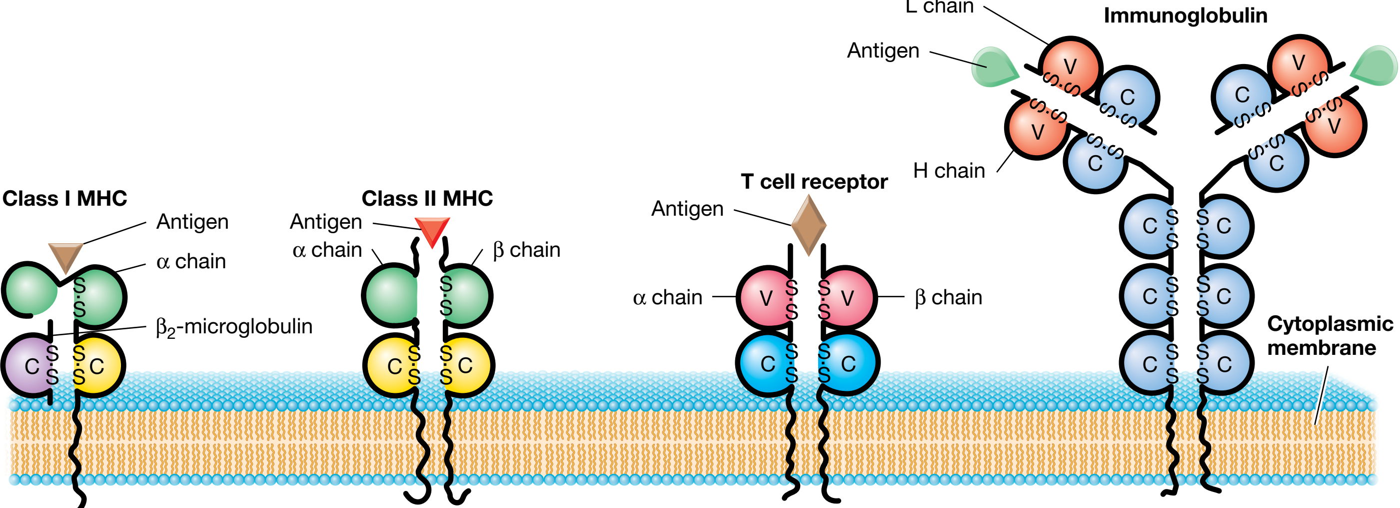


Figure 27.20 Immunoglobulin gene superfamily proteins. Constant domains have homologous amino acid sequences and higher-order structures. The Ig-like C domains in each protein chain indicate evolutionary relationships that identify the proteins as members of the Ig gene superfamily. The V domains of Igs and TCRs are also Ig domains, but the peptide-binding domains of MHC class I and class II proteins are not because their structures vary considerably from the basic features of the Ig domain.

domains bind to the accessory CD8 protein on Tc cells, and homologous MHC II C domains bind CD4 on Th cells. As we have discussed, such interactions are critical steps for T cell activation and initiation of adaptive immunity (Section 27.5).

The variable (V) domains of TCR and Ig molecules are about the same length as the C domains, but the structures of V domains can vary considerably from one another and from C domains. Ig and TCR V domains interact specifically with a nearly limitless variety of antigens. By contrast, the V domains of MHC proteins have evolved independently of Ig and TCR V domains. MHC V domains interact with foreign peptides that share a common motif (Section 27.6), resulting in the MHC–peptide complex recognized by the TCR.

MINIQUIZ

- Distinguish among the functions of the CDR1, CDR2, and CDR3 segments of the T cell receptor.
- Identify diversity-generating mechanisms unique to TCRs as compared to diversity-generating mechanisms in Igs.
- Describe and compare the structural features of Ig gene superfamily constant and variable domains.

27.8 T Cell Diversity

Antigen-reactive T cells consist of multiple T cell subsets having different functional properties. **T-cytotoxic (Tc) cells**, also called *cytotoxic T lymphocytes* (*CTLs*), are CD8 T cells (Section 27.5) that recognize the peptide–MHC I complex on an infected cell. By contrast, **T-helper (Th) cells**, which can differentiate into several more specialized Th cell subsets, are CD4 T cells that interact with peptide–MHC II complexes on the surface of APCs and function to activate macrophages and stimulate antibody-mediated immunity.

T-Cytotoxic Cells

When a Tc cell interacts with a foreign peptide on an infected cell, it kills the peptide-bearing target cell through an antigen-specific mechanism. For example, a viral peptide embedded in MHC I, displayed on a virus-infected cell, marks the cell for interaction and killing by a Tc cell whose TCRs recognize the viral antigen.

Contact between a Tc cell and the target cell is required to initiate killing of the infected cell (Figure 27.21). The point of initial contact is between the TCR and the peptide–MHC I complex. The CD8 protein on the Tc cell then binds the MHC I protein, strengthening the interaction. On contact with the target cell, granules in the Tc cell migrate to the contact site, where the contents of the granules are released (degranulation). The

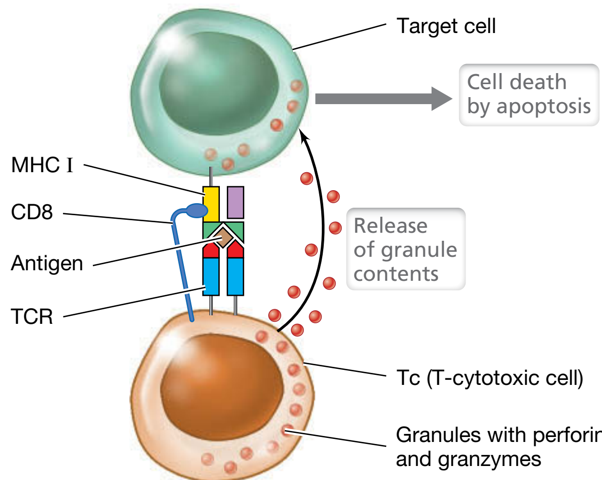


Figure 27.21 T-cytotoxic cells. When the TCR on a Tc cell binds MHC I–peptide complexes on any cell, the Tc cell releases granules that contain perforin and granzymes, cytotoxins that perforate the target cell and cause apoptosis, respectively.

TABLE 27.4 **T-helper cell subsets**

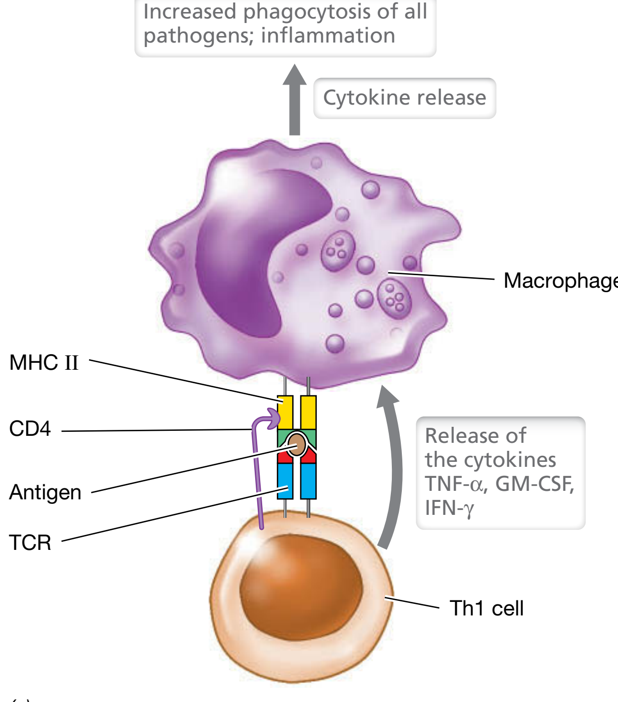
Characteristic	Th1	Th2	Th17	Treg
Antigen-presenting cell	Macrophage	B cell	Activated dendritic cell	Nonactivated dendritic cell
Major cytokines produced	IL-2, IFN-γ, TNF-α	IL-4, IL-5	IL-17, IL-6	IL-10, TGF-β
Cellular effects	Activation of T cells (IL-2) and macrophages	Activation of B cells	Activation and recruitment of neutrophils	Suppression of adaptive immune cells
Systemic effects	Cell-mediated immunity	Antibody-mediated immunity	Amplification of innate immunity	Control of Th immunity

granules contain perforin and granzymes (Section 27.5). Perforin enters the membrane of the target cell and combines to form a transmembrane pore through which granzymes then enter the target cell. Granzymes are cytotoxins that induce *apoptosis* (programmed cell death), characterized by organized killing and degradation of the target cell from within. Tc cells kill only those cells displaying the foreign antigen because the granules are released only at the contact surface between the Tc cell and the target cell bearing peptide–MHC I. Cells lacking the peptide recognized by the Tc cells do not make contact and are not killed.

Different Classes of T-Helper Cells

Interactions with APCs drive CD4 Th cells to differentiate into several subsets, each producing unique combinations of cytokines that recruit effector cells (Table 27.4). Macrophages (Section 26.4) play a central role as APCs in cell-mediated immunity. As illustrated in Figure 27.22*a*, macrophages engulf, process, and present antigen to **Th1 cells**. Th1 cells produce IL-2, a cytokine that promotes growth and activation of other T cells, and activate macrophages through the cytokines interferon gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), and granulocyte–monocyte colony-stimulating factor (GM-CSF) (Figure 27.22*a*). Th1-activated macrophages take up and kill foreign cells more efficiently than nonactivated macrophages (Figure 27.22*b*) and also kill tumor cells because the macrophages recognize tumor-specific antigens expressed by cancer cells as nonself.

Activated macrophages also produce several cytokines and chemokines that function as proinflammatory mediators and



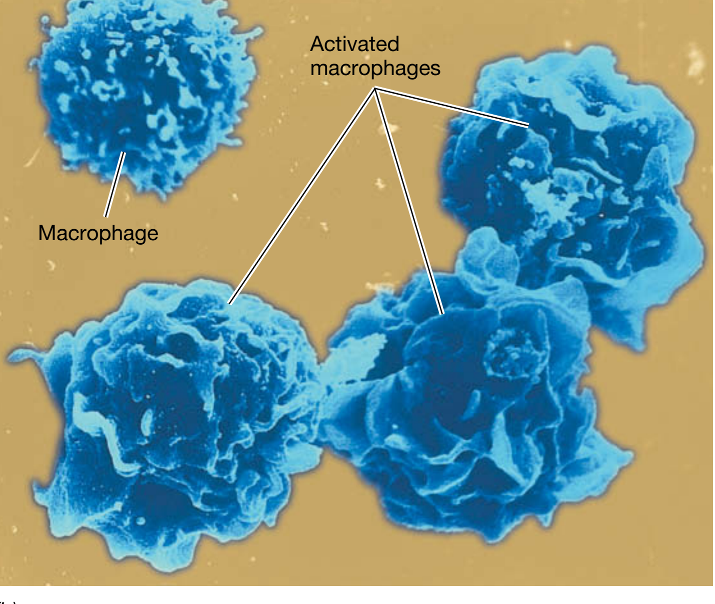


Figure 27.22 Th1 cells and macrophage activation. *(a)* Th1 cells (T-inflammatory cells) are activated by antigens presented on macrophages in the context of MHC II protein. Activated Th1 cells produce cytokines that activate macrophages, leading to increased phagocyte activity and inflammation. *(b)* Activated macrophages are generally larger than resting macrophages and have a ruffled surface, often with cytoplasmic extensions that "feel" for pathogens. In addition to carrying out more aggressive phagocytosis, activated macrophages express genes that encode bactericidal enzymes found in lysosomes, such as proteases and enzymes that produce reactive oxygen species, all of which are designed to quickly kill ingested microorganisms inside the phagolysosome (Figure 26.10).

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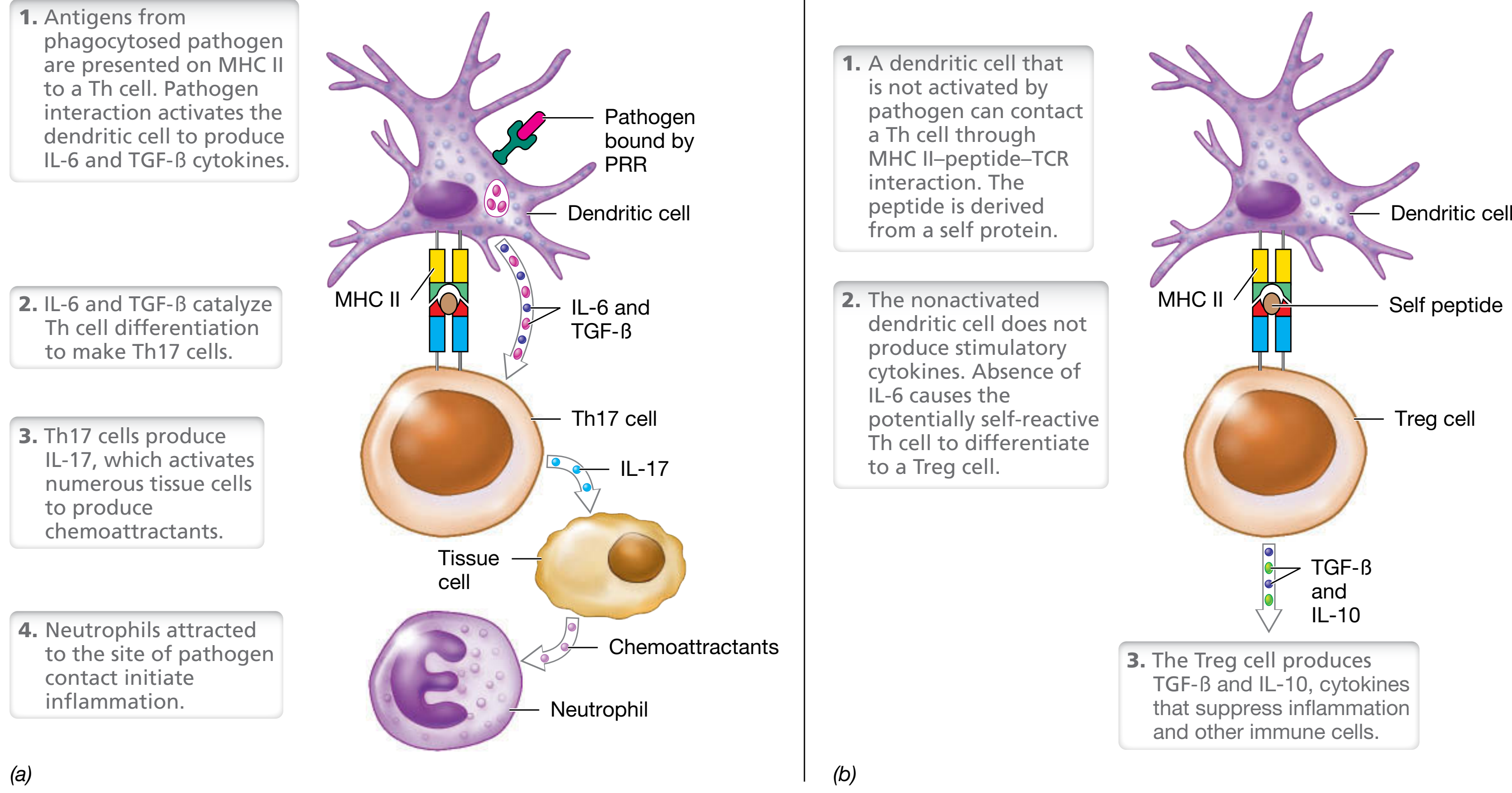


Figure 27.23 Th17 and Treg cells. *(a)* Th17 cells interact with pathogen-stimulated dendritic cells to draw neutrophils to the site of pathogen invasion, leading to inflammation and pathogen control. *(b)* Treg cells interact with nonactivated dendritic cells and respond by making immunosuppressive cytokines that control reactions to self antigens.

chemoattractants, respectively. Table 27.5 summarizes the source and activities of these and other important immune cytokines and chemokines. In the case of organ or tissue transplants, Th1-activated macrophages can actually harm the host if the host's Th1 cells recognize nonself MHC proteins on the transplant and trigger macrophage activation and transplant destruction (Section 27.6).

TABLE 27.5 Major immune cytokines and chemokines

Cytokine (chemokine)	*Major producer cells*	*Major target cells*	*Major effect*
IL-4[a]	Th2	B cells	Activation, proliferation, differentiation, IgG1 and IgE synthesis
IL-5	Th2	B cells	Activation, proliferation, differentiation, IgA synthesis
IL-2	Naive T cells, Th1, and Tc	T cells	Proliferation (often autocrine)
IFN-γ[b]	Th1	Macrophages	Activation
GM-CSF[c]	Th1	Macrophages	Growth and differentiation
TNF-α[d]	Th1	Macrophages	Activation, production of proinflammatory cytokines
	Macrophages	Vascular epithelium	Activation, inflammation
IL-1β	Macrophages	Vascular epithelium, lymphocytes	Activation, inflammation
IL-6	Macrophages, dendritic cells	Lymphocytes	Activation
IL-12	Macrophages, endothelial cells	NK cells, naive T cells	Activation, enhances differentiation to Th1
IL-17	Th17	Neutrophils	Activation
CXCL8 (chemokine)	Macrophages	Neutrophils, basophils, T cells	Chemotactic factor
CCL2 (MCP-1[e]) (chemokine)	Macrophages	Macrophages, T cells	Chemotactic factor, activator

[a]IL, interleukin; [b]IFN, interferon; [c]GM-CSF, granulocyte–monocyte colony-stimulating factor; [d]TNF, tumor necrosis factor; [e]MCP, macrophage chemoattractant protein.

Th2 cells play a pivotal role in B cell activation and antibody production. As we have discussed (Section 27.3), B cells make antibodies, and differentiated B cells are coated with antibodies (BCRs) that are antigen receptors. When antigen binds the BCR (Figure 27.6), the antibody-bound antigen is taken into the B cell by endocytosis and degraded. Peptides from the degraded antigen are then loaded into the B cell's MHC II protein for presentation to a Th2 cell. The Th2 cell responds by producing IL-4 and IL-5, cytokines that activate the B cell (Table 27.5) and cause it to proliferate and differentiate into plasma cells that produce and secrete antibodies specific to the presented antigen (Section 27.3).

Antigen presentation by dendritic cells (Section 26.4) plays a critical role in the development of other Th cell subsets, including Th17 and Treg cells. **Th17 cells** are important in the first stages of the adaptive immune response. Undifferentiated, or *naive*, Th cells differentiate into Th17 cells through the activity of dendritic cells. When dendritic cells encounter pathogens, they present antigen and secrete IL-6 and transforming growth factor-β (TGF-β), cytokines that catalyze differentiation of naive Th cells to Th17 cells (**Figure 27.23*a***). Th17 cells then produce IL-17, a cytokine that activates other tissue cells to produce cytokines and chemokines that attract proinflammatory neutrophils to the site of infection (Table 27.5). Thus, the function of Th17 cells is to produce IL-17, starting a cascade that draws neutrophils to infection sites. By recruiting neutrophils, Th17 cells amplify innate immunity triggered by interaction of a pathogen with the dendritic cell.

Treg cells are important in control of immunity. Undifferentiated Th cells remain so unless they are stimulated to mature by certain cytokines, as is the case for IL-6 stimulation to produce Th17 cells. However, in the absence of a pathogen, Th cells can still interact with dendritic cells through MHC II–peptide–TCR (Figure 27.23*b*). In this case, the peptide is usually a self peptide, and an immune response to it could cause an autoimmune disease. However, because the dendritic cells did not interact with a pathogen, they cannot produce IL-6 to promote Th17 differentiation. Instead, the absence of IL-6 pushes differentiation to Treg cells that make IL-10 and TGF-β, two cytokines that suppress immunity and inflammation. In the presence of self antigens and in the absence of IL-6, Treg cells shut down the immune response and inhibit inflammation. This is important for controlling immune responses to self antigen and preventing autoimmunity.

MINIQUIZ

- Describe the mechanism used by Tc cells to recognize infected host cells.
- Describe the effector system (the cell-killing mechanism) used by Tc cells.
- Compare and contrast the roles and activities of the different Th cells. What cytokines do they produce, and which effector cells do these cytokines act upon?

V • Immune Disorders and Deficiencies

In some cases, reactions of the adaptive immune response can damage the host. For example, **hypersensitivity** is an immune response that results in host damage and, in some cases, even host death. Hypersensitivities are grouped according to the antigens and the mechanisms that produce disease. Likewise, exposure to *superantigens*, proteins produced by certain bacteria and viruses that initiate massive inflammatory responses, also causes severe immune reactions that result in host damage. To conclude this chapter we consider both of these along with immunodeficiency, a condition in which a host's immune response is either absent or insufficient to effectively fight infections.

27.9 Allergy, Hypersensitivity, and Autoimmunity

Antibody-mediated **immediate hypersensitivity** is more commonly called *allergy*. Cell-mediated hypersensitivities also cause allergy-like diseases, but because of the delayed onset of symptoms, cell-mediated reactions are termed **delayed-type hypersensitivity (DTH)**. **Autoimmunity** is a harmful immune reaction directed against self antigens. These hypersensitivities are categorized as type I, II, III, or IV based on immune effectors, antigens, and symptoms (**Table 27.6**).

Immediate Hypersensitivity

Immediate (type I) hypersensitivity is caused by the release of substances that either increase or decrease blood pressure or heart rate (vasoactive products) from mast cells coated with IgE

TABLE 27.6 Hypersensitivity

Classification	*Description*	*Immune mechanism*	*Time of latency*	*Examples*
Type I	Immediate	IgE sensitization of mast cells	Minutes	Reaction to bee venom (sting) Hay fever
Type II	Cytotoxic[a]	IgG interaction with cell surface antigen	Hours	Drug reactions (penicillin)
Type III	Immune complex	IgG interaction with soluble or circulating antigen	Hours	Systemic lupus erythematosus (SLE)
Type IV	Delayed type	Th1 inflammatory cell activation of macrophages	Days (24–48 h)	Poison ivy Tuberculin test

[a]Autoimmune diseases may be caused by type II, type III, or type IV reactions.

(Figure 27.24). Immediate hypersensitivity reactions occur within minutes after exposure to an *allergen*, the antigen that caused the type I hypersensitivity. Depending on the individual and the allergen, immediate hypersensitivity reactions can be mild allergic reactions or can cause a life-threatening reaction called *anaphylaxis*.

About 20% of the population suffers from immediate hypersensitivity allergies to pollens, molds, animal dander, certain foods (strawberries, nuts, and shellfish), insect venoms, dust mites, and other agents. Most allergens enter the body at the surface of mucous membranes, such as the lungs or the gut. Initial exposure to allergens stimulates Th2 cells to produce cytokines that induce B cells to make IgE antibodies. The allergen-specific IgE antibodies bind to IgE receptors on mast cells (Figure 27.24). Mast cells are nonmotile granulocytes (Section 26.4) associated with the connective tissue adjacent to capillaries throughout the body. With any subsequent exposure to the immunizing allergen, the mast cell–bound IgE molecules bind the antigen. Cross-linking of IgEs by an antigen triggers *degranulation*—the release of soluble allergic mediators from the mast cells. These mediators cause allergic symptoms within minutes of antigen exposure. After initial sensitization by an allergen, the allergic individual responds to each subsequent reexposure to the allergen.

The principal chemical mediators released from mast cells are histamine and serotonin, modified amino acids that cause rapid dilation of blood vessels and contraction of smooth muscle, initiating symptoms ranging from mild local discomfort to systemic *anaphylactic shock*. Local symptoms typically include mucus production, rash, sneezing, itchiness, watery eyes, and hives (Figure 27.25). Symptoms of anaphylactic shock may include vasodilation (causing a sharp drop in blood pressure) and asthma due to smooth muscle constriction in the lungs. Severe anaphylaxis is treated immediately with the hormone epinephrine to counter smooth muscle contraction, increase blood pressure, and promote breathing. Less serious allergic symptoms may be treated with *antihistamines*, which are drugs that neutralize histamine, or with anti-inflammatory steroids. Finally, immunization with increasing doses of the allergen may shift antibody production from IgE to IgG and IgA. The IgG and IgA interact with the allergens, thereby blocking antigen binding to IgE on sensitized mast cells. This process, called *desensitization*, inhibits IgE production and stops allergic symptoms.

Delayed-Type Hypersensitivity

Delayed-type (type IV) hypersensitivity (DTH) is cell-mediated hypersensitivity characterized by tissue damage due to inflammation initiated by Th1 cells (Table 27.6). DTH symptoms appear several hours after secondary exposure to the eliciting antigen, with a maximal response usually occurring in 24 to 48 hours. Typical DTH antigens include chemicals that are not normally immunogens but become so when they covalently bind to skin proteins, creating new antigens and eliciting a DTH response. Hypersensitivity to these newly created antigens is known as *contact dermatitis* and results in, for example, skin reactions to poison ivy (Figure 27.26), jewelry, cosmetics, latex, and other chemicals that react with host tissues. Several hours after a second or subsequent exposure to the antigen, the skin feels itchy at the site of contact. Erythema (reddening) and edema (swelling) appear, often with localized tissue destruction in the form of blistering, and reach a maximum in several days. The delayed onset and the progress of the inflammatory response are the hallmarks of the DTH reaction. As discussed below, certain self antigens may also elicit DTH responses, resulting in autoimmune disease.

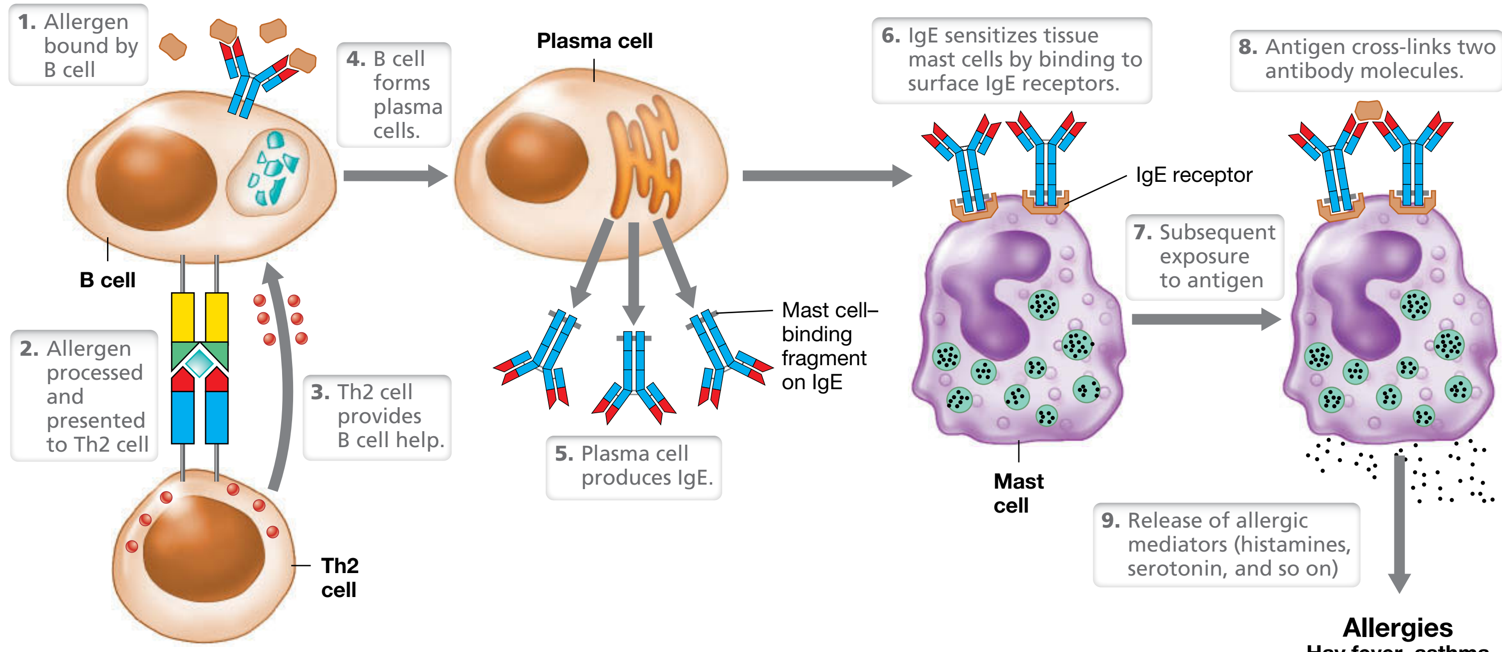


Figure 27.24 Immediate hypersensitivity. Certain antigens, such as pollens, stimulate IgE production. IgE binds to mast cells by means of a high-affinity surface receptor and arms the mast cell. Antigen cross-links surface IgE, causing release of soluble mediators, including histamine. These mediators produce symptoms ranging from mild allergic symptoms to life-threatening anaphylaxis.

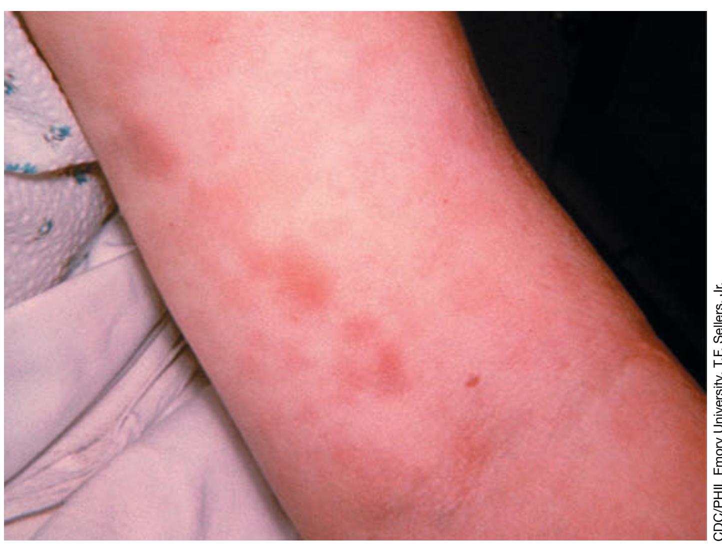


Figure 27.25 Hives due to immediate hypersensitivity. The raised, red areas are typical symptoms after contact with allergens that cause immediate hypersensitivity.

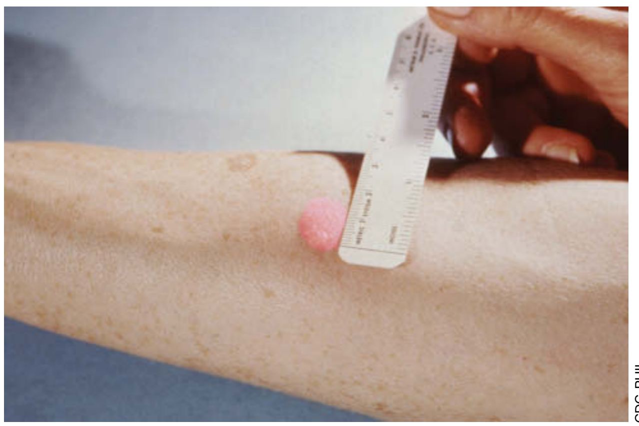


Figure 27.27 Th1 cells and macrophage activation. This tuberculin test shows a positive reaction. Macrophages activated by antigen-specific Th1 cells caused the localized, delayed-type reaction to a tuberculosis antigen, tuberculin, at the site of injection. The raised area of inflammation on the forearm is about 1.5 cm in diameter.

Another example of delayed-type hypersensitivity is the development of protective immunity to the bacterium that causes tuberculosis, *Mycobacterium tuberculosis* (Section 30.4). The German physician turned microbiologist Robert Koch discovered this cellular immune response during his classic studies on tuberculosis over a century ago (Section 1.10). When antigens derived from the bacterium are injected subcutaneously into a person previously infected with *M. tuberculosis*, a skin reaction called the *tuberculin reaction* develops within 24–48 hours (Figure 27.27). Local Th1 cells stimulated by the introduced *M. tuberculosis* antigens release cytokines that attract and activate large numbers of macrophages, which in turn produce a characteristic local inflammation, including induration (hardening), edema, erythema, pain, and heating of the skin. The activated macrophages then ingest and destroy the invading antigen. The DTH-based tuberculin skin test is used to test for a current or previous infection with *M. tuberculosis* or previous immunization with the tuberculosis vaccine (Section 28.5).

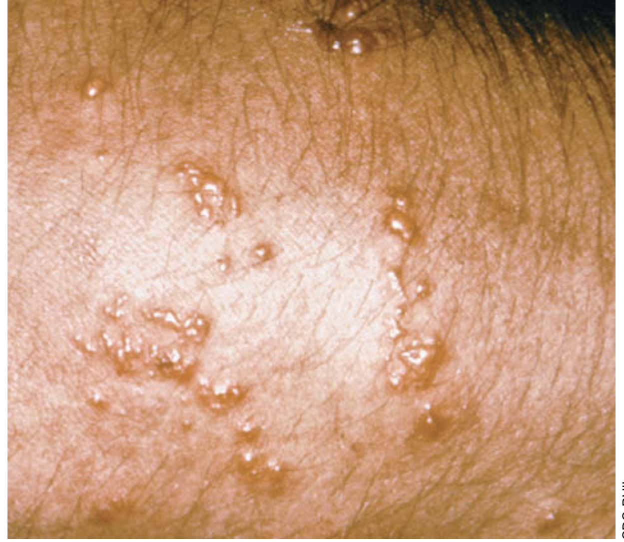


Figure 27.26 Delayed-type hypersensitivity. Poison ivy blisters on an arm. The raised rash appears 24–48 hours after exposure to plants of the genus *Rhus* as a result of macrophage activation by Th1 cells sensitized to *Rhus* antigens.

A number of other infectious diseases due to intracellular pathogens elicit DTH reactions. These include bacterial diseases such as leprosy, brucellosis, and psittacosis; viral diseases such as mumps; and fungal diseases such as coccidioidomycosis, histoplasmosis, and blastomycosis. Visible, antigen-specific skin responses similar to the tuberculin reaction occur after injection of antigens derived from the pathogens, indicating pathogen exposure and Th1-mediated immunity.

Autoimmunity

As lymphocytes develop, T and B cells that react with self antigens are normally eliminated (Section 27.1). Autoimmune diseases result when these cells are instead activated to produce immune reactions against self proteins (Table 27.7). For example, Th1-mediated DTH can cause autoimmune responses directed against self antigens, as in the case of the Th1-mediated response to brain-derived antigens in allergic encephalitis. In type 1 (juvenile) diabetes mellitus, Th1 cells directed to antigens on pancreatic cells cause reactions that destroy the insulin-producing beta cells in the pancreas.

Many autoimmune diseases, however, are caused by **autoantibodies**, antibodies that interact with self antigens, many of which are organ-specific. For example, in *Hashimoto's disease* (hypothyroidism), autoantibodies are made against thyroglobulin, a protein product of the thyroid gland that assists in the synthesis of thyroid hormones. In Hashimoto's disease, antibodies to thyroglobulin bind complement proteins (Section 26.9), leading to local inflammation and the destruction of host cells, the hallmarks of a type II hypersensitivity disease (Table 27.7).

Systemic lupus erythematosus (*SLE*) is an autoimmune disease caused by a type III hypersensitivity. This disease and others like it are caused by autoantibodies directed against soluble, circulating self antigens. In SLE, the antigens include nucleoproteins and DNA. Autoantibodies to these bind to soluble proteins, producing insoluble immune complexes, and disease symptoms result when these circulating antigen–antibody complexes deposit in different

TABLE 27.7 Some autoimmune diseases of humans

Disease	Organ, cell, or molecule affected	Mechanism (hypersensitivity type[a])
Type I diabetes (insulin-dependent diabetes mellitus)	Pancreas	Cell-mediated immunity and autoantibodies against surface and cytoplasmic antigens of beta cells of pancreatic islets (II and IV)
Myasthenia gravis	Skeletal muscle	Autoantibodies against acetylcholine receptors on skeletal muscle (II)
Goodpasture's syndrome	Kidney	Autoantibodies against basement membrane of kidney glomeruli (II)
Rheumatoid arthritis	Cartilage	Autoantibodies against self IgG antibodies, which form complexes deposited in joint tissue, causing inflammation and cartilage destruction (III)
Hashimoto's disease (hypothyroidism)	Thyroid	Autoantibodies to thyroid surface antigens (II)
Male infertility (some cases)	Sperm cells	Autoantibodies agglutinate host sperm cells (II)
Pernicious anemia	Intrinsic factor	Autoantibodies prevent absorption of vitamin B_{12} (III)
Systemic lupus erythematosus (SLE)	DNA, cardiolipin, nucleoprotein, blood clotting proteins	Autoantibody response to various cellular constituents results in immune complex formation (III)
Addison's disease	Adrenal glands	Autoantibodies to adrenal cell antigens (II)
Allergic encephalitis	Brain	Cell-mediated response against brain tissue (IV)
Multiple sclerosis	Brain	Cell-mediated and autoantibody response against central nervous system (II and IV)

[a]See Table 27.6.

body tissues, such as the kidney, lungs, and spleen. In the tissues, the antibodies bind complement, resulting in inflammation and local, often severe, tissue damage. Type III hypersensitivities are also called *immune complex disorders* (Table 27.6).

Organ-specific autoimmune diseases are sometimes more easily controlled clinically than diseases that affect multiple organs. For example, the product of organ function, such as thyroxine in autoimmune hypothyroidism or insulin in type I diabetes, can often be supplied in pure form from another source. SLE, rheumatoid arthritis, and other autoimmune diseases that affect multiple organs and sites can often be controlled only by general immunosuppressive therapy, such as the use of steroid drugs. Unfortunately, the immunosuppression associated with these treatments significantly increases the chance of developing opportunistic infections. Recently, however, therapies that employ monoclonal antibodies (Section 28.5) have emerged as promising alternative treatment strategies for autoimmune diseases. For example, adalimumab (Humira) is a monoclonal antibody that neutralizes tumor necrosis factor alpha (TNF-α), an inflammatory cytokine linked to several autoimmune diseases including rheumatoid arthritis, Crohn's disease, and psoriasis. Similarly, the monoclonal antibody belimumab (Benlysta) targets B cell activating factor, a cytokine that stimulates B cell maturation but is overexpressed in SLE patients, causing the persistence of B cells that produce autoantibodies.

Heredity influences the incidence, type, and severity of autoimmune diseases. Many autoimmune diseases correlate strongly with the presence of certain MHC proteins (Section 27.6). Studies of model autoimmune diseases in mice support such a genetic link, but the precise conditions necessary for developing autoimmunity may also depend on other factors such as prior infections, gender, age, and health status. Women, for example, are about ten times more likely to develop SLE than are men. It is thus likely that the balance between a normal immune response and autoimmunity is tipped by a combination of factors in which genetic predisposition plays a central role.

MINIQUIZ

- Discriminate between immediate hypersensitivity and delayed-type hypersensitivity with respect to antigens and immune effectors.
- Provide examples and mechanisms for an antibody-mediated autoimmune disease directed against a specific organ and one involving circulating immune complexes.

27.10 Superantigens and Immunodeficiency

Extremes in the adaptive immune response, whether overactive or deficient, can have devastating effects on the host. Some exotoxins, called *superantigens*, damage host cells indirectly by subverting the immune system so that T cells and their cytokine products destroy host tissues through an exaggerated immune response. By contrast, some diseases—either genetic or infectious—cause *immunodeficiency*, resulting in increased susceptibility of the host to infectious diseases. We consider these opposing extremes in the adaptive immune response here.

Superantigens

Superantigens are proteins that upon exposure to the immune system activate many more T cells than normal and are therefore capable of eliciting an unusually strong immune response

(**Figure 27.28**). A variety of viruses and bacteria produce superantigens. For example, streptococci and staphylococci, especially certain strains of *Streptococcus pyogenes* and *Staphylococcus aureus*, produce several different extremely potent superantigens (Sections 30.2 and 30.9).

Superantigen interaction with TCRs differs from conventional antigen–TCR binding (Figure 27.18). Whereas typical foreign antigens, presented by MHC proteins, bind to a TCR at a defined antigen-binding site, superantigens bind to sites on TCR and MHC proteins that are outside the antigen-specific binding site (**Figure 27.29**). A superantigen binds to all TCRs with a shared common structure, and many different TCRs share the same structure outside the antigen-binding site. Whereas less than 0.01% of all available T cells interact with a conventional foreign antigen in a typical immune response, some superantigens can bind up to 25% of all T cells in the body! These interactions mimic conventional antigen presentation and stimulate large numbers of T cells to grow and divide. As in normal responses, the activated T cells produce cytokines that stimulate phagocytes and other immune cells. However, the extensive cytokine production by the large population of superantigen-activated T cells triggers a widespread cell-mediated response characterized by systemic inflammatory reactions. The resulting fever, diarrhea, vomiting, mucus production, and even systemic shock may be fatal in extreme cases. The clinical symptoms of superantigen shock are indistinguishable from those of septic shock, a condition in which a bacterial infection has spread throughout the body (Section 26.8).

One of the most common superantigen diseases is *Staphylococcus aureus* food poisoning, characterized by fever, vomiting, and diarrhea, and caused by one of several superantigen staphylococcal enterotoxins (Section 32.8). *S. aureus* also produces the superantigen responsible for *toxic shock syndrome* (Figure 27.28). *Streptococcus pyogenes* produces erythrogenic toxin, the superantigen responsible for scarlet fever (Section 30.2).

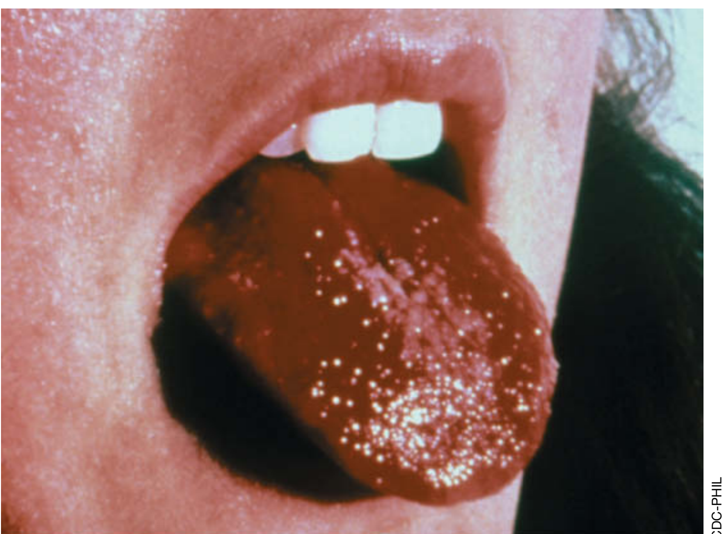


Figure 27.28 Toxic shock syndrome. This individual exhibits "strawberry tongue," a symptom of toxic shock syndrome caused by a *Staphylococcus aureus* superantigen.

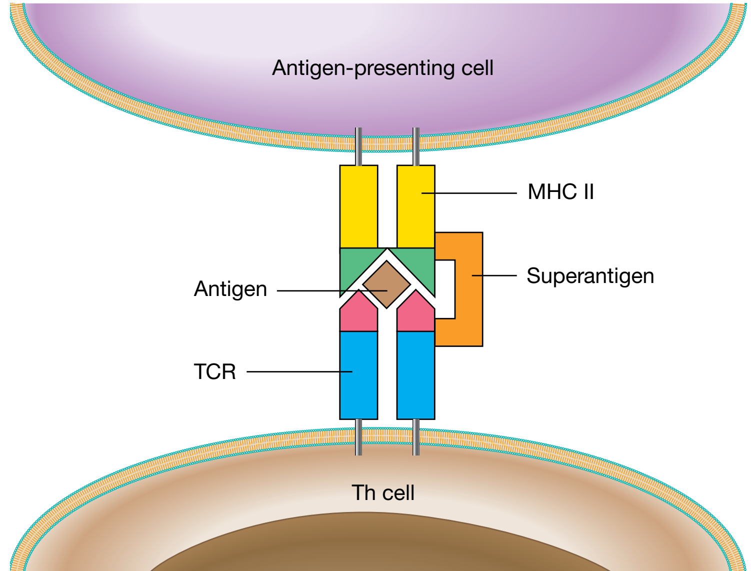


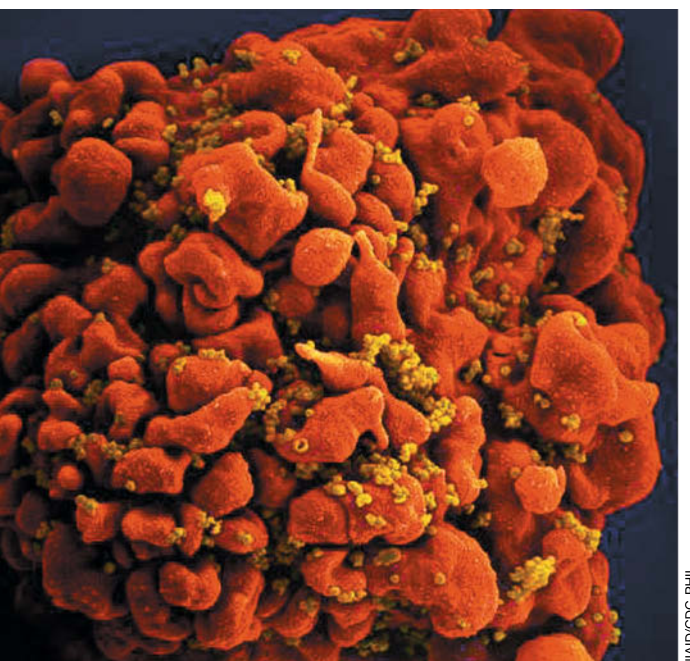
Figure 27.29 Superantigens. Superantigens bind to conserved regions of both the MHC and TCR proteins at positions outside the normal binding site. Superantigens interact with large numbers of T cells, causing large-scale T cell activation, cytokine release, and systemic inflammation.

Immunodeficiency

Active adaptive immunity is critical for infectious disease resistance. We know this because of problems caused by genetic defects and diseases that affect the adaptive immune system. For example, animals that cannot produce antibodies because of genetic defects in their B cells acquire serious infections from extracellular pathogens, especially bacteria. In addition, animals with genetic defects that prevent development of T cells suffer from recurrent infections with viruses and other intracellular pathogens.

Severe combined immune deficiency syndrome (*SCID*) is a genetic disorder that prevents proper formation of either B or T cells. Individuals with SCID essentially have no effective adaptive immunity. A lack of proper T cell function directly results in a deficiency of cell-mediated immunity and indirectly causes a loss of antibody-mediated immunity because B cell activation against most antigens is dependent upon the presence of functional Th cells. Unless patients receive supportive therapy, such as a bone marrow transplant and antibiotic treatments, SCID eventually causes death from multiple recurrent infections. The transplantation of compatible bone marrow tissue provides the afflicted individual with hematopoietic stem cells (Sections 26.3 and 26.4) that are free of the genetic defects that cause SCID.

Recently, gene therapy has also shown promise as a curative treatment option for some forms of SCID. In this procedure, defective genes in hematopoietic stem cells are replaced through a transduction process using viral vectors (Section 11.7). As with bone marrow transplantation, successful gene therapy restores function of the adaptive immune system. However, a major challenge in the use of this procedure is the risk of the patient developing cancer, especially leukemia. This is because the viral vectors used to introduce the functional genes are typically attenuated



NIAID/CDC-PHIL

Figure 27.30 HIV infection of a Th cell. Colorized scanning electron micrograph of a large number of HIV virions (yellow) attacking a Th cell (red).

retroviruses or adenoviruses; such viruses readily infect host cells to facilitate the necessary transduction, but they also contain or may activate *oncogenes*, which disrupt control of cell division and induce tumorigenesis. However, promising new forms of "gene editing" that do not require viral vectors are now on the horizon (Section 12.12), and thus safe genetic therapies for SCID may be developed in the not too distant future.

In some cases, immunodeficiency is not the result of a genetic disorder but rather is caused by microbial infection. The best-studied example of this is the loss of the adaptive immune response due to *acquired immunodeficiency syndrome* (*AIDS*). Human immunodeficiency virus (HIV) infects host cells that express the CD4 cell surface protein. The virus initially infects macrophages but later attacks and replicates primarily in Th cells (Figure 27.30). Once infected, Th cells cease growing and dividing before they eventually die. Therefore, in untreated cases, HIV infection causes gradual depletion of Th cells, resulting in a lack of effective immunity and the eventual onset of AIDS (Section 30.15 and Figure 30.44).

In most AIDS cases, the actual cause of patient death is not directly attributed to HIV but rather to any of a variety of secondary microbial infections caused by *opportunistic pathogens*. Many of these infections are caused by fungal, bacterial, and viral pathogens that only rarely cause serious disease symptoms in individuals that have a fully functional immune system. The deficiency of adaptive immunity in AIDS patients allows these opportunistic pathogens to colonize and invade body tissues, leading to additional diseases. We discuss the pathogenicity, symptoms, treatment, and other aspects of HIV/AIDS in more detail in Chapter 30.

MINIQUIZ

- Describe the binding site for superantigens on T cells and APCs. How does this relate to the activation of a much larger population of T cells than normal upon exposure to superantigens?
- Compare and contrast the immunodeficiency observed in SCID patients to that of AIDS patients. What cell types are affected by each condition?
- In the absence of treatment, what is the prognosis for individuals afflicted with SCID and AIDS?

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Chapter Review

I • Principles of Adaptive Immunity

27.1 The adaptive immune response is characterized by *specificity* for the antigen, the ability to respond more vigorously when reexposed to the same antigen (*memory*), and the acquired inability to interact with self antigens (*tolerance*). Tolerance in lymphocytes is acquired through a selection process. Immature T cells that do not interact with MHC–peptide (positive selection) or that react strongly with self antigens (negative selection) are eliminated by clonal deletion in the thymus. T cells that survive positive and negative selection leave the thymus and can participate in the immune response. B cell reactivity to self antigens is controlled through clonal deletion and anergy.

Q Why is it necessary for immature T lymphocytes to undergo a two-step selection process through which antigen-reactive cells are first selected followed by the elimination of cells that react strongly with self-antigens?

27.2 Immunogens are foreign macromolecules that induce an immune response. Immunogens initiate an immune response when introduced into a suitable host. Antigens are molecules recognized (bound) by antibodies or TCRs. Adaptive immunity develops naturally and actively through immune responses to infections, or naturally and passively through antibody transfer from the placenta or breast milk. Artificial passive immunity occurs when

antibodies or immune cells are transferred from an immune individual to a nonimmune individual. Immunization induces artificial active immunity and is widely used to prevent infectious diseases.

Q Immunity can be of many types: natural active immunity, natural passive immunity, artificial active immunity and artificial passive immunity. Which of these forms of immunity confer immune memory?

II • Antibodies

27.3 Antibody production is initiated when an antigen contacts an antigen-specific B cell. The B cell then processes the antigen and presents it to an antigen-specific Th2 cell. The Th2 cell becomes activated, producing cytokines that signal the B cell to clonally expand and differentiate to produce soluble, antigen-specific antibodies. Each antibody (immunoglobulin) protein consists of two heavy and two light chains. The antigen-binding site is formed by the interaction of the variable regions of one heavy and one light chain. Each antibody class has different structural characteristics, expression patterns, and functional roles. Activated B and T cells can live for years as memory cells and can rapidly expand and differentiate to produce high titers of antibodies after reexposure to antigen.

Q Describe the structural and functional differences among the five major classes of antibodies. What cellular and molecular interactions take place in the production of antibodies?

27.4 The antigen-binding site of Ig is composed of the V (variable) domains of one heavy chain and one light chain. Each V region contains three complementarity-determining regions, or CDRs, that are folded together to form the antigen-binding site. Immunoglobulin diversity is generated by several mechanisms. Somatic recombination of gene segments allows shuffling of the various Ig gene segments. Random reassortment of the heavy- and light-chain genes, imprecise joining of VDJ and VJ gene segments, and hypermutation mechanisms contribute to nearly unlimited immunoglobulin diversity.

Q Which Ig chains are used to construct a complete antigen-binding site? Which domains? Which CDRs? Calculate the total number of germ-line-encoded V_H and V_L domains that can be constructed from the available Ig genes.

III • The Major Histocompatibility Complex (MHC)

27.5 T cells, with their TCRs, bind peptide antigens presented by MHC proteins on infected cells or APCs. Class I MHC proteins are expressed on all nucleated cells and present endogenous antigenic peptides to TCRs on Tc cells. Class II MHC proteins are expressed only on APCs. They function to present exogenously derived peptide antigens to TCRs on Th cells. These interactions activate T cells to kill antigen-bearing cells or to promote inflammation or antibody production.

Q Describe the basic structure of class I and class II major histocompatibility complex (MHC) proteins. In what functional ways do they differ?

27.6 Class I and class II MHC genes are highly polymorphic, and the many allelic variations challenge successful tissue transplantation. Different alleles of MHC class I and class II genes encode proteins that bind and present different peptide subsets, each characterized by a specific structural motif.

Q Polymorphism implies that each different MHC protein binds a different peptide motif. For the MHC class I polymorphisms, how many different MHC proteins are expressed in an individual? How many by the entire human population?

IV • T Cells and Their Receptors

27.7 T cell receptors bind to peptide antigens presented by MHC proteins. The CDR3 regions of both the α chain and the β chain bind to the antigen epitope; the CDR1 and CDR2 regions bind to the MHC protein. VDJ gene segments encode the β-chain V domain of TCRs, and VJ gene segments encode the α-chain V domain. TCR diversity, generated by a variety of mechanisms, is nearly unlimited. The Ig gene superfamily encodes proteins that are evolutionarily, structurally, and functionally related to immunoglobulins. The antigen-binding Igs, TCRs, and MHC proteins are members of this family.

Q What diversity-generating mechanisms function to produce the nearly unlimited variety of antigen-specific TCRs? What structural and functional features are common to proteins classified within the Ig gene superfamily?

27.8 T-cytotoxic (Tc) cells recognize antigens on virus-infected host cells and tumor cells through antigen-specific TCRs. Antigen-specific recognition triggers killing via perforins and granzymes. T-helper (Th) cells differentiate into several subsets. Through the action of cytokines, Th1 inflammatory cells activate macrophage effector cells; Th2 cells activate B cells. Th17 cells are activated by pathogen-activated dendritic cells and secrete IL-17 to recruit neutrophils to the site of infection. Treg cells produce cytokines that suppress adaptive immunity.

Q What mechanism do Tc cells use to identify and destroy infected cells in the body? How do Th cells differ from Tc cells, and how do the different subsets of Th cells differ from each other?

V • Immune Disorders and Deficiencies

27.9 Hypersensitivity is the induction by foreign antigens of antibody-mediated or cell-mediated immune responses

that damage host tissue. In autoimmunity, the immune response is directed against self antigens. Damage to host tissue is caused by the inflammation produced by immune mechanisms.

Q How do immediate and delayed-type hypersensitivites differ from one another in terms of immune effectors, target tissues, antigens, and clinical outcome?

27.10 Superantigens are components of certain bacterial and viral pathogens that bind outside the antigen-specific binding site of TCRs and, therefore, activate large numbers of T cells. Superantigen-activated T cells may produce diseases characterized by systemic inflammatory reactions. Immunodeficiency is the inability to generate a proper immune response, resulting in recurrent, uncontrollable infections by opportunistic pathogens. Some of the most severe immunodeficiency syndromes are SCID, caused by a genetic disorder, and AIDS, caused by HIV infection.

Q How do superantigens differ from conventional antigens in terms of initial T cell activation and clinical outcome? How does immunodeficiency resulting from SCID differ from that caused by HIV infection and AIDS?

Application Questions

1. Antibodies of the IgA class are probably more prevalent than those of the IgG class. Explain this and define the benefits this may have for the host.
2. Although genetic recombination events are important for generating significant diversity in the antigen-binding site of Igs, postrecombination somatic events may be even more important in achieving overall Ig diversity. Do you agree or disagree with this statement? Explain.
3. Polymorphism implies that each different MHC protein binds a different peptide motif. However, for the MHC class I proteins, only 6 peptide motifs can be recognized in an individual, whereas over 6000 motifs can be recognized by the entire human population. What advantage does recognition of multiple motifs have for the individual? What potential advantage does recognition of the extremely large number of motifs have for the population? Can everyone process and present the same antigens?
4. What problems would arise if a person had a hereditary deficiency that resulted in an inability to present antigens to Tc cells? What would the problems be if the person had a deficiency in presenting antigen to Th1 cells? To Th2 cells? To all T cells? What molecules might be deficient in each situation? Could a person having any one of these deficiencies survive in a normal environment? Explain for each.

Chapter Glossary

Antigen a molecule capable of interacting with specific components of the immune system and that often functions as an immunogen to elicit an adaptive immune response

Autoantibody an antibody that reacts to self antigens

Autoimmunity a harmful immune reaction directed against self antigens

B cell receptor (BCR) a cell-surface antibody that acts as an antigen receptor on a B cell

CD4 coreceptor a protein found on Th cells that interacts with MHC II on an antigen-presenting cell

CD8 coreceptor a protein found exclusively on Tc cells that interacts with MHC I on a target cell

Clonal anergy the inability to produce an immune response to specific antigens due to the neutralization of effector cells

Clonal deletion for T cell selection in the thymus, the killing of useless or self-reactive clones

Clone a copy of an antigen-reactive lymphocyte

Complementarity-determining region (CDR) a varying amino acid sequence within the variable domains of immunoglobulins or T cell receptors where contacts with antigen are made

Delayed-type hypersensitivity (DTH) an inflammatory allergic response mediated by Th1 lymphocytes

Epitope the portion of an antigen that reacts with a specific antibody or T cell receptor

Human leukocyte antigen (HLA) an antigen-presenting protein encoded by a major histocompatibility complex gene in humans

Hypersensitivity an immune response leading to damage to host tissues

Immediate hypersensitivity an allergic response mediated by vasoactive products released from IgE-sensitized mast cells

Immune memory (memory) the capacity to respond more quickly to second and subsequent exposures to an eliciting antigen

Immunogen a molecule capable of eliciting an adaptive immune response

Immunoglobulin gene superfamily a family of genes that are evolutionarily, structurally, and functionally related to immunoglobulins

Memory cell a long-lived B or T lymphocyte responsive to a specific antigen

MHC class I protein an antigen-presenting molecule found on all nucleated vertebrate cells

MHC class II protein an antigen-presenting molecule found on macrophages, B cells, and dendritic cells

Motif in antigen presentation, a specific amino acid sequence found in all peptides that bind to a given MHC protein

Negative selection in T cell selection, the deletion of T cells that interact strongly with self antigens in the thymus (*see also* clonal deletion)

Polygeny the occurrence of two or more genetically, structurally, and functionally related gene loci due to an evolutionary gene duplication event

Polymorphism in a population, the occurrence of multiple alleles for a gene locus at a higher frequency than can be explained by recent random mutations

Positive selection in T cell selection, the growth and development of T cells that interact with self MHC–peptide in the thymus

Primary immune response the production of antibodies or immune T cells on first exposure to antigen; the antibodies are mostly of the IgM class

Secondary immune response the enhanced production of antibodies or immune T cells on second and subsequent exposures to antigen; the antibodies are mostly of the IgG class

Somatic hypermutation the mutation of immunoglobulin genes at rates higher than those observed in other genes

Specificity the ability of the immune response to interact with particular antigens

Superantigen a pathogen product capable of eliciting an inappropriately strong inflammatory immune response by stimulating greater than normal numbers of T cells

T cell receptor (TCR) an antigen-specific receptor protein on the surface of T cells

T-cytotoxic (Tc) cell a lymphocyte that interacts with MHC I–peptide complexes through its T cell receptor and produces cytotoxins that kill the interacting target cell

T-helper (Th) cell a lymphocyte that interacts with MHC II–peptide complexes through its T cell receptor and produces cytokines that act on other cells. Th subsets include **Th1** cells that activate macrophages; **Th2** cells that activate B cells; **Th17** cells that activate neutrophils; and **Treg** cells that suppress adaptive immunity

Tolerance the acquired inability to produce an immune response to particular antigens

Vaccination (immunization) the inoculation of a host with inactive or weakened pathogens or pathogen products to stimulate protective active immunity

28

Clinical Microbiology and Immunology

I The Clinical Microbiology Setting 867

II Isolating and Characterizing Infectious Microorganisms 869

III Immunological and Molecular Tools for Disease Diagnosis 876

IV Prevention and Treatment of Infectious Diseases 886

microbiologynow

Bacteriophages: Tiny Allies in the Fight against Antibiotic-Resistant Bacteria

For reasons that will unfold in this chapter, it is surprisingly common for patients admitted into a healthcare facility to acquire an infection during their treatment that is unrelated to their original illness or condition. In the United States, about 1 of every 10 hospitalized individuals acquires a new infection as a result of exposure to the clinical environment, and limiting these *healthcare-associated infections* (HAIs) is a constant challenge for medical personnel.

Patient treatments that require invasive medical procedures are a common cause of HAIs, and catheter-associated urinary tract infections (CAUTIs) are among the most difficult to control. Gram-negative enteric bacteria cause the vast majority of CAUTIs, with the major causative agents being *Escherichia coli* and *Proteus mirabilis*. Many strains of these bacteria produce attachment structures, such as fimbriae and pili, which allow them to colonize the surface of the catheter (see photo of technician preparing a catheter) and potentially establish a urinary tract infection. It is estimated that more than 100 million urinary catheters are fitted each year to relieve urinary retention and incontinence, creating a need for reliable and effective measures for prevention and treatment of CAUTIs.

CAUTIs are routinely treated using antibiotics, typically sulfamethoxazole–trimethoprim (SMZ-TMP), ampicillin, or a fluoroquinolone, such as ciprofloxacin, but pathogen resistance to these drugs is becoming increasingly common. Infections that were once treated successfully through a straightforward antibiotic regimen become lingering problems when the causative agents show multidrug resistance. However, a new and potentially quite effective approach to CAUTI prevention is in development and may have great practical application.

In a recent report, researchers isolated two novel, lytic bacteriophages that specifically attack strains of *Proteus* spp., especially *P. mirabilis*, the cause of more than 40% of all CAUTIs in the United States. They then combined the viruses to create a "phage cocktail" that was applied to the surface of silicone catheters. Using culture techniques, epifluorescence, and scanning electron microscopy, the researchers were able to show that colonization and biofilm formation of *P. mirabilis* was substantially reduced in phage-coated catheters. In separate studies, similar results were obtained against pathogenic strains of *Escherichia coli* using *E. coli*–specific phages, and therefore the use of phage-coated urinary catheters for the control and prevention of CAUTIs shows exciting promise.

Source: Melo, L.D.R., et al. 2016. Development of a phage cocktail to control *Proteus mirabilis* catheter-associated urinary tract infections. *Front. Microbiol. 7:* 1024 doi:10.3389/fmicb.2016.01024. Photo courtesy of Marion General Hospital, Marion Indiana.

Clinical microbiology is that subdiscipline of microbiology concerned with identifying pathogenic microbes and advising the medical provider on treatment. Clinical laboratories must identify pathogens safely, efficiently, and reliably. The clinical microbiologist examines patient samples using direct observation, culture, immunological assays, and molecular tools to identify pathogens. Identification of pathogens guides disease control by targeting antimicrobial drugs to specific pathogens.

I • The Clinical Microbiology Setting

28.1 Safety in the Microbiology Laboratory

Clinical laboratories handle dangerous materials, and thus laboratory workers must adhere to strict safety protocols to prevent the spread of infectious agents. Standard laboratory practices for handling clinical samples have been established to minimize the risk of accidental laboratory infections. One only has to be reminded of the 2014 Ebola hemorrhagic fever outbreak in West Africa (Sections 29.7 and 30.12) to appreciate how treating infected persons without paying rigorous attention to every safety detail can endanger the lives of medical personnel.

Laboratory Safety

The clinical laboratory has potential biohazards for all personnel and is especially dangerous for untrained personnel or those who do not employ the necessary precautions. All laboratories that handle human or primate tissue must have an occupational exposure control plan for handling bloodborne pathogens. This plan is specifically designed to protect workers from infection by hepatitis B virus (HBV, the cause of infectious hepatitis, Section 30.11) and human immunodeficiency virus (HIV, the cause of acquired immunodeficiency syndrome [AIDS], Section 30.15). The occupational exposure plan limits infection by all pathogens and typically includes the use of appropriate *personal protective equipment* (PPE), such as a lab coat, gloves, eye protection, and face mask (**Figure 28.1**).

Proper training and enforcement of established safety procedures can prevent most accidental infections, which usually do not result from identifiable exposures like culture spills but instead from routine handling of patient specimens. Infectious aerosols generated during microbiological procedures are the most common causes of laboratory infections. Clinical laboratories follow the safety rules outlined in **Table 28.1** to minimize laboratory infections. These general standards apply to all laboratories that handle potentially infectious agents and are the basis for all aspects of healthcare infection control. However, as discussed next, laboratories that handle particularly dangerous or transmissible agents adhere to additional rules and procedures to ensure a safe work environment.

Biological Containment and Biosafety Levels

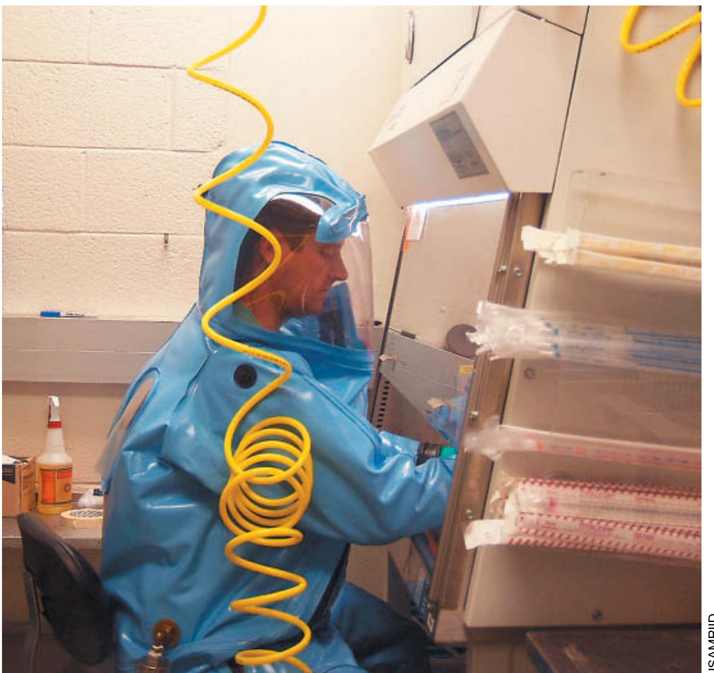
The level of containment used to prevent accidental infections or accidental environmental contamination (escape) in clinical, research, and teaching laboratories must be proportional to the biohazard potential of the organisms handled in the laboratory. Laboratories are classified according to their containment capabilities from least to greatest by their *biosafety level* (*BSL*), designated as *BSL-1, BSL-2, BSL-3*, or *BSL-4* (**Figure 28.2**). Personnel in laboratories working at all biosafety levels must follow standard laboratory practices that ensure basic cleanliness and limit contamination (Table 28.1). The precautions, equipment, and operational costs increase with each biosafety level.

CDC/PHIL

Figure 28.1 Standard apparel for clinical laboratory safety. This technician is wearing proper personal protective equipment (PPE) for a clinical laboratory, including gloves, eye protection, lab coat, and face mask.

TABLE 28.1 Microbiology laboratory safety standards

Rule	*Implementation*
Restrict access	Only laboratory workers and trained support personnel have access.
Practice good personal hygiene	Eating, drinking, applying cosmetics, and manipulating contact lenses are forbidden in the laboratory. Hand washing prevents spread of pathogens.
Use personal protective equipment (PPE)	Lab coats, gloves, eye protection, and respirators are recommended or required depending on the pathogens being handled.
Vaccinate	Personnel must be vaccinated against agents to which they may be exposed.
Handle specimens safely	Assume all clinical specimens are infectious and handle appropriately.
Decontaminate	After use or exposure, decontaminate specimens, surfaces, and materials by disinfecting, autoclaving, or incinerating.



USAMRIID

Figure 28.2 Conducting research in a BSL-4 (biosafety level 4) laboratory. BSL-4 is the highest level of biological control, affording maximum worker protection and pathogen containment. The researcher has a whole-body sealed suit with an outside air supply and ventilation system. Air locks control all access to the laboratory. All material leaving the laboratory is autoclaved or chemically decontaminated.

Most colleges and universities have BSL-1 and BSL-2 facilities for teaching and research. Standard clinical laboratories operate at BSL-2. The specialized physical requirements for BSL-3 facilities limit them to major clinical centers and research settings. Because BSL-4 facilities must ensure total isolation and physical containment of pathogens, only about fifty BSL-4 laboratories are operational worldwide. Most BSL-4 laboratories are associated with government facilities, such as the Centers for Disease Control and Prevention (CDC; Atlanta, Georgia, USA) and the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID; Fort Detrick, Maryland, USA).

MINIQUIZ

- The use of personal protective equipment (PPE) is required for clinical laboratory technicians. What protective apparel does PPE include?
- Identify and discuss the standard safety procedures adopted by microbiology laboratories. Under what biosafety level do most clinical laboratories operate? Where are most BSL-4 laboratories located?

28.2 Healthcare-Associated Infections

The universal safety measures described in the previous section are implemented to contain infectious agents and prevent their transmission. But despite such precautions, the accidental transfer of pathogens to individuals in healthcare facilities is rather common (see page 866 for more on this).

Mechanisms of Transfer of Healthcare-Associated Infections

A **healthcare-associated infection (HAI)**, also called a *nosocomial infection* (from the Latin *nosocomium*, meaning "hospital"), is an infection acquired by a patient during a stay at a healthcare facility (clinic, hospital, rehabilitation facility, etc.). HAIs cause significant morbidity (incidence of *disease* in a population) and mortality (incidence of *death* in a population). An estimated 10% of patients admitted to healthcare facilities in the United States acquire HAIs, and up to 2 million HAIs occur annually, leading directly or indirectly to about 75,000 deaths. Some of the common risk factors for acquiring infectious diseases in healthcare settings are summarized in Table 28.2.

Some HAIs are acquired from patients with communicable diseases, but others are caused by pathogens that are selected and maintained within the hospital environment, spread by cross-infection from patient to patient or from healthcare personnel. Healthcare-associated pathogens are often present as normal microbiota in either patients or healthcare staff. Therefore, healthcare facilities are high-risk environments for the spread of infections because these facilities concentrate individuals who have infectious disease or are at risk for acquiring infectious disease because of underlying health conditions. Such conditions often lead to a compromised immune system and increased susceptibility to pathogens. The frequency of HAIs at different sites of the body is shown in Figure 28.3.

Common Causative Agents of HAIs

Most HAIs are caused by a relatively short list of pathogens (Table 28.3), but many other infectious agents can cause HAIs. *Staphylococcus aureus* is one of the most important and widespread HAI pathogens (Section 30.9). It is the most common

TABLE 28.2 Risk factors for hospital-acquired infections (HAIs)

Risk factor for HAI	*Rationale*
Patients	Patients are already ill or immunocompromised
Newborn infants and the elderly	Not fully immune competent
Infectious disease patients	Pathogen reservoirs
Patient proximity	Increases cross-infection
Healthcare personnel	Can transfer pathogens between and among patients; healthcare personnel may be asymptomatic disease carriers
Medical procedures (blood draws, etc.)	Breaching the skin barrier can introduce pathogens
Surgery	Exposes internal organs, may introduce pathogens, and causes stress, which lowers resistance to infection
Anti-inflammatory drug treatment	Lower resistance to infection
Antibiotic treatment	May select for resistant and opportunistic pathogens

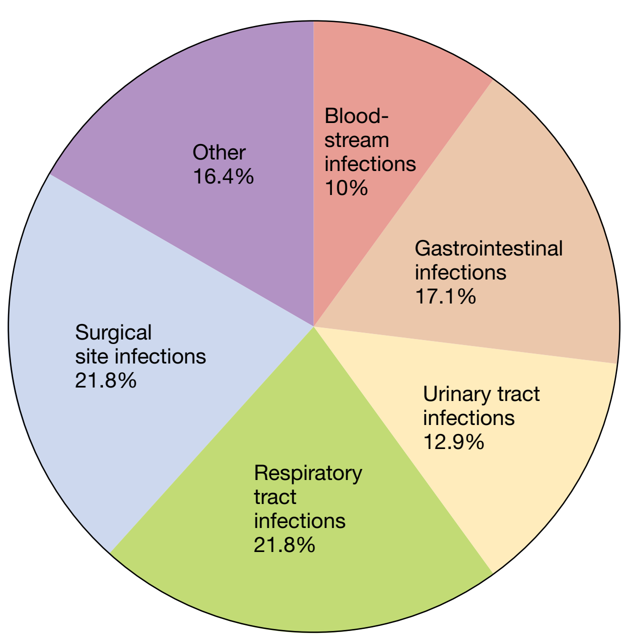


Figure 28.3 Frequency of healthcare-associated infections (HAIs) in different sites of the body. Up to 2 million HAIs occur annually in the United States. Data are from the Centers for Disease Control and Prevention.

cause of pneumonia, the third most common cause of blood infections, and is particularly problematic in nurseries. Many hospital strains of *S. aureus* are unusually virulent and are resistant to common antibiotics, making treatment especially difficult (see Explore the Microbial World: MRSA—A Formidable Clinical Challenge). The staphylococci are the most common cause of bloodborne HAIs and are also prevalent in pus-forming wound infections.

Staphylococcus and *Enterococcus*, as well as *Escherichia coli, Klebsiella pneumoniae*, and various other *Enterobacteriaceae*, all have the potential to cause HAIs, but they are also members of the normal microbiota of most individuals, making it essentially impossible to eliminate these potential pathogens from healthcare settings. In addition, these organisms can acquire multidrug resistance by horizontal gene flow (Chapter 11). Pathogens that are not part of the normal microbiota, such as species of *Acinetobacter* and *Mycobacterium*, can be eliminated from the healthcare environment. These pathogens are carried into the healthcare facilities by infected individuals or, in the case of some mycobacteria, as environmental contaminants that enter in dust and air.

Prevention of HAIs involves cooperation between the healthcare facility infection-control team and the rest of the facility staff, including direct healthcare workers and supporting staff, such as housekeeping. Infection control starts with management of incoming patients at the point of entry to the healthcare facility; incoming patients should be assessed for possible infections and isolated as necessary to prevent spread of infections to staff and other patients. From this point, the healthcare facility staff employs standard procedures that limit infection, applying the same general precautions outlined for laboratory technicians in Table 28.1.

MINIQUIZ

- Why are patients in healthcare facilities especially susceptible to pathogens?
- How can the spread of HAIs be controlled?

II • Isolating and Characterizing Infectious Microorganisms

The growth and observation of pathogens from patient specimens are important strategies for identification of the causative agent of an infectious disease. Identification leads to antimicrobial drug susceptibility testing and development of a specific treatment plan. We begin by looking at methods for collecting, culturing, and identifying pathogens, followed by methods used to determine drug susceptibility.

28.3 Workflow in the Clinical Laboratory

Collecting specimens from infectious patients and subsequently culturing pathogens using a variety of growth media is a necessary and routine practice in clinical medicine. Combined with microscopic observation, these methods allow for direct detection and identification of causative agents of disease.

Collecting Specimens and Detecting and Culturing Pathogens

Proper clinical diagnosis of infectious diseases requires that pathogens be identified from tissue or fluid samples using a variety of microbiological, immunological, and molecular biological techniques (Figure 28.4). Patient specimens must be collected aseptically from the site of the infection, and the sample size must be large enough to ensure an inoculum sufficient for growth. In addition, the requirements for organism survival, such as oxic versus anoxic conditions, must be maintained at all times, and the sample should be processed quickly to avoid degradation. Sterile swabs are often used to obtain samples from infected areas, including wounds, nares, or throat (Figure 28.5), and the swab is then used to inoculate a suitable growth medium.

Many pathogens can be detected by direct means using one or more of several diagnostic tests. For example, the microscopic observation of gram-negative diplococci, especially inside neutrophil inclusions, in a urethral exudate sample is diagnostic for infection with *Neisseria gonorrhoeae* (Figure 28.6*a*), the causative agent of the sexually transmitted disease gonorrhea (Section 30.13). However, the reliability of any diagnostic test depends on both the *specificity* and the *sensitivity* of the test. **Specificity** is the ability of the test to recognize a single pathogen. High specificity reduces the likelihood of a *false-positive* result. **Sensitivity** defines the smallest quantity of a pathogen or a pathogen product that can be detected. High sensitivity minimizes the likelihood of a false-negative reaction. For the detection of *N. gonorrhoeae*, the specificity of Gram-stained smears of urogenital exudates is high for both men and women (≥95%), so false-positive tests for gonorrhea are rare. By contrast, the sensitivity of Gram-stained smears of

TABLE 28.3 Common healthcare-associated pathogens

Pathogen	*Common infection sites and diseases*	*Micrographs*[b]
[a]*Acinetobacter*	Wound/surgical site, bloodstream, pneumonia, urinary tract	*Acinetobacter*
Burkholderia cepacia	Pneumonia	*B. cepacia*
Clostridium difficile *C. sordellii*	Gastrointestinal tract Pneumonia, endocarditis, arthritis, peritonitis, myonecrosis	*C. difficile*
[a]*Enterobacteriaceae*, carbapenem-resistant, especially *Escherichia coli* and *Klebsiella pneumoniae*	Urinary tract, pneumonia, wound/surgical site, [c]bloodstream	*E. coli* *Klebsiella*
[a]Vancomycin-resistant *Enterococcus*	Wound/surgical site, bloodstream, urinary tract	*Enterococcus*
Hepatitis	Chronic liver infection	Hepatitis B virus
Human immunodeficiency virus (HIV)	Immunodeficiency	HIV
Influenza virus	Pneumonia	Influenza virus
[a]*Mycobacterium tuberculosis* *M. abscessus*	Chronic lung infection Skin and soft tissue infections	*M. tuberculosis*
Norovirus	Gastroenteritis	Norovirus
[a]*Staphylococcus aureus* Methicillin-resistant (MRSA) and vancomycin-intermediate and -resistant strains	Bloodstream, pneumonia, endocarditis	*S. aureus*

[a]Antibiotic-resistant organisms that exhibit multiple drug resistance. Because of the promiscuous nature by which multiple drug-resistant plasmids can be transmitted, many of the pathogens listed as well as members of the normal microbiota could be or become drug resistant in the highly selective nature of the healthcare environment where antibiotics are used routinely and extensively. In addition to the pathogens listed, other extremely pathogenic agents (such as Ebola virus) could be localized to an isolation unit of a healthcare facility, thus making that part of the facility especially dangerous for disease transmission.
[b]All inset photographs are colorized scanning or transmission electron micrographs obtained from CDC/PHIL. Additional micrograph credits (numbers run top to bottom): 1–5, 10, and 12, Janice Haney Carr; 6, Peta Wardell; 7, Erskine Palmer; 8, A. Harrison and P. Feorino; 9, Frederick Murphy; 11, Charles D. Humphrey.
[c]Many urinary tract hospital-associated infections are caused by the gram-negative enteric bacterium *Proteus mirabilis* (see page 866).

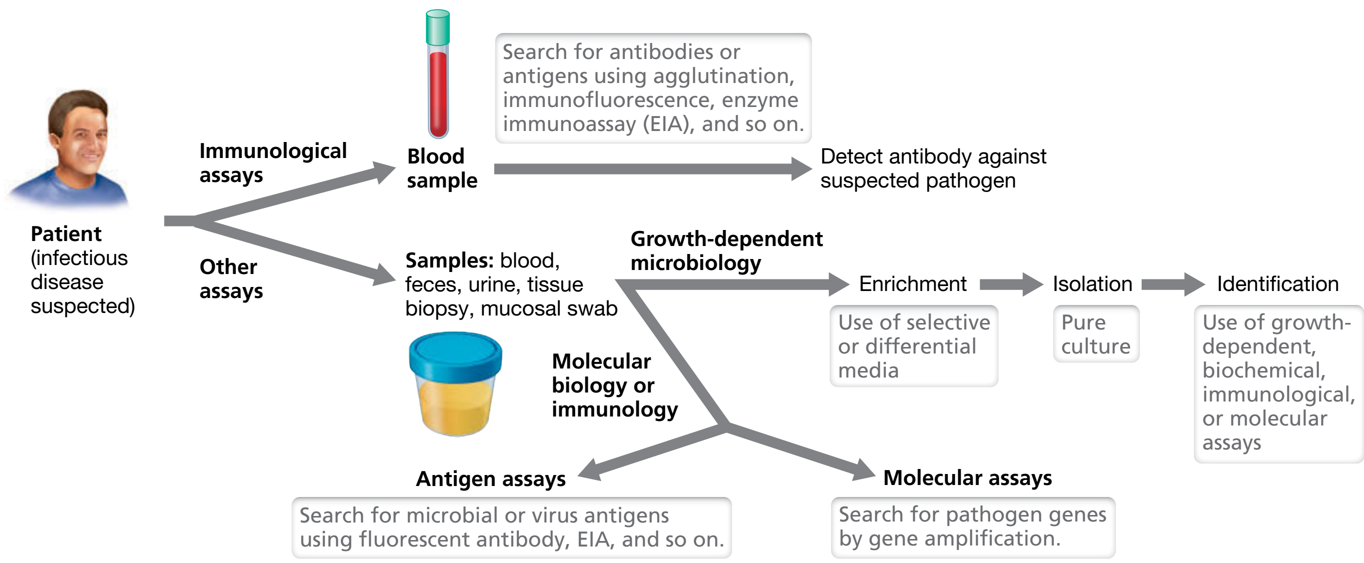


Figure 28.4 Laboratory identification of microbial pathogens. The flowchart shows alternative paths for identifying pathogens or pathogen exposure in the clinical laboratory.

urogenital exudates for the detection of *N. gonorrhoeae* is about 80% greater for men than for women. Thus, in suspected cases of gonorrhea in females, *false-negative* Gram stains are relatively common. Females must therefore be examined by more sensitive methods, including culture techniques (Figure 28.6*b*), to establish or confirm a diagnosis of gonorrhea.

Specific pathogens can be selectively grown, isolated, and identified from patient specimens using specialized growth media and incubation conditions to establish an *enrichment culture* (Sections 19.1 and 19.2). For primary enrichment, clinical samples are inoculated on general-purpose media, such as *blood agar* (**Figure 28.7*a***) and *chocolate agar* (so called because it contains heat-lysed blood, making it brown in color; Figure 28.7*b*), which support the growth of a variety of microorganisms. To aid in the isolation and identification of specific pathogens, medical technologists use various *selective* and *differential* growth media. **Selective media** are specialized culture media that contain inhibitory agents for the purpose of allowing some organisms to grow but not others. **Differential media** allow identification of organisms based on the appearance of the culture after growth. For example, eosin–methylene blue (EMB) agar is a selective

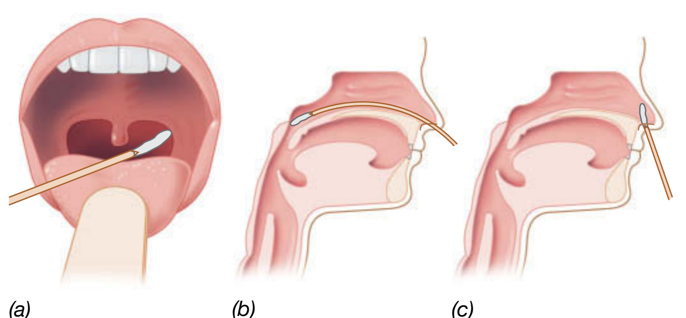


Figure 28.5 Specimens from the upper respiratory tract. *(a)* Throat swab. *(b)* Nasopharyngeal swab passed through the nose. *(c)* Swabbing the inside of the nose.

medium because the methylene blue it contains inhibits the growth of gram-positive bacteria. EMB agar is also a differential medium because it distinguishes gram-negative bacteria that can ferment lactose from those that cannot. Lactose fermenters, such as *Escherichia. coli*, acidify the medium and produce dark colonies that may have a reflective metallic green sheen; non–lactose "fermenters, such as *Pseudomonas aeruginosa*, produce opaque or translucent colonies (Figure 28.7*c*).

Blood and Cerebrospinal Fluid Specimens

Pathogens in liquid tissue samples, such as blood and cerebrospinal fluid (CSF), are routinely detected using automated culture

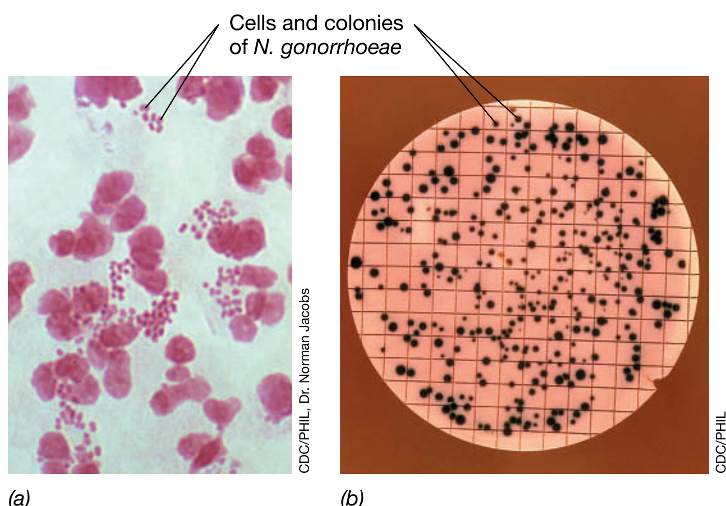


Figure 28.6 Identification of *Neisseria gonorrhoeae*, the cause of gonorrhea. *(a)* Cells of gram-negative *N. gonorrhoeae* within human polymorphonuclear leukocytes (neutrophils) from a urethral exudate. *(b)* Colonies of *N. gonorrhoeae* growing on a filter placed on Thayer–Martin agar. Oxidase reagent, which turns colonies dark purple if they contain cytochrome *c*, has been added to the filter. The dark color of the colonies shows that *N. gonorrhoeae* is oxidase-positive.

MRSA—A FORMIDABLE CLINICAL CHALLENGE

Staphylococcus aureus is an opportunistic human pathogen that colonizes an estimated one-third of the population. In such individuals, this gram-positive bacterium usually exists as an innocuous member of the normal microbiota of the skin and mucous membranes, especially in the nasal epithelium. Although most complications from staphylococci occur in the form of local skin infections, *S. aureus* may in some cases invade other tissues of the body, including the bloodstream, where a host of virulence factors it produces may transform this bacterium from a harmless commensal to a serious pathogen capable of causing life-threatening disease.

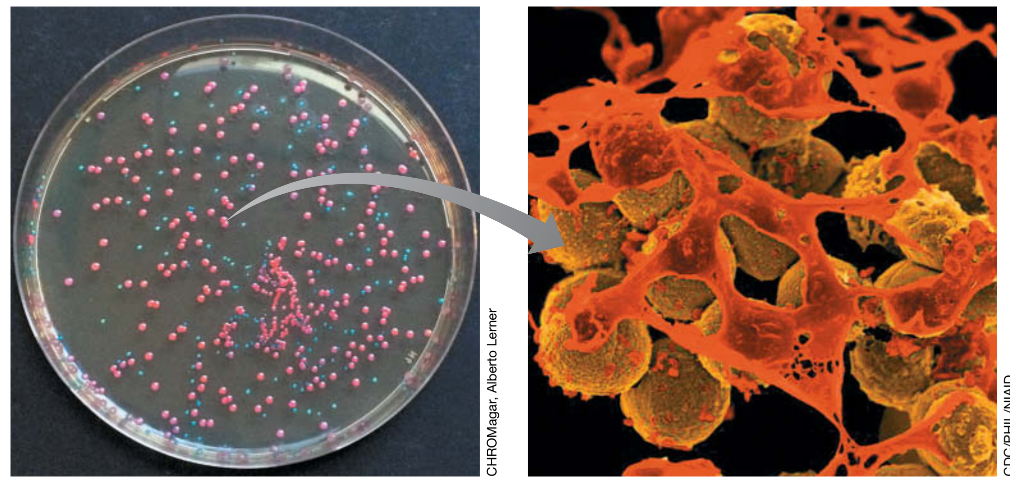


Figure 1 **Methicillin-resistant *Staphylococcus aureus* (MRSA).** Colonies of MRSA on this chromogenic agar medium (left) appear pink, whereas colonies of other bacteria appear blue. The colorized scanning electron micrograph on the right shows cells of MRSA (yellow) embedded in a matrix of cellular debris (orange).

Virulence factors of *S. aureus* include coagulase and clumping factor, two proteins that precipitate blood plasma components and promote the formation of a protective matrix around the bacterial cells that inhibits the activity of phagocytes at sites of infection. In addition, protein A is a surface protein on *S. aureus* that obstructs opsonization by antibodies and complement (Section 26.9), effectively camouflaging the pathogen and further impeding phagocytosis. *S. aureus* also produces several potent exotoxins, including α-hemolysin (lyses blood cells), enterotoxin B (causes food poisoning), and toxic shock syndrome toxin-1 (causes life-threatening toxic shock).

Many *S. aureus* infections are effectively treated using β-lactam antibiotics, such as various penicillins, but the number of strains resistant to these drugs is increasing as global use of antibiotics remains extremely high (see Figure 28.28*a*). Infections caused by multidrug-resistant strains of *S. aureus* are often treated with *methicillin*, a powerful semisynthetic β-lactam drug that is among the last treatment options available in these cases. However, an alarming percentage of *S. aureus* strains have also developed resistance to methicillin in recent years. These methicillin-resistant *S. aureus* (MRSA) strains have been especially prevalent as healthcare-associated infections.

Clinical diagnosis of MRSA infections depends upon either culture-based techniques, which often include the use of chromogenic agar media (**Figure 1**) or nucleic acid–based tests (Section 28.8). Confirmation of infection must be followed by antibiotic susceptibility testing (Section 28.4) to determine the best treatment strategy. Typically, treatments are case-specific and require a non-β-lactam antibiotic such as vancomycin, tetracycline, or sulfa drugs (Section 28.10), often administered intravenously.[1]

The global incidence of MRSA infection remains high; almost 80,000 cases are documented every year in the United States alone, and several regions of the world have reported increases in MRSA prevalence in recent years, especially in India.[2] Interestingly, the prevalence of healthcare-associated MRSA infections has steadily declined in recent years while that of community-associated MRSA infections has increased. The United States Centers for Disease Control and Prevention estimates that MRSA carriers comprise 2% of the population. Considering its prevalence and extensive multidrug resistance, MRSA may continue to prove a difficult pathogen to control.

[1]American Academy of Microbiology. 2015. FAQ: The threat of MRSA. AAM: Washington, D.C.
[2]Center for Disease Dynamics, Economics, and Policy. 2015. State of the world's antibiotics, 2015. CDDEP: Washington, D.C.

systems. For a suspected case of meningitis, a CSF specimen is obtained by a procedure called a *lumbar puncture* (spinal tap) in which 3–5 ml of fluid is collected drop-by-drop from a needle inserted between lumbar vertebrae. CSF is sterile and clear in a healthy individual, and therefore fluid turbidity and high leukocyte counts are indicators of infection. Similar to other liquid specimens (blood, urine, sputum, wound exudates, etc.), CSF is routinely examined by Gram staining and used to inoculate selective culture media.

The standard procedure for obtaining a blood sample is to aseptically draw 10–20 ml of blood from a vein and inject it into two culture bottles containing general-purpose growth media and an anticoagulant. One bottle is incubated aerobically, while the other is incubated anaerobically (**Figure 28.8**); both are kept at 35°C for several days. Automated culture systems (Figure 28.8*b*) detect growth by measuring turbidity or fluorescence and by periodically monitoring the consumption of O_2 or the production of CO_2. Most clinically significant bacteria are recovered within 2 days, but growth of some pathogens, including mycobacteria and certain fungi, may take 3 to 5 days or longer. Cultures that exhibit growth are Gram stained and then inoculated onto specialized media for isolation and identification.

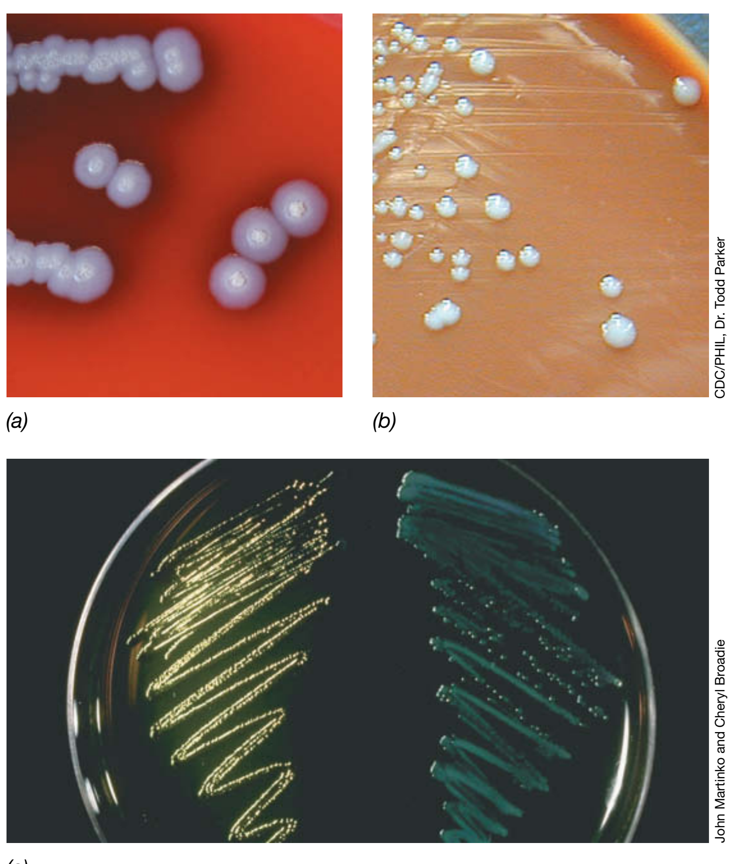


Figure 28.7 Enriched media. *(a) Burkholderia* growing on sheep blood agar (SBA); the red color is from blood suspended in the trypticase soy agar medium. *(b) Francisella tularensis* growing on chocolate agar; the brown color is due to heat-lysed blood in the trypticase soy agar medium. *(c) Escherichia coli*, a lactose fermenter (left), and *Pseudomonas aeruginosa*, a non–lactose fermenter (right) growing on eosin–methylene blue (EMB) agar. The reflective, greenish-yellow sheen of the colonies on the left identifies *E. coli* as a lactose fermenter.

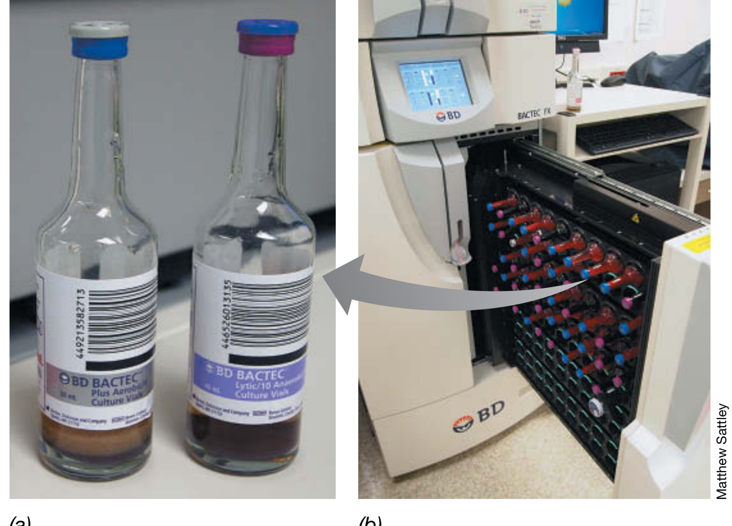


Figure 28.8 Growth-dependent diagnostic testing for blood infections. *(a)* Cultures to assay both aerobic (left vial) and anaerobic (right vial) bacterial growth are inoculated with an aseptically drawn patient blood sample. *(b)* After inoculation, both vials in part *a* are incubated in an automated system that measures growth, for example, by turbidity, production of CO_2, or fluorescence. Photos taken courtesy of Marion General Hospital, Marion, Indiana, USA.

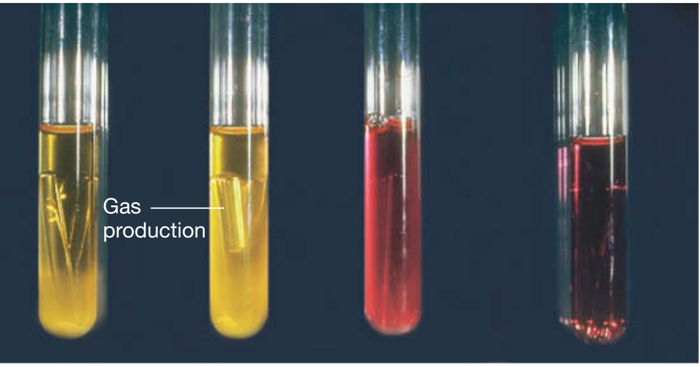
The most common pathogens found in blood include species of *Staphylococcus* and *Enterococcus*, but many other bacteria may cause blood infections. Computer databases are used to unambiguously identify clinical isolates by matching their metabolic reactions in various differential media to the biochemical patterns of known pathogens. The biochemical tests incorporated into differential media evaluate the presence or absence of enzymes that catabolize a specific substrate or substrates. Although hundreds of different biochemical tests are known, just a few key tests may be sufficient to identify some pathogens.

Urinary Tract and Fecal Cultures

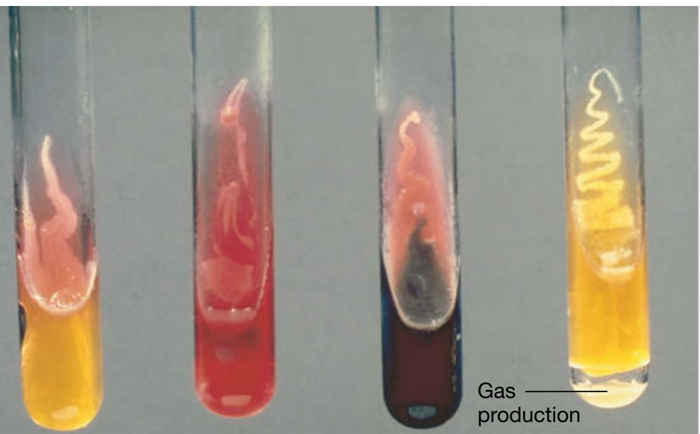
Urinary tract infections (UTIs) are common, especially in women. In most cases, microorganisms infect the urinary tract by ascending into the bladder from the urethra. UTIs, often introduced through the use of urinary catheters, are among the most common healthcare-associated infections. Direct microscopic examination of urine from a UTI patient usually shows the presence of abnormal numbers of bacteria in the urine. A Gram stain may be done directly on urine samples to identify the morphology of urinary tract pathogens, such as gram-negative rods (various enteric bacteria), gram-negative cocci (species of *Neisseria*), and gram-positive cocci (especially species of *Enterococcus*).

A significant UTI typically results in bacterial counts of 10^5 or more cells per milliliter of urine. The most common causative agents of UTIs are enteric bacteria, with *E. coli* accounting for about 90% of cases. Blood agar is often used for primary enrichment and isolation of urinary tract pathogens. Selective and differential enteric media, such as EMB or MacConkey agar, permit differentiation of gram-negative lactose fermenters from non–lactose fermenters (Figure 28.7*c*) and inhibit the growth of possible gram-positive contaminants, such as commensal staphylococci. Additional differential and/or selective media may be used to identify urinary tract (or other) pathogens via traditional culturing techniques (**Figure 28.9*a, b***) or rapid and convenient media kits (Figure 28.9*c*).

Proper collection of fecal samples is important for the isolation of intestinal pathogens. Fecal specimens become more acidic during storage, so delay between sampling and processing must be minimized, especially for the isolation of acid-sensitive pathogens, such as *Shigella* and *Salmonella*. The fecal sample is placed in a sterile, sealed container for transport to the laboratory. Feces containing blood or pus, as well as feces from patients with suspected foodborne or waterborne infections, are inoculated into suitable media for isolation of potential pathogens. For example, many laboratories use selective and differential media to identify *E. coli* O157:H7 and *Campylobacter* species, important intestinal pathogens typically acquired from contaminated food or water (Sections 32.11 and 32.12). Intestinal eukaryotic pathogens, such as *Giardia intestinalis* (Section 33.4), are identified by direct microscopic observation of parasite cysts in diluted feces or through antigen-detection assays (see Figure 28.18*c*) rather than by culturing.



(a)



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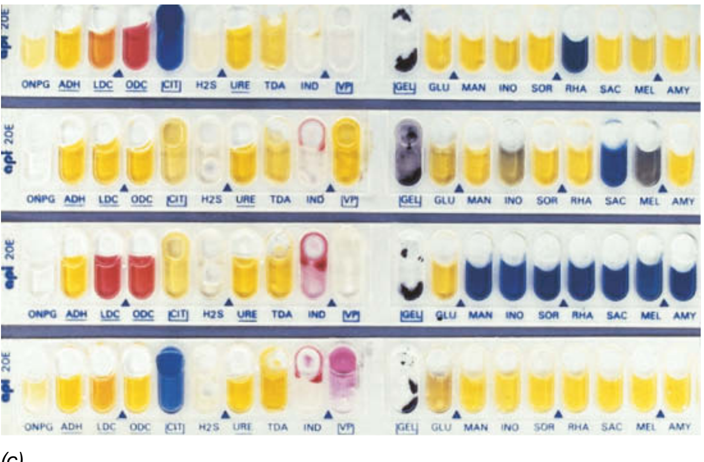


(c)

Figure 28.9 Growth-dependent diagnostic tests for clinical isolates. *(a)* Differential media to assess sugar fermentation. Acid production is indicated by a color change (from red to yellow) of the pH-indicating dye in the medium. The appearance of a bubble in the small, inverted inner tube indicates gas production from fermentation. *(b)* Diagnostic test for enteric bacteria using triple-sugar iron (TSI) agar. The medium contains glucose, lactose, and sucrose. Organisms able to ferment only glucose cause acid formation only in the bottom of the tube, whereas lactose- or sucrose-fermenting organisms cause acid formation throughout the slant. The breaking up of the agar in the bottom of the tube indicates gas formation. Blackening of the agar is due to the reaction of hydrogen sulfide (from either protein degradation or thiosulfate reduction) with ferrous iron in the medium. From left to right: Fermentation of glucose only (typical of *Shigella*); growth but no fermentation (typical of *Pseudomonas*); hydrogen sulfide formation (typical of *Salmonella*); fermentation of sugars with gas production (typical of *Escherichia coli*). *(c)* Miniaturized media kits allow rapid identification of clinical isolates by running many biochemical tests on specimen samples at the same time. Four separate strips, each with a different isolate, are shown.

Wounds and Abscesses

Infections associated with injuries such as animal bites, burns, or cuts are sampled to recover the relevant pathogen. The results must be interpreted carefully to differentiate between infection and contamination. Wound infections and abscesses often harbor a variety of normal microbiota, and swab samples from such lesions are frequently misleading. For abscesses and other purulent lesions, pus is aspirated with a sterile syringe and needle following disinfection of the skin surface. Internal purulent lesions are sampled by biopsy or from tissues removed in surgery. Gram stains are prepared directly from these specimens and examined microscopically.

Pathogens commonly associated with wound infections are *Staphylococcus aureus*, enteric bacteria, *Pseudomonas aeruginosa*, and anaerobes, such as species of *Bacteroides* and *Clostridium*. Because of the varied oxygen requirements of these bacteria, samples must be obtained, transported, and cultured under anoxic as well as oxic conditions. The major isolation media are blood agar, selective media for enteric bacteria, and enrichment media containing additional supplements and reducing agents for obligate anaerobes. Widely used tools for the detection of methicillin-resistant *Staphylococcus aureus* (MRSA) in skin infections are *chromogenic* agar media. These selective and differential media contain a chromogenic substrate that, when metabolized, causes MRSA to produce distinctly colored colonies (see Explore the Microbial World: MRSA—A Formidable Clinical Challenge).

Genital Specimens and Culture for Gonorrhea

Sexually transmitted infections (STIs) that cause a purulent urethral discharge, especially in males, are classified as either nongonococcal or gonococcal urethritis. Nongonococcal urethritis is usually caused by *Chlamydia trachomatis* (⇌ Section 30.14), *Ureaplasma urealyticum*, or *Trichomonas vaginalis* (⇌ Section 33.4). Gonococcal urethritis is caused by *Neisseria gonorrhoeae* (⇌ Section 30.13).

Cells of *N. gonorrhoeae* are gram-negative diplococci, a morphology not normally found in microbiota of the urogenital tract. Therefore, a Gram stain of a urethral, vaginal, or cervical smear revealing such cells, often surviving inside neutrophils (Figure 28.6*a*), is diagnostic for gonorrhea. Chocolate agar, a nonselective enriched medium, is often used for specimens suspected to contain *N. gonorrhoeae*. A selective medium used for isolation of *N. gonorrhoeae* is modified Thayer–Martin (MTM) agar (Figure 28.6*b*). This medium incorporates the antibiotics vancomycin, nystatin, trimethoprim, and colistin to suppress the growth of normal microbiota. These antibiotics have no effect on *N. gonorrhoeae* or *Neisseria meningitidis*, a cause of bacterial meningitis (⇌ Section 30.5).

Inoculated plates are incubated in a humid atmosphere containing 3–7% CO_2 for 24–48 hours and then tested for their oxidase reaction (Figure 28.6*b*). Oxidase-positive, gram-negative diplococci growing on MTM or chocolate agar are presumed to be gonococci if the inoculum was derived from the urogenital tract. Definitive identification of *N. gonorrhoeae* requires determination of carbohydrate utilization patterns and immunological or nucleic acid probe tests. Laboratory testing of urogenital samples for *N. gonorrhoeae* (and the often-associated *C. trachomatis*) is often done using DNA amplification via polymerase chain reaction (PCR) or other molecular methods.

Culture of Anaerobic Pathogens

The identification of obligately anaerobic bacteria from patient specimens requires special isolation and culture methods (Section 5.14). In general, media for anaerobes do not differ greatly from those used for aerobes, except that they (1) are usually richer in organic constituents, (2) contain reducing agents (usually cysteine or thioglycolate) to remove O_2, and (3) contain a redox indicator to show that conditions are anoxic. Collection, handling, and processing of specimens must exclude exposure to air because oxygen is toxic to obligate anaerobes. Samples collected by syringe aspiration or biopsy must be immediately transferred to a sealed tube containing O_2-free gas, usually with a dilute salt solution containing a reducing agent and a redox indicator to monitor O_2 contamination. Specimens are then used to inoculate anoxic media in an automated culture system or in an anoxic "glove box" filled with O_2-free gases, usually a mixture of N_2 and H_2.

Several habitats in the body, including portions of the oral cavity and the lower intestinal tract, are anoxic and support the growth of anaerobic normal microbiota. Other parts of the body may also become anoxic if injury or disease reduces the blood supply to certain tissues, a condition called *ischemia*. These anoxic sites can then be colonized by obligate anaerobes. Although potentially pathogenic anaerobic bacteria are part of the normal microbiota, their numbers are kept in check through competition with other members of the microbial community. Under certain conditions, however, normally benign anaerobes may become opportunistic pathogens. A key example is *Clostridium difficile* (Table 28.3); this usually harmless member of the normal microbiota of the lower intestinal tract commonly emerges as a healthcare-associated pathogen when extended antibiotic therapy destroys competing microbes (Section 24.10).

MINIQUIZ

- What are the key points necessary for proper collection of clinical specimens, and why is it important that diagnostic tests for these specimens are both highly specific and sensitive?
- Identify culture methods and conditions used for blood, wound, urine, fecal, and genital specimens. Of what importance are selective and differential growth media in pathogen detection, and what special conditions must be maintained for the isolation of anaerobic pathogens?

28.4 Choosing the Right Treatment

Pathogens isolated from clinical specimens are identified to confirm medical diagnoses and to guide antimicrobial therapy. Appropriate and effective treatment for many pathogens is based on current experience and practices. For some pathogens, however, decisions about appropriate antimicrobial therapy must be made on a case-by-case basis. Such pathogens include those for which antimicrobial drug resistance is common (for example, gram-negative enteric bacteria), those that cause life-threatening disease (for example, meningitis caused by *Neisseria meningitidis*), and those that require bactericidal rather than bacteriostatic drugs (Section 5.17) to prevent disease progression and tissue damage. Bactericidal agents are indicated, for example, for bacterial endocarditis (infection of the inner tissues of the heart, such as the heart valves), where total and rapid killing of the pathogen is critical for patient survival.

Minimum Inhibitory Concentration

Antimicrobial susceptibility is measured by determining the smallest amount of agent needed to completely inhibit the growth of the tested organism in vitro (in laboratory culture), a value called the **minimum inhibitory concentration (MIC)**. The traditional way to determine the MIC for a given agent against a given organism is to prepare a series of culture tubes inoculated with the same number of microorganisms. Each tube contains the growth medium with an increasing concentration of the antimicrobial agent. After incubation, the tubes are checked for visible growth (turbidity), and the MIC is the lowest concentration of the agent that completely inhibits the growth of the test organism (Section 5.17 and Figures 5.37 and 5.38).

In modern practice, the MIC of a given antimicrobial agent is typically determined using microliter amounts of media and reagents. For example, a miniaturized version of the MIC test uses a microtiter method with twofold dilutions of several antibiotics in medium inoculated with a standard amount of the test organism (**Figure 28.10*a***). In clinical microbiology laboratories, tests for routine MIC determinations are usually automated using instruments that also allow for species identification of pure cultures obtained from patient specimens (Figure 28.10*b, c*).

Measuring Antimicrobial Susceptibility

The standard assay for antimicrobial activity is the *disc diffusion test* (Figure 28.10*d–g*). A Petri plate containing an agar medium is inoculated by evenly spreading a suspension of a pure culture of the suspected pathogen on the agar surface. Known amounts of different antimicrobial agents infused into filter-paper discs are then placed on the surface of the agar. The agents then diffuse from the discs into the agar during incubation, establishing a gradient; the farther the chemicals diffuse away from each disc, the lower is the concentration of the agent. At some distance from each disc, the effective MIC is reached. Beyond this point the microorganism is able to grow, but closer to the disk, growth is absent. A *zone of inhibition* forms with a diameter proportional to the concentration, solubility, diffusion coefficient, and overall effectiveness of the antimicrobial agent in the disc.

In addition to the disc diffusion test, Figure 28.10*g* depicts antibiotic susceptibility using the *epsilometer test* (Etest). This assay uses a plastic strip infused with a predefined concentration gradient of an antimicrobial agent. When applied to the surface of an inoculated agar plate, the gradient transfers from the strip to the agar and remains stable throughout the incubation period, during which an elliptical zone of inhibition centered along the axis of the strip develops. The concentration of the antimicrobial agent (in μg/ml) is read at the point where the ellipse edge intersects the precalibrated test strip, providing a precise MIC (Figure 28.10*g*).

Assuming culture conditions are standardized, different antimicrobial agents can be compared to determine which is most effective

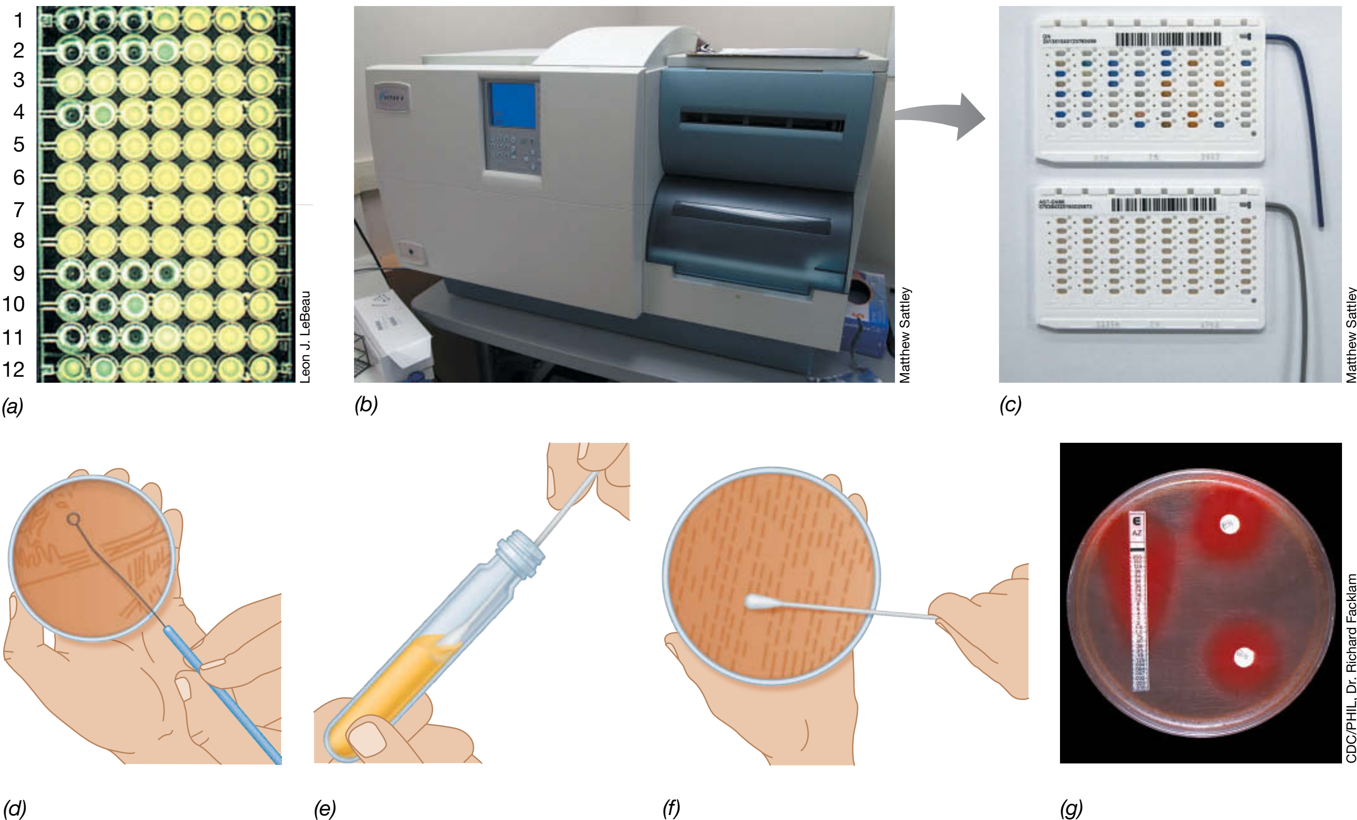


Figure 28.10 Antibiotic susceptibility testing. *(a)* Antibiotic susceptibility of a pathogen as determined by the broth dilution method in a microtiter plate. The organism is *Pseudomonas aeruginosa*. Each row has a different antibiotic in a series of concentrations. The highest concentration of antibiotic is in the well at the left; serial twofold dilutions are made in the wells to the right. In rows 1 and 2, the third well has the lowest concentration of antibiotic that shows no visible growth. In row 3, the antibiotic is ineffective at the concentrations tested because there is growth in all wells. *(b, c)* An automated system for identifying clinical isolates and determining their antibiotic susceptibility. Card wells (panel *c*) are inoculated using the attached capillary tube. Following internal incubation, computer-scanned results are available in less than 24 hours. Photos taken courtesy of Marion General Hospital, Marion, Indiana (USA). *(d)* For the disc diffusion test, colonies from a pure culture of the pathogen are transferred to a liquid medium and mixed. *(e, f)* A sterile swab is dipped into the bacterial suspension and streaked evenly over the entire surface of a suitable agar medium. *(g)* Discs containing known amounts of different antibiotics are placed on the inoculated agar surface. After incubation, zones of inhibition are measured, and antibiotic susceptibility is determined using a standardized chart of zone sizes. For the epsilometer test (Etest, AB BIODISK, Solna, Sweden), a plastic strip containing an antibiotic gradient (in μg/ml) indicates the MIC at the point where the elliptical zone of inhibition meets the strip. In this example, the MIC for azithromycin (AZ) is 1.0 μg/ml.

against the isolated pathogen. The Clinical and Laboratory Standards Institute (www.clsi.org) is responsible for developing, establishing, and constantly updating consensus standards for antimicrobial testing. Hospital infection-control microbiologists produce and examine susceptibility data to generate periodic reports called *antibiograms*. These reports define the susceptibility of clinically isolated organisms to the antibiotics in current use. Antibiograms are used to monitor control of known pathogens, to track the emergence of new pathogens, and to identify the emergence of antibiotic resistance at the local level.

MINIQUIZ

- Describe the disc diffusion test and the Etest for antimicrobial susceptibility. For an individual organism and an antimicrobial agent, what do the results signify?
- What is the value of antimicrobial drug susceptibility testing for the microbiologist, the physician, and the patient?

III • Immunological and Molecular Tools for Disease Diagnosis

Culture methods for some pathogens, including many viruses and some pathogenic bacteria, are not routinely available, are unreliable, or are prohibitively difficult or expensive to perform. In such cases, *growth-independent* diagnostic methods are used in clinical, reference, and research laboratories to detect specific pathogens or their products. These include a variety of immunological and molecular assays that can yield a relatively quick and reliable means of identifying individual pathogens or host exposure to pathogens in the absence of cultured organisms.

28.5 Immunoassays and Disease

Many immunoassays use antibodies specific for pathogens or their products for in vitro tests designed to detect specific infectious agents. Patient immune responses, discussed in Chapters 26

and 27, can also be monitored to obtain evidence of pathogen exposure and infection.

Serology and Antibody Titers

The study of antigen–antibody reactions in vitro is called **serology**. Serological assays detect pathogen-induced antibodies in patient serum and are the basis for a number of diagnostic tests. The *specificity* of the antibody–antigen reactions associated with serological tests allows one to pinpoint an exposure to a single pathogen, assuming the antigen used to detect antibodies is unique to the pathogen in question. Moreover, serological tests vary considerably in their *sensitivity*, that is, in the amount of antibody necessary to detect antigen. For example, passive *agglutination* reactions (see Section 28.6) are fast and easy to perform but require antibody concentrations of up to 6 nanograms (ng, 10^{-9}g) per ml. By contrast, the very sensitive but more technically demanding *enzyme immunoassay* (*EIA*) tests require as little as 0.1 ng of antibody per ml and can detect as little as 0.1 ng of antigen (Section 28.7).

If an individual is infected with a suspected pathogen, the immune response—antibodies to that pathogen—should become elevated. Strong evidence for infection can therefore be obtained by determining the *antibody titer* directed against antigens produced by the suspected pathogen. The **titer** is a quantitative measure of antibody level and is defined as the highest dilution (lowest concentration) of serum at which an antigen–antibody reaction is observed (**Figure 28.11**).

A positive antibody titer indicates previous infection or exposure to a pathogen. For pathogens rarely found in a population, such as the life-threatening hantaviruses (Section 31.2), a single positive test for a pathogen-specific antibody may indicate active infection. In most cases, however, the mere presence of antibody does not indicate active infection. Antibody titers typically remain detectable for long periods after a previous infection has been resolved. To link an acute illness to a particular pathogen, it is essential to show a *rise* in antibody titer in serum samples taken from a patient during the acute disease and later during the convalescent phase of the disease. Frequently, the antibody titer is low during the acute stage of the infection and rises during convalescence (Figure 28.11). A rise in antibody titer is strong circumstantial evidence that the illness is due to the suspected pathogen.

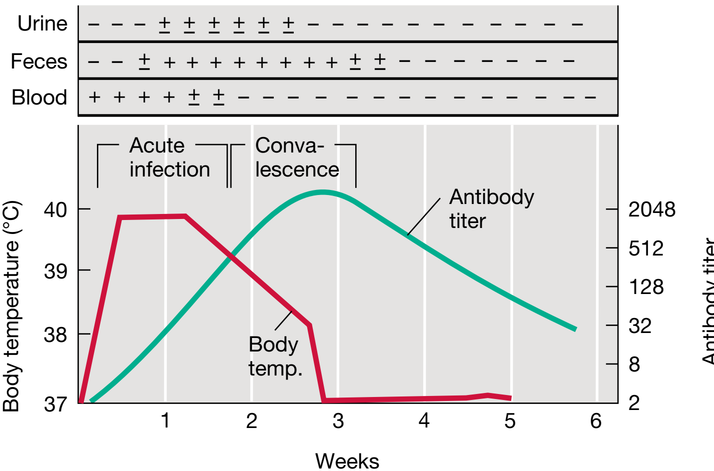


Figure 28.11 Pattern of infection and immunity in untreated typhoid fever patients. Body temperature indicates acute disease progression over time. Antibody titer is shown as the reciprocal of the highest serial dilution causing agglutination of *Salmonella enterica* (*typhi*) (Section 32.5). The presence of bacteria in blood, feces, and urine was determined from cultures (–, no bacteria; ±, low numbers of bacteria; +, high numbers of bacteria). Bacteria clear from the blood as the antibody titer rises, whereas clearance from feces and urine requires more time. Body temperature drops to normal as the antibody titer rises.

Skin Tests

A number of pathogens induce a delayed-type hypersensitivity (DTH) response mediated by Th1 cells (Section 27.9). For these pathogens, skin testing may be useful to determine exposure. As an example, a commonly used skin test is the *tuberculin test*, which consists of an intradermal injection of a soluble extract from cells of *Mycobacterium tuberculosis*. A positive inflammatory reaction at the site of injection within 48 hours indicates current infection or previous exposure to (or vaccination against) *M. tuberculosis*. This test identifies responses caused by pathogen-specific inflammatory Th1 cells (Figure 27.27). Skin tests are routinely used to aid in diagnosis of tuberculosis, Hansen's disease (leprosy), and some fungal diseases because the antibody responses for intracellular and fungal infections are often weak or undetectable.

If a pathogen is extremely localized, there may be little induction of a systemic immune response and no rise in antibody titer or skin test reactivity, even if the pathogen is proliferating profusely at the site of infection. A good example is the infection of urogenital mucosal surfaces with the bacterium *Neisseria gonorrhoeae*. Gonorrhea does not elicit a systemic or protective immune response, there is no serum antibody titer or skin test reactivity, and reinfection of individuals is common (Section 30.13).

Monoclonal Antibodies

An expanding area of research with broad application in the diagnosis and treatment of disease is the development and use of **monoclonal antibodies (mAbs)**. In contrast to *polyclonal antibodies*, which occur as a mixture of immunoglobulins produced by many individual B cells and directed at numerous antigenic determinants on a pathogen, mAbs are derived from a B cell clone sensitized to a single antigenic determinant. Thus, an in vitro B cell clone culture produces monospecific mAbs that can be collected for diagnostic or therapeutic purposes. However, antibody-producing B cells are relatively short-lived and normally die within several weeks in cell culture. To produce long-lived B cell clones for commercial mAb production, antibody-producing B cells are fused with *myelomas*, tumorigenic B cells that divide and grow indefinitely (**Figure 28.12**). The "immortal" cell lines that result from this fusion are hybrid cells, appropriately called *hybridomas*. The hybrid cell lines share the biological properties of both fusion partners; they grow indefinitely in vitro and produce antibodies.

To produce a particular mAb, a mouse is immunized with the antigen of interest. Antigen-specific B cells then proliferate over several weeks and begin to produce antibodies in the mouse. B cells are then removed from the mouse and mixed with myeloma cells (Figure 28.12), but only a small number of these fuse into antibody-producing hybridomas. The hybridomas are isolated from unfused cells using a selective *HAT* medium, so called because it contains the metabolites hypoxanthine (H) and thymidine (T),

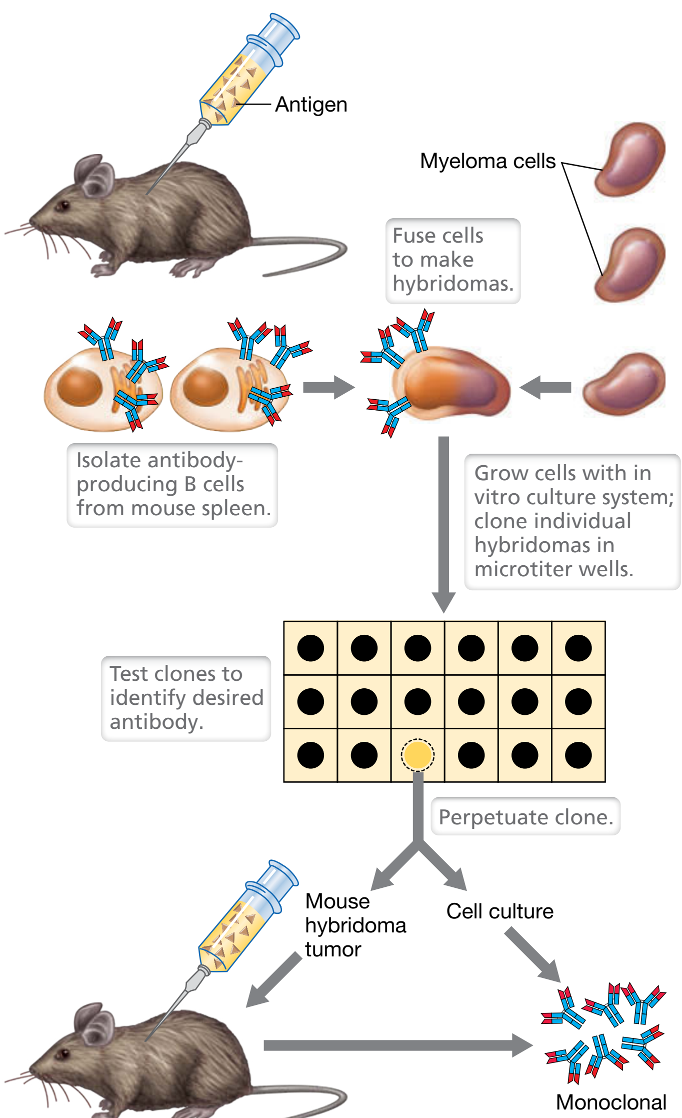


Figure 28.12 Production of monoclonal antibodies (mAbs). The hybridoma can be indefinitely cultured or passed through animals as a tumor. The hybridoma cells are stored as frozen tumor cells that can be thawed and grown in tissue culture or in a suitable animal host.

as well as the cell poison aminopterin (A). The myelomas are unable to grow in this medium because they lack an enzyme that allows them to use the H and T metabolites to circumvent the poisonous effect of aminopterin. Unfused B cells can produce the necessary enzyme, but they die after several days because they cannot divide in culture. Only the fused hybridomas, which combine the properties of both cell types, are able to both produce the necessary enzyme and grow indefinitely.

An enzyme immunoassay (Section 28.7) can be used to identify hybridomas that produce the desired mAb. From a typical fusion, several distinct clones are isolated, each making a different mAb. Once the clones of interest are identified, they can be grown either in the mouse as an antibody-producing tumor or in continuous cell culture. Antibodies can then be harvested from either source.

Commercial production of mAbs has replaced polyclonal antibodies for many immunodiagnostic applications because mAbs are highly specific bioreagents that can be generated with a high degree of reproducibility. Clinical diagnostic tests that use mAbs include immunological typing of bacterial pathogens, identification of cells containing foreign surface antigens (for example, a virus-infected cell), and highly specific blood and tissue typing. Because of their remarkable specificity, mAbs are also used to detect and treat human cancers. Malignant cells often contain tumor-specific antigens on their surfaces, and therefore mAbs prepared against these antigens specifically target the cancer cells and can be used to deliver toxins directly to them. The use of anticancer mAbs has great potential as an alternative to nonspecific chemical and radiation treatments that damage healthy cells as well as cancer cells.

MINIQUIZ

- Explain the reasons for changes in antibody titer for a single infectious agent, from the acute phase through the convalescent phase of the infection.
- Describe the method, time frame, and rationale for the tuberculin skin test. What component of the immune response does this test detect?
- What advantages do monoclonal antibodies have compared to polyclonal antibodies? How are mAbs produced?

28.6 Precipitation, Agglutination, and Immunofluorescence

Many clinically useful immunological reactions, including precipitation reactions and agglutination reactions, yield a product visible to the naked eye. Some other reactions are visualized microscopically when fluorescent dyes attached to specific antigens react with their specific antibody. We consider examples of these now.

Precipitation

Precipitation results from the interaction of a soluble antibody with a soluble antigen to form an insoluble complex. Tests can be done in liquid test tubes (or capillary tubes) or, as shown in Figure 28.13, in agarose gel. Antigens that have more than one antibody-binding epitope can cross-link the bivalent antibodies that recognize them, causing a precipitate to develop from the aggregated antibody–antigen complexes. Precipitation occurs maximally when there are optimal proportions of the two reacting substances. The presence of either excess antigen or excess antibody results in the formation of soluble immune complexes (Figure 28.13).

Precipitation reactions carried out in agarose gels, called *immunodiffusion tests* (Figure 28.13 inset), are especially useful for diagnosing fungal infections, including coccidioidomycosis, histoplasmosis, blastomycosis, and paracoccidioidomycosis (Section 33.2). For these tests, prepared antigen and patient antisera containing antibodies are loaded into separate wells cut into the agarose gel. The reagents diffuse outward from the wells and form precipitation bands where antibody interacts with

UNIT 6

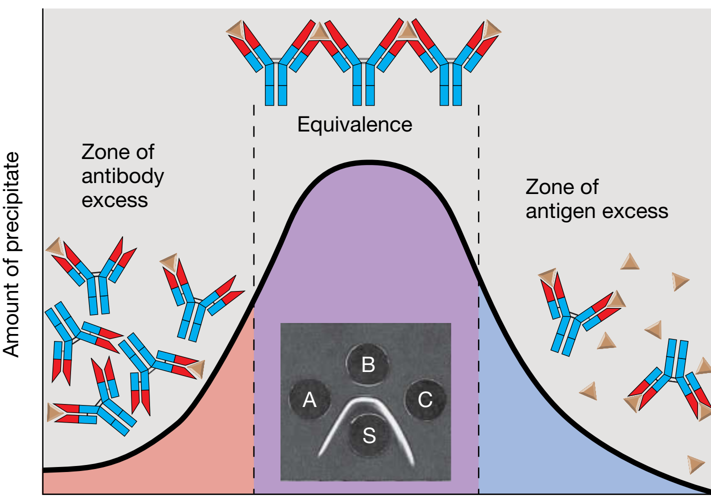


Figure 28.13 Precipitation reactions between soluble antigen and antibody. The extent of precipitation is a function of antigen and antibody concentration. Inset photo: Precipitation in agarose gel (immunodiffusion). Well *S* contains antibodies to cells of *Proteus mirabilis*. Wells *A, B*, and *C* contain soluble extracts of *P. mirabilis*. An insoluble precipitation band forms where antibody and antigen concentrations are equivalent. Immunoprecipitation photo courtesy of C. Weibull, W.D. Bickel, W.T. Hashius, K.C. Milner, and E. Ribi.

antigen in optimal proportions (Figure 28.13). Unfortunately, precipitation reactions are not very sensitive; visible precipitation requires microgram quantities of specific antibody rather than the nanogram quantities of more sensitive diagnostic tests. Consequently, with the exception of clinical diagnostic testing for fungal infections, precipitation assays are typically used only in research and reference laboratories.

Agglutination

Agglutination is a reaction between antibody and particle-bound antigen resulting in visible clumping of the particles. Agglutination tests can be done in test tubes, in microtiter plates, or by mixing reagents on glass or coated paper slides. Agglutination tests are quick to perform, inexpensive, highly specific, and reasonably sensitive, making them suitable for large-scale use in clinical applications. Standardized agglutination tests are used for the identification of blood group (red blood cell) antigens (**Figure 28.14*a***), as well as pathogens and pathogen products. To determine blood groups, blood samples are mixed with either anti-A antisera or anti-B antisera and the agglutination of red blood cells, called *hemagglutination*, is assessed (Figure 28.14).

Agglutination is often assessed using rapid assays that employ small (0.8-μm diameter) latex beads coated with a specific antigen. The beads are mixed with patient serum on a slide and incubated for a short period. If patient antibody binds the antigen on the bead surface, the milky white latex suspension will become visibly clumped, indicating a positive agglutination reaction and exposure to the pathogen.

Latex agglutination is also used to detect bacterial surface antigens by mixing cells from a bacterial colony with antibody-coated latex beads. For example, a commercially available suspension of latex beads coated with antibodies to protein A and clumping

(a)

Blood type	Percentage of U.S. population	Serum	
		Anti A	Anti B
O	48	No aggl.	No aggl.
A	32	Aggl.	No aggl.
B	16	No aggl.	Aggl.
AB	4	Aggl.	Aggl.

(b)

Figure 28.14 Direct agglutination of human red blood cells: ABO blood typing. *(a)* A drop of whole blood was mixed with antigen-specific antisera for each reaction. The reaction on the left shows no agglutination with antibody, typical of blood type O. The reaction in the center shows the diffuse agglutination pattern indicative of blood type B. The reaction on the right shows the strong agglutination pattern with large, clumped aggregates typical of blood type A. *(b)* Table of expected blood typing results for people in the United States.

factor, two proteins found exclusively on the surface of *Staphylococcus aureus* cells, is specific for identification of clinical isolates of *S. aureus* (**Figure 28.15**). Latex bead assays take less than a minute and can be used directly on clinical samples, such as the exudate from a purulent infection. Latex bead agglutination assays have also been developed to identify other common pathogens, such as *Streptococcus pyogenes, Neisseria gonorrhoeae, Escherichia coli* O157:H7, and the fungus *Candida albicans*.

Immunofluorescence

Antibodies containing conjugated fluorescent dyes can be used to detect antigens on intact cells. Such **fluorescent antibodies** are

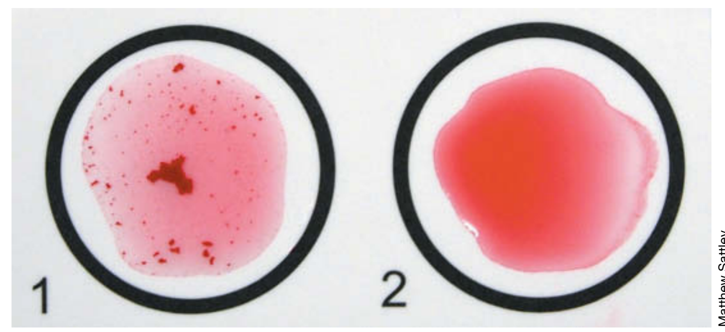


Figure 28.15 Latex bead agglutination test for *Staphylococcus aureus*. In circle 1, a loopful of material from a bacterial colony was mixed in a suspension of microscopic, red latex beads coated with antibodies to two antigens found exclusively on the surface of *Staphylococcus aureus* cells. The bright red clumps indicate positive agglutination, confirming the colony as *S. aureus*. Circle 2 is a negative control showing the uniform red color expected in the absence of agglutination.

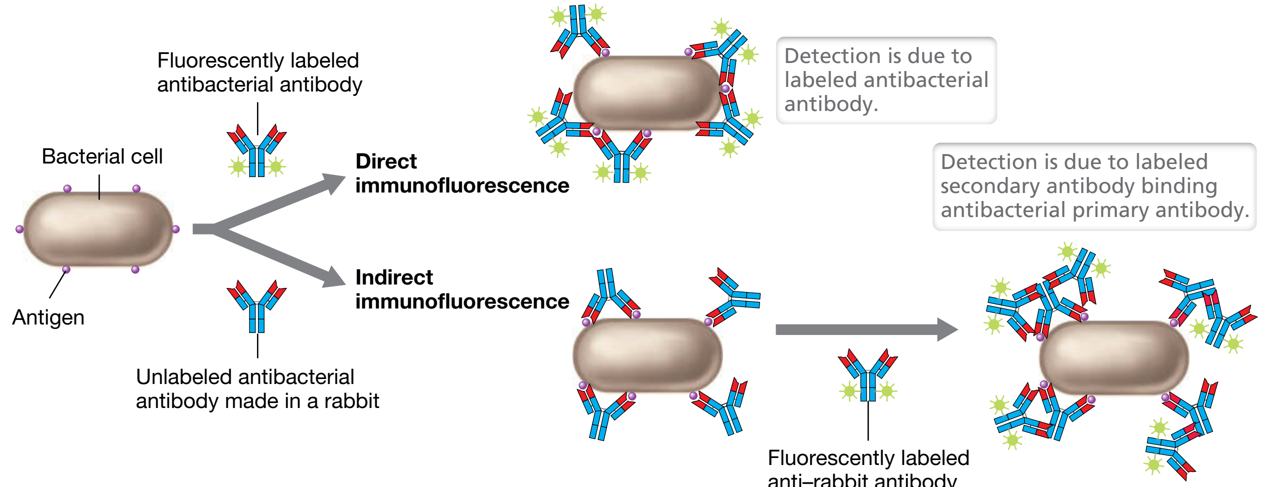
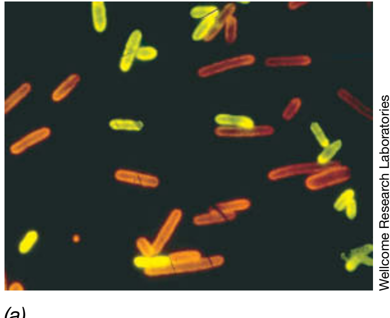


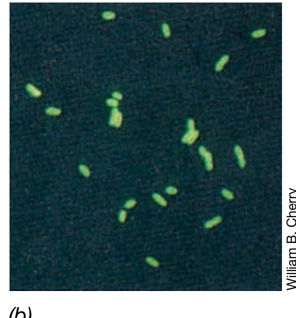
Figure 28.16 Fluorescent antibody methods for detection of microbial surface antigens. Note how indirect immunofluorescence requires a labeled secondary antibody that binds to the primary antibody.

widely used for diagnostic and research applications. Fluorescent antibody staining methods can be either direct or indirect (Figure 28.16). In the *direct method*, the antibody that interacts with the surface antigen is itself covalently linked to the fluorescent dye. In the *indirect method*, the presence of a nonfluorescent primary antibody on the surface of a cell is detected by the use of a fluorescent secondary antibody directed against the nonfluorescent antibody.

Fluorescent dyes typically conjugated to antibodies include rhodamine B, which fluoresces red-orange, and fluorescein isothiocyanate, which fluoresces yellow-green (Figure 28.17). Once the fluorescent antibodies have bound to cell surface antigens, the complex can be visualized using a fluorescence microscope (Figure 1.20). The cell-bound fluorescent antibodies emit their characteristic fluorescent color when excited with light of particular wavelengths. Fluorescent antibodies can be used for tasks as varied as identifying a microorganism directly in a patient specimen (Figure 28.17) to enumerating T cells in the blood of patients with human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS).



(a)



(b)

Figure 28.17 Fluorescent antibody identification of bacteria. *(a)* Cells of *Clostridium septicum* were stained with antibody conjugated with fluorescein isothiocyanate, which fluoresces yellow-green. Cells of *Clostridium chauvoei* were stained with antibody conjugated with rhodamine B, which fluoresces red-orange. *(b)* Immunofluorescently stained cells of *Legionella pneumophila* (legionellosis) from biopsied lung tissue. Individual cells are 2–5 μm in length and were stained green with antibodies coupled to fluorescein isothiocyanate.

Fluorescent antibodies applied directly to infected host tissues may permit disease diagnosis long before culture methods yield a suspected pathogen (Figure 28.18). For example, a presumptive diagnosis of legionellosis (Legionnaires' disease), a form of infectious pneumonia (Section 32.4), can be confirmed by staining biopsied lung tissue directly with fluorescent antibodies specific for cell wall antigens of *Legionella pneumophila* (Figure 28.17*b*), the causative agent of the disease. Immunofluorescence assays are also used to help diagnose infections from viral pathogens, such as Epstein–Barr virus (EBV) (Figure 28.18*a*); fungal pathogens, such as *Aspergillus* (Figure 28.18*b*); and gastrointestinal parasitic protozoa, such as *Giardia intestinalis* (Figure 28.18*c*).

MINIQUIZ

- How is the bivalence of antibodies significant for a precipitation reaction, and under what conditions does precipitation occur maximally?
- What are the advantages and disadvantages of agglutination tests versus fluorescent antibody assays? How are the latter used to identify specific cells in complex mixtures, such as blood?

28.7 Enzyme Immunoassays, Rapid Tests, and Immunoblots

Enzyme immunoassays (EIAs), which include *enzyme-linked immunosorbent assays* (*ELISAs*), are immunodiagnostic tools widely used in clinical microbiology and research. EIAs are particularly useful because they are inexpensive, produce no hazardous waste, and are highly specific and sensitive (they can detect as little as 0.1 nanograms of antigen or antibody). *Rapid tests* are similar to EIAs except that results can often be reported within minutes instead of hours. Many rapid tests are given as "point-of-care" tests but are generally not as specific or sensitive as EIAs.

The comparatively complex and time-consuming **immunoblot (Western blot)** uses immobilized pathogen proteins as antigens

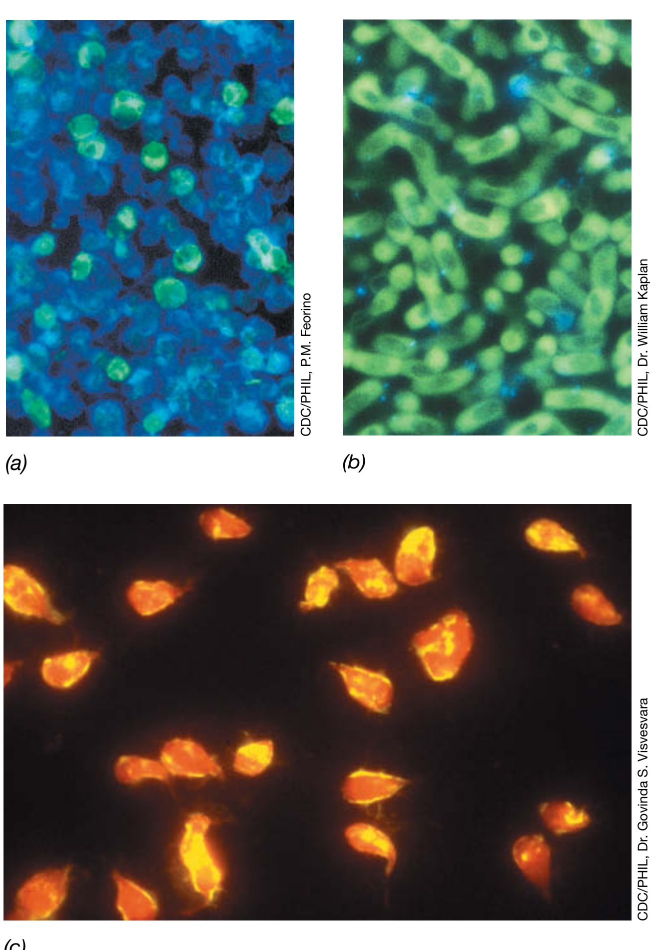


Figure 28.18 Fluorescent antibody identification of pathogens. *(a)* Detection of cells infected with Epstein–Barr virus (EBV) using indirect immunofluorescence. The green-stained cells are infected with EBV, which causes mononucleosis and lymphoma. *(b)* Detection of *Aspergillus* mold in a case of aspergillosis (Section 33.1) using fluorescein-conjugated antibodies. *(c)* Detection of the waterborne intestinal parasite *Giardia intestinalis* (Section 33.4) using indirect immunofluorescence.

to bind antibodies from patient specimens, providing highly specific evidence for pathogen exposure. Immunoblots are often used to confirm results obtained from other serological tests, including rapid tests and EIAs.

EIA

In EIA tests, an enzyme is covalently attached to an antigen or antibody molecule, creating an immunological tool with high specificity and sensitivity. Enzymes typically bound to antigen or antibody include peroxidase, alkaline phosphatase, and β-galactosidase, all of which interact with specific substrates to form colored reaction products that can be detected in low amounts. Four EIA formats are commonly used for evaluation of specimens for infectious disease: *direct EIA* (detects antigen), *indirect EIA* (detects antibodies), *antigen sandwich EIA* (detects antibodies using a sandwich technique), and *combination EIA* (detects both antigen and antibodies). The principal features of each platform are illustrated in Figure 28.19.

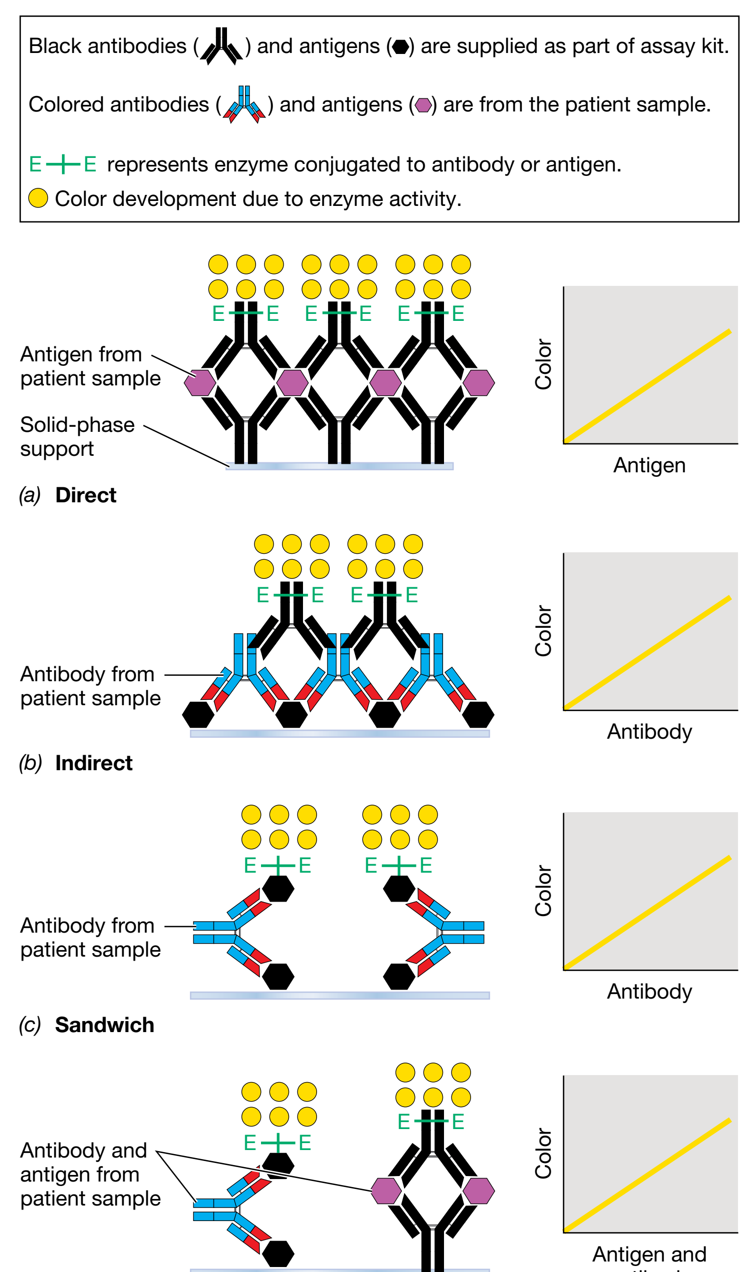


Figure 28.19 Enzyme immunoassays (EIAs). Patient samples are in color. Assay reagents are shown in black. All assays are fixed to a solid-phase support (light blue). Enzymes bound to antigen or antibody convert substrate to a colored product, shown in yellow. In each assay, the amount of colored product is proportional to the amount of pathogen-specific antibody or antigen derived from a patient sample. *(a)* The *direct EIA* uses immobilized pathogen-specific antibody and enzyme-labeled pathogen-specific antibody to detect pathogen antigen in patient samples such as blood. *(b)* The *indirect EIA* uses immobilized pathogen antigen and enzyme-labeled antibody directed to immunoglobulin to detect pathogen-specific antibodies in patient samples such as blood. *(c)* The *sandwich EIA* uses immobilized pathogen antigen and enzyme-labeled pathogen antigen to detect pathogen-specific antibodies in patient samples such as blood. The sandwich EIA is more sensitive than the direct or indirect EIA methods. *(d)* The *combination EIA* uses both the sandwich and direct assays in one platform to identify antibody and antigen in patient samples, maximizing sensitivity. In all cases, it is the enzymatic conversion of a colorless substrate to a colored product, measured spectrophotometrically, that enables quantification of specific antigen and/or antibody.

Direct EIAs are designed to detect antigens, such as virus particles in a blood or fecal sample (Figure 28.19*a*). Antibodies to a pathogen antigen are coated onto a support, such as a plastic microtiter plate, and the patient sample is then added. After antigen in the sample binds to the antibody, a second antibody, specific to the same antigen and coupled to an enzyme, is added. Finally, enzyme substrate is added, and the enzyme converts the substrate to its colored product in proportion to the amount of patient antigen bound by the enzyme–antibody complex. Direct EIAs are useful for detecting bacterial exotoxins, such as those produced by *Vibrio cholerae* and *Staphylococcus aureus* (Section 25.6), as well as a variety of viruses, including those that cause influenza and hepatitis.

Indirect EIAs are used to detect antibodies to pathogens in body fluids (Figure 28.19*b*). The indirect test starts with pathogen antigen immobilized on a supportive matrix. Patient serum is added, and antibodies (if present) bind to the antigen. Next, an antibody–enzyme complex specific for the patient antibodies is added. Finally, the addition of the enzyme substrate results in the development of a colored product that is proportional to the concentration of patient antibody in the sample. Indirect EIAs are used to detect serum antibodies to a wide variety of bacterial, viral, and eukaryotic pathogens.

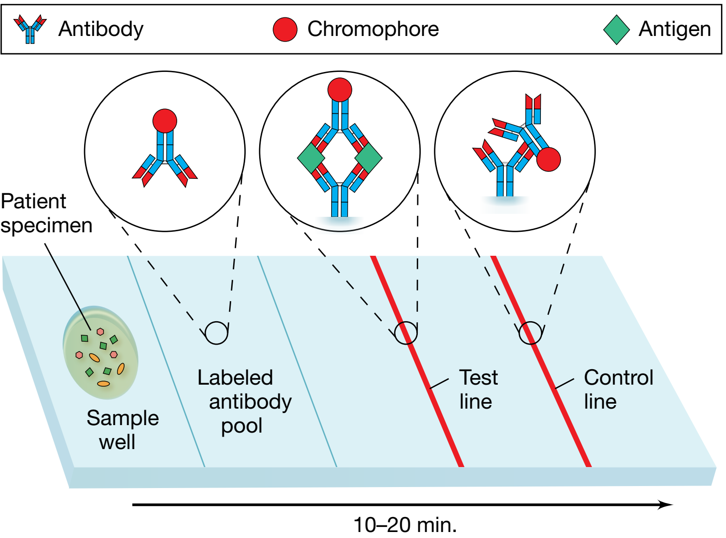
The antigen sandwich EIA also detects antibodies to pathogens in body fluids (Figure 28.19*c*). The sandwich test starts with pathogen antigen immobilized on a support. Patient serum is added, and antibodies (if present) bind to the antigen. Next, the same antigen coupled to enzyme is added, followed by the addition of the enzyme substrate, resulting in the development of colored product that is proportional to the concentration of patient antibody in the sample. This method is especially sensitive because it detects pathogen-specific antibody irrespective of antibody class. This method is often used for HIV screening (the third-generation HIV test) because it can detect IgM produced during the primary immune response to HIV as soon as four weeks after infection. By contrast, most indirect EIAs use anti-IgG as the enzyme-conjugated antibody, delaying observation of antibodies to HIV until the secondary antibody response at least five weeks after infection (Figure 27.10).

The combination EIA, shown in Figure 28.19*d*, makes use of a direct EIA to detect pathogen antigen and the sandwich method to detect pathogen-specific antibodies, both on a single matrix. This method, used for a fourth-generation HIV test, is more sensitive than the third-generation sandwich test; antigen can be detected as little as 2.5 weeks after HIV infection, reducing the time to treatment.

Rapid Tests

Rapid immunoassay procedures use reagents adsorbed to a fixed support material, such as paper strips or plastic membranes. These point-of-care tests cause a color change on the strip within minutes and serve as rapid diagnostic aids for a variety of infectious diseases, including HIV/AIDS.

For most rapid tests, a body fluid (generally urine, blood, saliva, or sputum) is applied to a sample well in a reagent–support matrix. To detect antigen in patient samples, for example to determine infection by *Streptococcus pyogenes* (strep throat), the matrix contains soluble antibodies that are specific to the antigen in question and conjugated to a colored molecule called a *chromophore* (**Figure 28.20**). As the liquid sample diffuses through the matrix, patient antigens (if present) bind the chromophore-labeled antibodies. Capillary action pulls each labeled antigen–antibody complex through the matrix, where it contacts a single line of fixed antibodies. The labeled antigen–antibody complex binds a fixed antibody and becomes immobilized. As the concentration of labeled complex increases, the chromophore becomes visible as a line of color along the fixed antibody, indicating a positive test for the antigen. Labeled antibodies not bound by antigen concentrate at a second line of fixed antibodies that are specific to the labeled antibody rather than the antigen, thus forming a second colored line that serves as the control.



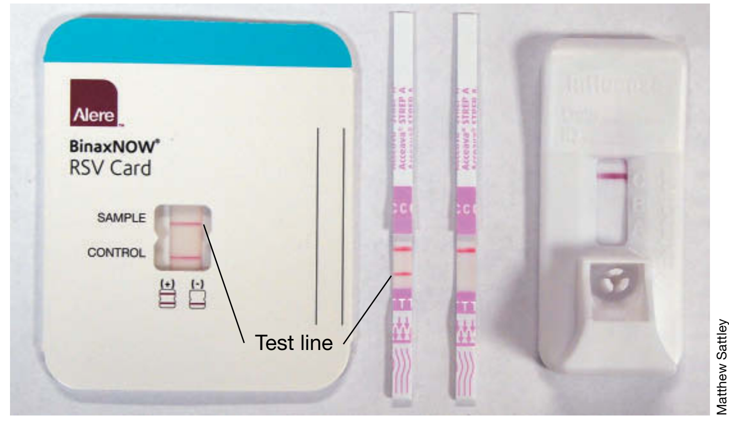


Figure 28.20 Rapid tests. *(a)* A patient specimen containing a mixture of antigens is applied to the sample well of a support matrix. Capillary action pulls the liquid sample through the matrix, and specific antigen (if present) binds soluble, chromophore-labeled antibodies. The labeled antigen–antibody complexes diffuse through the matrix and bind a line of fixed antibodies. A colored line becomes visible as the concentration of labeled complex builds, indicating a positive test for the antigen. Unbound labeled antibodies bind a second line of fixed antibody as a control. *(b)* From left to right, rapid tests for respiratory syncytial virus (RSV), group A streptococci (GAS), and influenza A/B. The RSV test and the left GAS test show a test line, indicating positive reactions. The right GAS test and the influenza A/B test show only a control line, indicating negative reactions. Photos taken courtesy of Marion General Hospital, Marion, Indiana (USA).

These tests are valuable for point-of-care analysis and provide rapid diagnostic results that can be reported almost immediately, avoiding the need for delays in patient care or for follow-up visits to obtain test results. The drawback to rapid tests, however, is that they are often less specific or less sensitive than more elaborate assays for the same pathogens. As a result, rapid tests often need to be confirmed by EIA or other tests, such as the immunoblot, discussed next.

Immunoblots

Immunoblot methodology requires the *separation* of proteins on a polyacrylamide gel, the *transfer* (blotting) of the proteins from the gel to a nitrocellulose or nylon membrane, and finally, the *identification* of the proteins using specific antibodies (**Figure 28.21a**). The HIV immunoblot (Figure 28.21*b*) can be used to accurately diagnose HIV infection, but although it is highly specific, it is generally not used as a screening tool because it is less sensitive, more time consuming, and more expensive than the HIV EIA. However, because the HIV EIA occasionally yields false-positive results, the immunoblot method is often used to confirm positive EIA tests.

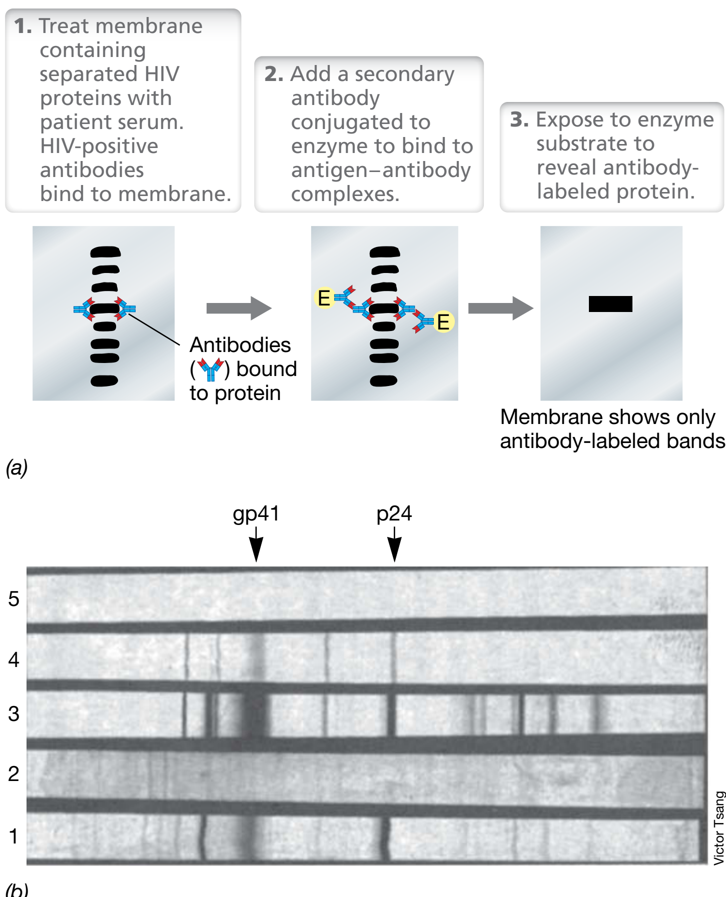


Figure 28.21 The immunoblot (Western blot) and its use in the diagnosis of human immunodeficiency virus (HIV) infection. *(a)* Protocol for an immunoblot. *(b)* The molecules p24 (capsid protein) and gp41 (envelope glycoprotein) are diagnostic for HIV. Lane 1, positive control serum (from known AIDS patients); lane 2, negative control serum (from healthy volunteer); lane 3, strong positive from patient sample; lane 4, weak positive from patient sample; lane 5, reagent blank to check for background binding.

The HIV immunoblot procedure is similar to immunoblot methods used to diagnose infection by other pathogens. In general, immunoblots are used to detect pathogen-specific antibody in patient samples.

To perform the HIV immunoblot, membrane strips containing fixed HIV proteins are incubated with the patient serum sample. If the sample is HIV-positive, patient antibodies will bind to the HIV proteins on the membrane. To detect whether antibodies from the serum sample have bound to HIV antigens, a detecting antibody, anti–human IgG conjugated to an enzyme, is added to the strips. If the detecting antibody binds, the activity of the conjugated enzyme, after addition of substrate, will form a colored band on the strip at the site of antibody binding. The patient is HIV-positive if the positions of the bands in the patient sample match those of a positive control; a negative control serum is also analyzed and must show no bands (Figure 28.21*b*). As the test is mostly used to confirm positive EIA results for HIV (or correct false positives), variations in band intensity do not affect interpretation of the results.

MINIQUIZ

- Compare direct, indirect, sandwich, and combination EIAs with respect to their ability to identify infection with a particular pathogen.
- Compare the advantages and disadvantages of EIA, rapid tests, and immunoblots with respect to speed, sensitivity, and specificity.

28.8 Nucleic Acid–Based Clinical Assays

In Section 12.1 we discussed how the polymerase chain reaction (PCR) amplifies nucleic acids, forming multiple copies of target sequences. PCR techniques can employ primers for a pathogen-specific gene to examine DNA derived from suspected infected tissue, even in the absence of an observable or culturable pathogen. As a result, PCR-based tests are widely used in the clinical lab for pathogen identification, and they are particularly useful for identifying viruses and other intracellular pathogens that are difficult or impossible to culture using current techniques. PCR methods are extremely sensitive and do not depend on pathogen isolation or growth, and no detection of an immune response to the pathogen is required. Instead, microbe-specific nucleic acid sequences are detected in the assays.

Nucleic Acid Hybridization and Amplification

Nucleic acid hybridization (Section 12.1) is the central theme of nucleic acid–based molecular methods. In clinical medicine, hybridization methods are employed to identify specific pathogens in patient samples by using unique nucleic acid *probes* to detect the presence of specific DNA sequences. Nucleic acid probes are single-stranded DNA molecules having a sequence complementary to that of a gene of interest. A DNA probe oligonucleotide may be less than 100 base pairs or up to several kilobases in length. If a microbe from a clinical specimen contains DNA or RNA sequences complementary to the probe, the probe will hybridize (following

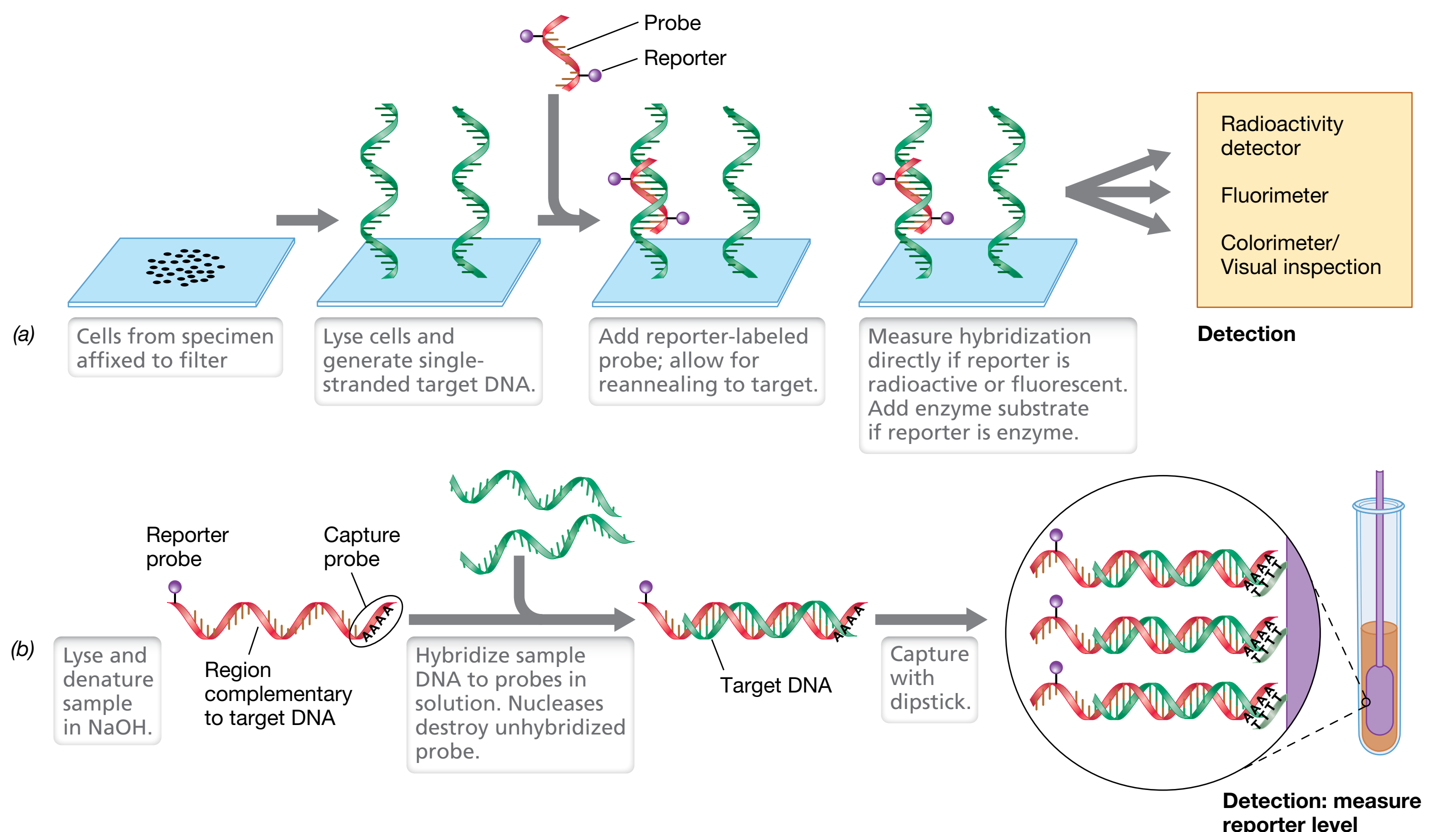


Figure 28.22 Nucleic acid probe methodology in clinical diagnostics. *(a)* Membrane filter assay. The reporter can be a radioisotope, a fluorescent dye, or an enzyme. *(b)* Dipstick assay. Dual reporter and capture probes are used. The capture probe contains a poly(A) tail that hybridizes to a poly(T) oligonucleotide affixed to the dipstick. Binding of the target DNA–reporter complex is usually detected as a visible color change.

appropriate sample preparation to yield single-stranded target molecules), forming a double-stranded molecule (Figure 28.22). To detect the hybridization reaction, the probe is labeled with a reporter molecule, which is usually a fluorescent compound, but radioisotopes or enzymes may also be used.

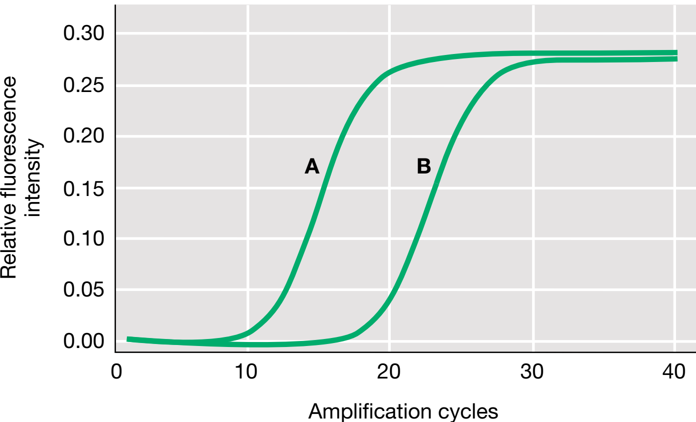
To carry out a probe assay, samples are treated with strong alkali, usually sodium hydroxide (NaOH), to lyse the cells and partially denature the pathogen DNA, forming single-stranded DNA molecules (Figure 28.22). Incubation at an appropriate temperature facilitates formation of a stable duplex between target DNA and probe DNA. The extent of hybridization is measured using the reporter molecule attached to the probe. Some assays use two-component probes that function as both a *reporter* probe and a *capture* probe; the addition of a sequence tag allows the hybridized molecule to be affixed to a matrix, usually a dipstick, for detection purposes (Figure 28.22*b*).

Nucleic acid hybridization also plays a critical role in the various PCR-based techniques used to amplify target DNA or RNA molecules. PCR analysis begins with the extraction of DNA or RNA from the sample to be tested. Next, the nucleic acid must be amplified using appropriate gene-specific nucleic acid *primers*. These short oligonucleotides (typically 15–27 base pairs in length) are not used as probes but instead function to jump-start DNA polymerase during PCR amplification of pathogen-specific genes. Lastly, the amplified nucleic acid product (the *amplicon*) is visualized, a procedure that may involve gel electrophoresis or, more often in clinical laboratories, fluorescence. The presence of the appropriate amplified gene segment confirms the presence of the pathogen.

Quantitative and Reverse Transcription PCR

Many clinical PCR tests employ *quantitative real-time PCR* (*qPCR*). This process uses fluorescent probes to label PCR amplicons, thereby allowing the accumulation of target DNA to be visualized. Because probe fluorescence increases upon binding to DNA, the level of fluorescence increases proportionally as the target DNA is amplified. The fluorescent probes may be either nonspecific or specific for the target DNA. For example, the dye SYBR Green binds *nonspecifically* to double-stranded DNA and fluoresces only when bound. When added to the PCR mixture, SYBR Green fluorescence indicates the presence of double-stranded DNA produced by the amplification process (Figure 28.23*a*). By contrast, *gene-specific* fluorescent probes, made by attaching a fluorescent dye to a short DNA probe specific to a target sequence, fluoresce only when double-stranded DNA of the correct sequence accumulates.

Because qPCR amplification can be monitored continuously via fluorescence, visualization by gel electrophoresis is not necessary to confirm amplification. Using modern instrumentation, such as that shown in Figure 28.23*b*, detection of a gene diagnostic for a particular pathogen in a clinical sample may be performed in about 2 hours. Moreover, by monitoring the *rate* of fluorescence increase in the PCR reaction, it is possible to accurately determine



(a)

(b)

Matthew Sattley

Figure 28.23 Quantitative real-time polymerase chain reaction (qPCR) for clinical diagnostics. *(a)* DNA extracted from a gram-negative bacterial culture was monitored for expression of 16S rRNA (curve A) and *npt* (curve B), a kanamycin resistance marker, using gene-specific primers. The fluorescent dye SYBR Green was added to the PCR mixture and used to visualize amplified DNA as it formed. The curve on the left (A) had 0.15 fluorescence units after 15 cycles, while the curve on the right (B) had 0.15 fluorescence units after 22 cycles, indicating that the 16S rRNA had a higher abundance of template DNA than *npt* in this strain. *(b)* qPCR instrumentation in a clinical laboratory. The single-use cartridges contain all necessary components for the qPCR reaction, including group-specific primers and fluorescent dyes, and pathogens can be identified in less than 2 hours. The cartridge on the left is specific for detection of group B streptococci (*Streptococcus agalactiae*). Photos taken courtesy of Marion General Hospital, Marion, Indiana (USA).

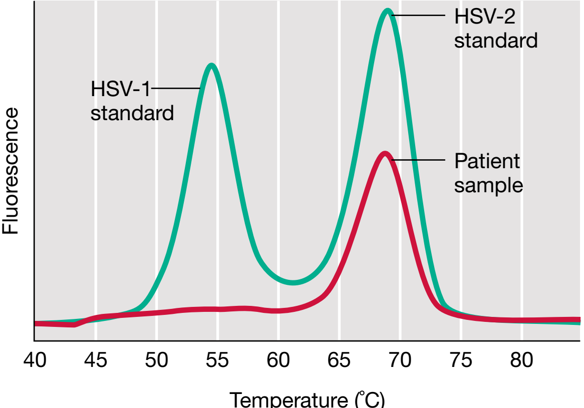


Figure 28.24 Qualitative PCR for the *pol* gene of HSV-1 (herpes simplex 1 virus) and HSV-2. DNA from a patient sample was assayed for the *pol* gene of both HSV-1 and HSV-2 following quantitative PCR (qPCR). Two fluorescent-labeled probes hybridize with an internal sequence of the amplified fragment of each viral genome during the PCR cycle. After hybridization to the template DNA, the probes are excited by a light source, and their fluorescence is measured. After the PCR cycle, each virus shows a distinct DNA melting curve. The melting profile in the patient sample (red) corresponds to the HSV-2 standard (green), indicating infection with HSV-2.

the amount of target DNA present in the original sample (Figure 28.23*a*). Thus, qPCR can be used to assess the abundance of a pathogen in a sample by quantifying a gene characteristic for that particular organism.

Another variation of basic PCR is *reverse transcription PCR* (*RT-PCR*), which uses pathogen-specific RNA to produce complementary DNA (cDNA) directly from patient samples (Section 12.1). This technology is especially useful for the detection of RNA viruses, including retroviruses such as HIV. The first step in RT-PCR is to use the enzyme reverse transcriptase to make a cDNA copy of an RNA sample. PCR is then used to amplify the cDNA. By isolating RNA in a sample and making cDNA copies of the corresponding gene(s), one can employ qPCR to monitor the expression of a particular gene from a pathogen. The amplified DNA can then be sequenced or probed for identification.

Qualitative PCR

Some diagnostic tests based on the qPCR format use a slightly different amplification protocol and an additional step to identify pathogen-associated genes. This method, called *qualitative PCR*, uses labeled hybridization primers that are incorporated into an amplicon product of a qPCR reaction.

In the example shown in **Figure 28.24**, the hybridization probes are targeted to the DNA *pol* gene of herpes simplex 1 and 2 viruses (HSV-1 and HSV-2) (Section 10.7). The amplicon is detected using two distinct hybridization probes labeled with fluorescent dyes. The probes hybridize to an internal sequence of the amplified fragment during the annealing phase of the PCR cycle. After hybridization to the template DNA, the probes are excited by a light source in the PCR instrument. The emitted fluorescence is then measured and, after the PCR cycle, a melting curve analysis is performed to differentiate between samples positive for HSV-1 and HSV-2. Because of nucleotide polymorphisms between the DNA *pol* genes of the two virus subtypes, the melting curve for HSV-1 is distinct from that of HSV-2 (Figure 28.24). The results are compared to internal assay control reactions, and an unambiguous diagnosis of viral infection can be obtained within hours.

MINIQUIZ

- What advantage(s) does nucleic acid amplification have over standard culture methods for identification of microorganisms? What are the disadvantages?
- How do quantitative PCR (qPCR) and qualitative PCR differ?

IV • Prevention and Treatment of Infectious Diseases

In the past several sections we have taken a tour of the clinical microbiology laboratory to learn some of the techniques used to accurately *diagnose* infectious diseases in a timely fashion. We now move on to a discussion of strategies used in clinical medicine to both *prevent* and *treat* infectious diseases.

28.9 Vaccination

A major means of disease prevention is **vaccination (immunization)**, in which deliberate exposure to an antigen triggers an adaptive immune response intended to protect an individual against future attack by a pathogen. The immunogen used to induce this artificial active immunity is called a **vaccine**. A summary of major diseases for which vaccines are available for human use is given in Table 28.4.

The Nature of Vaccines

Infants are immune to many common infectious diseases during the first 6 months of life because they acquire natural passive immunity from maternal antibodies transferred across the placenta or in breast milk (Section 24.7). However, it is recommended that infants be immunized against key infectious diseases as soon as possible so that their own active immunity can replace the temporary maternal passive immunity (Figure 28.25). As discussed in Section 27.3, a single exposure to antigen does not lead to a high antibody titer. After an initial immunization, a series of "booster" immunizations are given to produce a secondary response and a high antibody titer.

It is well established that the introduction of effective vaccines into a population has reduced the incidence of formerly epidemic childhood diseases, such as measles and mumps (Section 30.6), and has eliminated smallpox altogether (Section 29.5). However, lifelong immunity is rarely achieved by vaccination because the immune cells and antibodies induced by immunization gradually disappear from the body. On the other hand, natural infections may stimulate immune memory. In the complete absence of antigenic stimulation, the length of effective immunity varies considerably with different vaccines. For example, tetanus toxoid vaccine provides effective immunity for 10 years, but immunity induced by inactivated influenza virus vaccine generally disappears within a year or two.

To minimize the risk of infection or other adverse reactions, pathogens or pathogen products used in vaccines must be made harmless. To achieve this, many vaccines consist of pathogens killed either by heat treatment or by chemical agents. For example, formaldehyde is used to inactivate polio virions for preparation of the Salk polio vaccine. In addition, many exotoxins can be chemically modified to create a *toxoid*, a molecule that retains its antigenicity but is no longer toxic. Toxoid vaccines, such as the vaccine for *Clostridium tetani*, safely induce long-term protective immunity against the exotoxin. In other cases, antigens extracted from pathogens are purified and injected as a vaccine. Such is the case for some pneumococcal vaccines, which consist of a mixture of polysaccharide capsule antigens derived from the most common pathogenic strains of *Streptococcus pneumoniae*.

Immunization with intact pathogens is usually more effective than immunization with dead or inactivated material. It is often possible to isolate *attenuated strains* of pathogens, those that have lost their virulence but still retain the immunizing antigens. However, because attenuated strains of pathogens are still viable, some individuals may inadvertently acquire active disease from the vaccination. For example, serious cases of polio and smallpox have occurred, especially in immunocompromised individuals, from

TABLE 28.4 Vaccines for infectious diseases in humans

Bacterial diseases	*Type of vaccine used*
Anthrax	Toxoid
Cholera	Killed cells or cell extract (*Vibrio cholerae*)
Diphtheria	Toxoid
Haemophilus influenzae type b meningitis	Conjugated vaccine (polysaccharide of *Haemophilus influenzae* type b conjugated to protein)
Meningitis	Purified polysaccharide from *Neisseria meningitidis*
Paratyphoid fever	Killed bacteria (*Salmonella enterica* [*paratyphi*])
Pertussis	Killed bacteria (*Bordetella pertussis*) or acellular proteins
Plague	Killed cells or cell extract (*Yersinia pestis*)
Pneumonia (bacterial)	Purified polysaccharide from *Streptococcus pneumoniae* or polysaccharide–toxoid conjugate
Tetanus	Toxoid
Tuberculosis	Attenuated strain of *Mycobacterium tuberculosis*
Typhoid fever	Killed bacteria (*Salmonella enterica* [*typhi*])
Typhus	Killed bacteria (*Rickettsia prowazekii*)
Viral diseases	***Type of vaccine used***
Hepatitis A	Recombinant DNA vaccine
Hepatitis B	Recombinant DNA vaccine or inactivated virus
Human papillomavirus (HPV)	Recombinant DNA vaccine
Influenza (seasonal or H1N1)	Inactivated or attenuated virus
Japanese encephalitis	Inactivated virus
Measles and mumps	Attenuated virus
Polio	Attenuated virus (Sabin) or inactivated virus (Salk)
Rabies	Inactivated virus (human) or attenuated virus (animal)
Rotavirus	Attenuated virus
Rubella	Attenuated virus
Smallpox and monkeypox	Cross-reacting virus (vaccinia)
Varicella (chicken pox/ shingles)	Attenuated virus
Yellow fever	Attenuated virus

Immunizations against bacteria	Immunizations against viruses
Haemophilus Influenzae Type B (Hib)	Hepatitis A virus
Meningococcal (*Neisseria meningitidis*)	Hepatitis B virus
Pneumococcal (*Streptococcus pneumoniae*)	Human papillomavirus (HPV)
Tetanus, diphtheria, pertussis (DTaP, Tdap)	Influenza virus
	Inactivated poliovirus (IPV)
	Measles, mumps, rubella (MMR)
	Rotavirus
	Varicella virus (chicken pox)

Figure 28.25 Immunization recommendations for infants and children in the United States. The U.S. Centers for Disease Control and Prevention website (http://www.cdc.gov) has recommendations for timing and dose of immunizations for all age groups and for special populations, such as international travelers, women of childbearing age, and those with immunodeficiency or chronic disease.

attenuated vaccines. Nevertheless, although they are difficult to standardize and have a limited shelf life, attenuated vaccines tend to provide long-lasting immunity and a strong secondary booster response. By contrast, killed virus vaccines tend to provide short-lived immune responses with less long-term memory, but they are much more stable and easier to store.

Most bacterial vaccines are provided as antigens in an inactivated form, such as the toxoids that protect against tetanus and diphtheria. Inactivated bacterial vaccines induce antibody-mediated protection without exposing recipients to the risk of infection. However, variability in primary and secondary responses with each vaccine and individual make periodic reimmunization (boosters) necessary to establish and maintain immunity.

Synthetic and Genetically Engineered Vaccines

An alternative approach to vaccine development is to make use of genetic engineering tools (Chapter 12) to produce *synthetic peptides*. To make a vaccine, a genetic engineer can synthesize a peptide that corresponds to an antigen from an infectious agent. For example, the toxin from the foot-and-mouth disease virus, an important animal pathogen, must be modified from its native form to render it harmless for use as a vaccine. The toxin contains a peptide of 20 amino acids that is an important antigenic determinant in the protein, but the peptide is too small to be an effective vaccine by itself. Genetic engineers attached the small peptide to a larger, innocuous protein that acts as a carrier molecule, creating a *conjugate vaccine* against foot-and-mouth disease virus. This strategy has great promise for creating vaccines to a number of pathogens, especially because the complete genomic sequences of many pathogens are now known, providing the information necessary to identify the most likely antigenic parts of each.

Two widely available conjugate vaccines couple extracted bacterial polysaccharide to a protein toxoid, provoking a more robust immune response with better immune memory than injection of the polysaccharide antigen alone. One pneumococcal vaccine uses pneumococcal polysaccharide coupled to diphtheria toxoid (Figure 28.26). Likewise, the vaccine for *Haemophilus influenzae* type b (Hib) uses Hib polysaccharide coupled to tetanus toxoid. Although polysaccharide antigens typically provide only a primary response with little immune memory, the conjugated protein toxoids efficiently activate Th2 cells, resulting in a primary response followed by a strong secondary response and immune memory.

Genomic information is particularly useful for making viral vaccines. Genes that encode antigens from virtually any virus can be cloned into the vaccinia virus genome and expressed (Section 12.8). Inoculation with the antigen-producing vaccinia virus can induce immunity to the product of the cloned gene. Such a preparation is called a *recombinant-vector vaccine*, an example of which is the recombinant vaccinia–rabies vaccine used in animals. A second immunization strategy uses proteins made from cloned DNA as immunogens. After a pathogen gene is cloned and expressed in a suitable microbial host, the pathogen protein is harvested and used to produce a *recombinant-antigen vaccine*. For example, the current hepatitis B virus vaccine is a major hepatitis surface protein antigen (HbsAg) expressed by genetically modified yeast cells. Similarly, a vaccine effective against human papillomavirus (HPV) is also a recombinant-antigen vaccine made in yeast cells.

DNA Vaccines

A novel method of immunization is based on the expression of cloned genes in host cells. *DNA vaccines* are bacterial plasmids that contain cloned DNA that encodes the antigen of interest. Typically, the vaccine is injected intramuscularly into a host animal. Once host cells take up the plasmid, the DNA is transcribed and translated to produce immunogenic proteins, triggering a conventional immune response of activated T cells and antibodies (Chapter 27) directed to the protein encoded by the cloned DNA.

DNA vaccine strategies provide considerable advantages over conventional immunization methods. For instance, because only a single pathogen gene is cloned into the plasmid and injected, there is no chance of an infection, as there might be with an attenuated

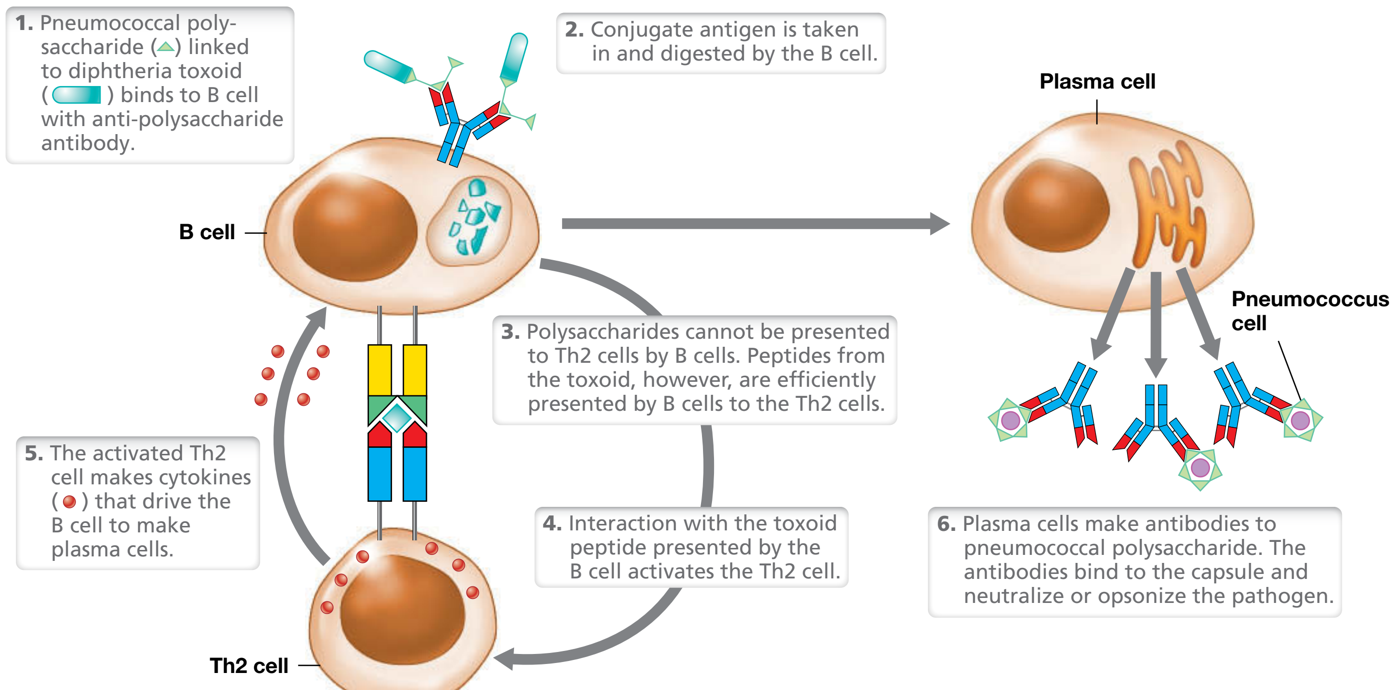


Figure 28.26 Conjugate vaccines. Conjugate vaccines, such as *Streptococcus pneumoniae* (pneumococcus) polysaccharide covalently linked to diphtheria toxoid (shown) or *Haemophilus influenzae* type B (Hib) polysaccharide coupled to tetanus toxoid provide effective immunity to polysaccharide antigens, which are poor immunogens in the absence of protein carriers.

vaccine. Genes for individual antigens, such as a tumor-specific antigen, can be cloned, targeting the immune response to a particular cell component. A single bioengineered plasmid encoding an antigen can be used to infect host cells and elicit a complete immune response, inducing both immune T cells and antibodies. In at least one case, an experimental DNA vaccine consisting of an engineered peptide–MHC I complex protected mice from infection with a cancer-producing papillomavirus.

MINIQUIZ

- Compare and contrast live attenuated vaccines, inactivated vaccines, and toxoids. Which of these has the greatest potential to cause active disease in the recipient? Which typically provides the longest-lasting immunity?
- Identify the advantages of alternative immunization strategies as compared to traditional immunization procedures.

28.10 Antibacterial Drugs

In cases where immunization is either not an option or fails to prevent disease, antimicrobial drug therapy is the primary weapon against infections. Antimicrobial drugs either kill or inhibit the growth of microorganisms in the host (in vivo) and are classified based on their molecular structure, mechanism of action (Figure 28.27), and spectrum of antimicrobial activity. **Antibiotics** are antimicrobial agents that are produced naturally by certain microorganisms, typically species of *Bacteria* or fungi. In addition to natural antibiotics, many other drugs are produced synthetically, but regardless of their origin, it is desirable that antimicrobial drugs exhibit **selective toxicity**—they inhibit or kill pathogens without adversely affecting the host. Although thousands of antibiotics are known, fewer than 1% are clinically useful, often because of problems with host toxicity or lack of uptake by host cells.

The susceptibility of individual microbes to different antimicrobial agents varies significantly. For example, gram-positive *Bacteria* are often sensitive to natural penicillin, whereas gram-negative *Bacteria* are generally resistant; thus, natural penicillin exhibits a relatively *narrow spectrum* of activity. By contrast, antibiotics that exhibit *broad-spectrum* activity, such as tetracycline, are generally effective against both groups. Although broad-spectrum antibiotics often find wider medical use than narrow-spectrum antibiotics, antibiotics with a limited spectrum of activity may be quite useful against certain pathogens, especially those that fail to respond to other antibiotics. A good example is vancomycin, a narrow-spectrum antibiotic that is an effective bactericidal agent for penicillin-resistant, gram-positive *Bacteria*, including certain enterococci, staphylococci, and clostridia.

Important targets of antibiotics in *Bacteria* include the cell wall, ribosomes, enzymes that facilitate nucleic acid synthesis or catalyze metabolic processes, and the cytoplasmic membrane (Figure 28.27). We provided an overview of antibiotic targets in Section 7.10 and now consider the mechanisms of drug activity in further detail.

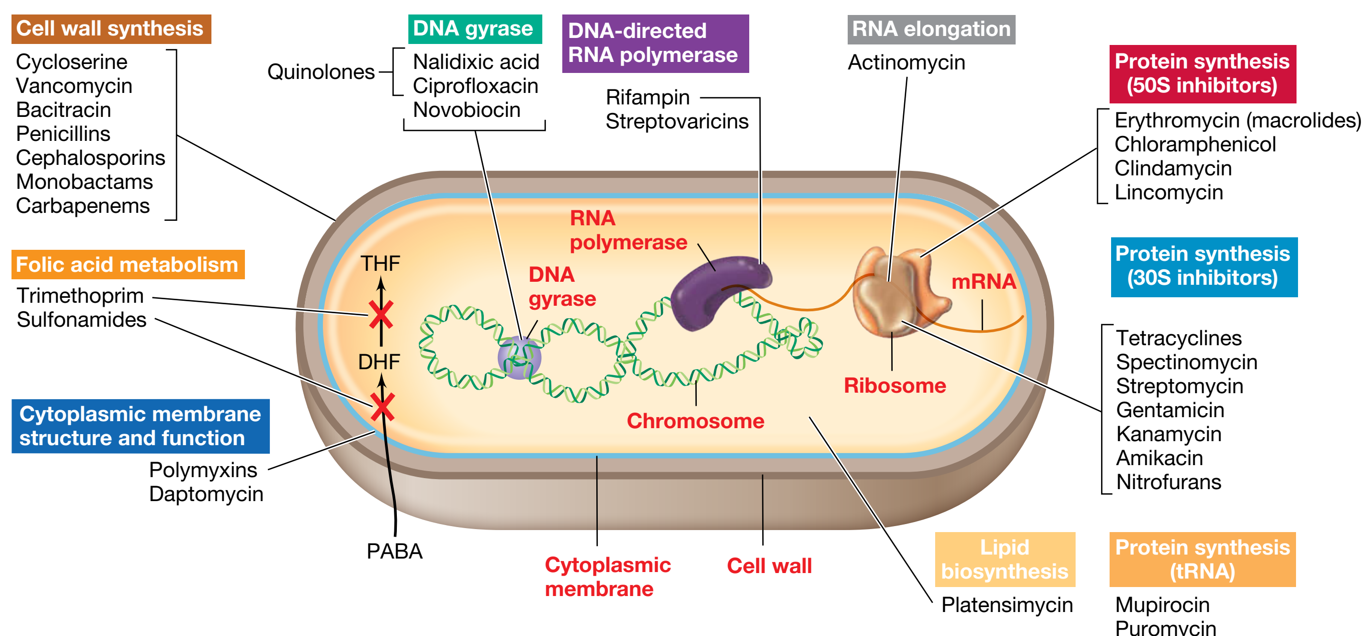


Figure 28.27 Mechanisms of action of major antibacterial agents. Agents are classified according to their target structures in the bacterial cell. THF, tetrahydrofolate; DHF, dihydrofolate; PABA, *p*-aminobenzoic acid.

The Cell Wall as a Drug Target

Worldwide, an estimated 100,000 metric tons of antibacterial drugs are manufactured and used annually (**Figure 28.28*a***), and the vast majority of these target the bacterial cell wall. **β-lactam antibiotics**, which include penicillins and cephalosporins, inhibit cell wall synthesis and account for nearly two-thirds of all antibiotics produced and used worldwide (Figure 28.28*b*). These antibiotics share a characteristic structural component, the *β-lactam ring* (**Figure 28.29**), and historically, they comprise some of our most effective weapons against many different types of bacterial infections.

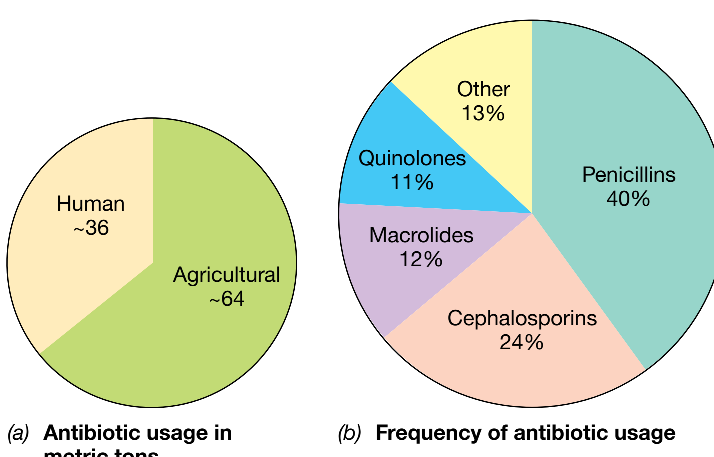


Figure 28.28 Annual worldwide production and use of antibiotics. *(a)* An estimated 100,000 metric tons of antimicrobial agents are manufactured worldwide per year, and nearly two-thirds of this amount is used in an agricultural context. *(b)* By far, the β-lactam antibiotics (penicillins and cephalosporins) constitute the most important and widely used class of antibiotic. "Other" includes trimethoprim combinations, tetracyclines, chloramphenicol, aminoglycosides, and all other antimicrobial drug classes. Data are from the Center for Disease Dynamics, Economics, and Policy, Washington D.C.

The first β-lactam antibiotic ever characterized was penicillin G, isolated from the fungus *Penicillium chrysogenum* in 1929 by Alexander Fleming. This new drug was dramatically effective for controlling staphylococcal and pneumococcal infections and was more effective for treating streptococcal infections than the synthetic sulfa drugs, discussed later in this section. Penicillin and other β-lactam antibiotics interfere with an important feature of bacterial cell wall synthesis called *transpeptidation*, the reaction that results in the cross-linking of two glycan-linked peptide chains (Section 2.4). Because peptidoglycan synthesis mechanisms are unique to *Bacteria*, the β-lactam antibiotics are highly selective and nontoxic to host cells.

Penicillin G is active primarily against gram-positive *Bacteria* because gram-negative *Bacteria* are impermeable to the antibiotic. However, chemical modification of the *N*-acyl group of penicillin G produces *semisynthetic* penicillins, such as ampicillin and carbenicillin, which have broader activity and are effective against certain gram-negative *Bacteria* (Figure 28.29). The structural differences in these semisynthetic penicillins allow them to be transported inside the gram-negative outer membrane (Section 2.5), where they inhibit cell wall synthesis. Penicillin G is also sensitive to *β-lactamases*, enzymes that destroy β-lactam antibiotics and are produced by many penicillin-resistant *Bacteria* (see Section 28.12). Oxacillin and methicillin are widely used β-lactamase-resistant semisynthetic penicillins (Figure 28.29).

Cephalosporins, produced by species of the fungus *Cephalosporium*, differ structurally from the penicillins. They retain the β-lactam ring but have a six-member dihydrothiazine ring joined to it instead of

UNIT 6

Natural penicillin (penicillin G)
Gram-positive activity; β-lactamase and acid-sensitive

CH_2–CO–N(H) … Thiazolidine ring … S … CH_3 … CH_3 … COOH … β-lactam ring

N-acyl group modification	Semisynthetic penicillins
OCH_3, –CO–, OCH_3	**Methicillin** acid-stable, β-lactamase-resistant
–CO–, N, O, CH_3	**Oxacillin** acid-stable, β-lactamase-resistant
–CH–CO–, NH_2	**Ampicillin** broadened spectrum of activity (especially against gram-negative *Bacteria*), acid-stable, β-lactamase-sensitive
–CH–CO–, COOH	**Carbenicillin** broadened spectrum of activity (especially against *Pseudomonas aeruginosa*), acid-stable but ineffective orally, β-lactamase-sensitive

Figure 28.29 Structure of selected penicillins. The red arrow (top panel) indicates the site within the β-lactam ring cleaved by most β-lactamases, enzymes that destroy penicillin and other β-lactam antibiotics. Although the *N-acyl* group (blue shading) varies among different penicillin drugs, all penicillins have a common nucleus consisting of a β-lactam ring and a thiazolidine ring. Whereas penicillin G must be injected, most acid-stable penicillins can be administered orally (carbenicillin is an exception).

the five-member thiazolidine ring of penicillins (Table 28.5 and Figure 28.29). The cephalosporins have the same mode of action as the penicillins; they bind irreversibly to transpeptidases and prevent the cross-linking of peptidoglycan. Clinically important cephalosporins are semisynthetic antibiotics with a broader spectrum of activity than the penicillins. In addition, cephalosporins are typically more resistant to β-lactamases. For example, ceftriaxone (Table 28.5) is highly resistant to β-lactamases and has replaced penicillin for treatment of gonorrhea because many *Neisseria gonorrhoeae* strains have become resistant to penicillin (see Section 28.12).

Some antimicrobial drugs are *growth factor analogs*. Growth factors are organic nutrients obtained from an organism's environment that are required by the organism for growth and survival (Section 3.1). A **growth factor analog** is a synthetic compound that is structurally similar to a growth factor, but subtle structural differences between the analog and the growth factor prevent the analog from functioning in the cell, thereby disrupting cell metabolism. *Isoniazid* (Table 28.5) is an important growth factor analog with a very narrow spectrum of activity. Effective against mycobacteria only, isoniazid is an analog of nicotinamide, a vitamin required for mycolic acid synthesis, which is, in turn, required to construct the mycobacterial cell wall. Isoniazid is the most effective drug for treatment of tuberculosis (Section 30.4), but isoniazid resistance in strains of *Mycobacterium tuberculosis* is increasing.

Protein Synthesis as a Drug Target

Some antibiotics inhibit bacterial pathogens by disrupting protein synthesis (translation), often through interactions with the ribosome that may include binding to ribosomal RNA (rRNA) (Figure 28.27). Most of these drugs target only bacterial ribosomes and, therefore, have no effect on the structurally distinct, cytoplasmic ribosomes of eukaryotic cells. However, because mitochondria and chloroplasts in *Eukarya* contain 70S ribosomes (Section 13.4), many antibiotics that inhibit protein synthesis in *Bacteria* also inhibit protein synthesis in these organelles. Nevertheless, these drugs are still medically useful because the eukaryotic 70S ribosomes are affected only at higher concentrations than are used for antimicrobial therapy.

The **aminoglycosides** are antibiotics that inhibit translation by targeting the 30S (small) subunit of the ribosome. Aminoglycosides contain amino sugars linked by glycosidic bonds, and clinically useful examples include streptomycin (produced by the bacterium *Streptomyces griseus*), kanamycin (Table 28.5), neomycin, and gentamicin. These broad-spectrum antibiotics are useful for treating infections caused by gram-negative *Bacteria*, but because of host toxicity effects that include kidney and hearing damage, aminoglycoside use has decreased since the development of the semisynthetic penicillins and tetracyclines (discussed next). With the exception of gentamicin, which is routinely used to combat *Pseudomonas* infections, and neomycin, which is commonly used in topical ointments, aminoglycosides are typically used only when other antibiotics fail.

Like the aminoglycoside antibiotics, **tetracyclines** interfere with the function of the 30S subunit of the ribosome. Tetracyclines are broad-spectrum antibiotics produced by several species of *Streptomyces*, and they inhibit most clinically relevant gram-positive and gram-negative *Bacteria*. The basic structure of tetracycline consists of a naphthacene ring system (Table 28.5), and side-chain substitutions (either natural or synthetic) to the various rings form new tetracycline analogs. Physicians are cautioned against the administration of tetracyclines to pregnant women and to young children because these antibiotics bind calcium in bones and teeth, weakening them and causing permanent staining of the latter (Figure 28.30). Tetracyclines are widely used in veterinary medicine, though, and in some countries, they are used as nutritional supplements for poultry and swine. However, because nonclinical use of medically important antibiotics can contribute to antibiotic resistance among pathogens, this practice is discouraged.

Macrolide antibiotics inhibit translation by targeting the 50S (large) subunit of the bacterial ribosome. Their basic structure contains a lactone ring bonded to sugars (as in erythromycin in Table 28.5), and variations in these constituents result in a diversity of macrolide antibiotics. Macrolides account for about 12% of global antibiotic production (Figure 28.28) and include, for example, erythromycin (produced by *Streptomyces erythreus*), clarithromycin, and azithromycin. The partial inhibition of protein synthesis by erythromycin, in particular, leads to preferential translation of some proteins and restricts translation of

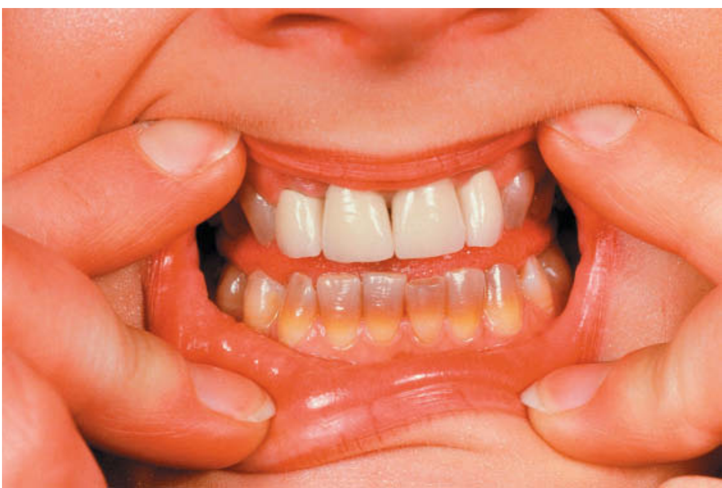


Figure 28.30 **Staining of teeth from the use of tetracycline.** Tetracycline binds calcium in developing bones and teeth, weakening them and causing permanent staining of tooth enamel. Therefore, tetracycline should not be administered to pregnant women or children unless absolutely necessary.

others, resulting in an imbalance in the proteome and potentially disrupting metabolic functions at all levels. Often used clinically in patients allergic to penicillin or other β-lactam antibiotics, erythromycin is particularly useful for treating legionellosis (Section 32.4).

Nucleic Acid Synthesis as a Drug Target

The **quinolones** are synthetic antibacterial compounds that disrupt bacterial metabolism by interfering with DNA gyrase, thus preventing the supercoiling and packaging of DNA in the bacterial cell (Section 4.1). Because DNA gyrase is found in all *Bacteria*, quinolones are effective for treating both gram-positive and gram-negative bacterial infections. *Fluoroquinolones*, such as ciprofloxacin (Table 28.5), are fluorinated derivatives of quinolones that are routinely used to treat urinary tract infections and have been widely used in the beef and poultry industries for prevention and treatment of respiratory diseases in animals. Ciprofloxacin is also the drug of choice for treating anthrax because some strains of *Bacillus anthracis*, the causative agent of anthrax (Section 31.8), are resistant to penicillin. The fluoroquinolone moxifloxacin is one of only a few drugs proven effective for treatment of tuberculosis. In combination with other anti-tuberculosis drugs, moxifloxacin may significantly reduce treatment time, a major problem with isoniazid-based treatments.

Some antibiotics disrupt transcription by inhibiting RNA synthesis (Figure 28.27). For example, rifamycins, such as *rifampin* (Table 28.5), inhibit RNA synthesis by binding to the β-subunit of RNA polymerase in *Bacteria*. Rifampin, often used in concert with isoniazid for treatment of tuberculosis, has the odd side effect of causing body secretions, including tears, urine, and sweat, to turn reddish-orange (Figure 28.31). *Actinomycin* inhibits RNA synthesis by combining with DNA and blocking RNA elongation. This agent binds most strongly to DNA at guanine–cytosine base pairs, fitting into the major groove in the double strand where RNA is synthesized.

Other Antibacterial Drug Targets

Like isoniazids, **sulfa drugs** (also called *sulfonamides*) are synthetic growth factor analogs. However, instead of affecting the cell wall, sulfa drugs block a key biosynthetic pathway in *Bacteria*. Sulfanilamide (Table 28.5), the simplest sulfa drug, is an analog of *p*-aminobenzoic acid (PABA), which is a component of the vitamin folic acid, a nucleic acid precursor. By mimicking PABA, sulfanilamide blocks the synthesis of folic acid, thereby inhibiting nucleic acid synthesis. Sulfanilamide is selectively toxic because bacteria synthesize their own folic acid, unlike humans and most animals, which obtain folic acid from their diet. Antimicrobial therapy with sulfamethoxazole (a sulfa drug) plus trimethoprim, a related folic acid synthesis competitor, is highly effective because the drug combination blocks two sequential steps in the folic acid synthesis pathway (Figure 28.27); resistance to this drug combination therefore requires two mutations in genes of the same pathway, a relatively rare event. However, resistance to sulfonamides is increasing as many formerly susceptible pathogens develop the ability to import folic acid from their environment.

Some antibiotics have atypical structures or targets. For example, the antibiotic *daptomycin* (Table 28.5) is produced by a species of *Streptomyces* and is a cyclic lipopeptide with a unique mode of action. Used mainly to treat infections by gram-positive *Bacteria*, including pathogenic streptococci and staphylococci, daptomycin binds specifically to bacterial cytoplasmic membranes, forms a

Figure 28.31 **Reddish-orange urine from rifampin usage.** The antibiotic rifampin is often administered to tuberculosis patients. However, a side effect of rifampin usage is that it turns urine and other body fluids reddish-orange.

TABLE 28.5 Selected antibacterial compounds

Mode of action	Antibiotic class	Example(s)	Representative structures
Inhibit cell wall synthesis	β-lactams	Penicillins, cephalosporins	Ceftriaxone
	Isoniazids	Isoniazid	Isoniazid
	Polypeptide antibiotics	Vancomycin, bacitracin	Vancomycin (see Figure 28.34)
Inhibit protein synthesis	Aminoglycosides	Streptomycin, kanamycin, gentamicin	Kanamycin
	Tetracyclines	Tetracycline, doxycycline	Tetracycline
	Macrolides	Erythromycin, azithromycin	Erythromycin
	Chloramphenicol	Chloramphenicol	Chloramphenicol

Chloramphenicol

Kanamycin

Tetracycline

Erythromycin

Dihydrothiazine ring

β-lactam ring

Ceftriaxone

Isoniazid

TABLE 28.5 *(continued)*

Mode of action	Antibiotic class	Example(s)	Representative structures
Inhibit nucleic acid synthesis	Quinolones and fluoroquinolones	Nalidixic acid, ciprofloxacin, moxifloxacin	Ciprofloxacin
	Rifamycins	Rifampin	Rifampin
Inhibit metabolite synthesis	Trimethoprim	Trimethoprim	Trimethoprim
	Sulfa drugs	Sulfanilamide, sulfamethoxazole	Sulfanilamide
Damage to the cell membrane	Lipid biosynthesis disruptor	Platensimycin	Platensimycin
	Membrane structure disruptor	Daptomycin	Daptomycin

Platensimycin

Daptomycin

Trimethoprim

Ciprofloxacin

Sulfanilamide

Rifampin

pore, and induces rapid depolarization of the membrane. The depolarized cell quickly loses its ability to synthesize macromolecules, such as nucleic acids and proteins, resulting in cell death. However, alterations in cytoplasmic membrane structure may lead to resistance.

Platensimycin (Table 28.5), produced by *Streptomyces platensis*, is an unusual antibiotic that inhibits fatty acid and lipid biosynthesis. Platensimycin is effective against a broad range of gram-positive *Bacteria*, including nearly untreatable infections caused by MRSA and vancomycin-resistant enterococci. Platensimycin has a unique mode of action, shows no host toxicity, and there is no known potential for development of resistance by pathogens. We discuss the discovery of platensimycin in Section 28.12.

MINIQUIZ

- Explain the concept of selective toxicity in terms of antimicrobial therapy.
- How does the activity of each antibiotic class lead to control of the affected pathogens?
- What are the sources of aminoglycosides, tetracyclines, macrolides, daptomycin, and platensimycin?

28.11 Antimicrobial Drugs That Target Nonbacterial Pathogens

Antiviral drugs often adversely affect the host as well as the pathogen because viruses depend on host cell biosynthetic machinery for their replication. As a result, selective toxicity for viruses is achieved only with agents that preferentially affect unique viral replication pathways or the assembly of viral components. In spite of these limitations, a number of drugs are more toxic for viruses than for the host, and a few agents specifically target individual viruses. Fungi, protozoa, and helminths (worms) pose special problems for the development of effective drugs because these pathogens are eukaryotic, and therefore their cellular biology is similar to that of humans.

Antiviral Drugs

The most successful and commonly used agents for antiviral chemotherapy are the *nucleoside analogs*. These drugs are **nucleoside reverse transcriptase inhibitors (NRTIs)**, which work by inhibiting elongation of the viral nucleic acid chain by a nucleic acid polymerase. The first NRTI to be widely used was *zidovudine*, also called *azidothymidine* (*AZT*) (Table 28.6) (Figure 30.48*a*), which effectively blocks reverse transcription and production of complementary DNA (cDNA) in HIV and other retroviruses (Section 30.15). It is structurally similar to thymidine but is a dideoxy derivative, lacking the 3′-hydroxyl group. Another widely used nucleoside analog, *acyclovir* (Table 28.6), resembles guanosine and has been successfully used to control the symptoms of genital herpes (Section 30.14).

Some antiviral agents target the key enzyme of retroviruses, reverse transcriptase. *Nevirapine* (Figure 30.48*b*), a **nonnucleoside reverse transcriptase inhibitor (NNRTI)**, binds directly to reverse transcriptase and inhibits reverse transcription (Table 28.6). Phosphonoformic acid, an analog of inorganic pyrophosphate, inhibits normal internucleotide linkages, preventing synthesis of viral nucleic acids. Because their action affects normal host cell nucleic acid synthesis, both NRTIs and NNRTIs usually induce some host toxicity.

TABLE 28.6 Antiviral compounds

Examples	Mechanism of action	Virus affected
Enfuvirtide	Blocks fusion of HIV with T lymphocyte membrane	HIV (human immunodeficiency virus)
α, β, γ-Interferon	Induces proteins that inhibit viral replication	Broad spectrum (host-specific)
Oseltamivir (Tamiflu®) and zanamivir (Relenza®)	Block active site of influenza neuraminidase	Influenza A and B
Nevirapine	Reverse transcriptase inhibitor	HIV
Acyclovir (Figure 30.42)	Viral polymerase inhibitor	Herpes viruses, *Varicella zoster*
Zidovudine (AZT) (Figure 30.48*a*)	Reverse transcriptase inhibitor	HIV
Ribavirin	Blocks capping of viral RNA	Respiratory syncytial virus, influenza A and B, Lassa fever
Cidofovir	Viral polymerase inhibitor	Cytomegalovirus, herpesviruses
Tenofovir (TDF)	Reverse transcriptase inhibitor	HIV
Indinavir, saquinavir (Figure 28.35)	Viral protease inhibitors	HIV

Other anti-retroviral drugs include **protease inhibitors** and *enfuvirtide*, a **fusion inhibitor** (Table 28.6). Protease inhibitors disrupt viral replication by binding the active site of HIV protease (see Figure 28.35), preventing this enzyme from processing large viral polyproteins into individual viral components (Section 10.11). Enfuvirtide is a 36-amino-acid synthetic peptide that binds to the gp41 membrane protein of HIV (Section 30.15); this stops the conformational changes necessary for the fusion of HIV with T lymphocyte membranes, thus preventing infection of host immune cells by HIV (Figure 30.43).

A single category of drugs effectively limits influenza infection. The *neuraminidase inhibitors* oseltamivir (Tamiflu) and zanamivir (Relenza) block the active site of neuraminidase in influenza A and B viruses, inhibiting virus release from infected cells. Zanamivir is used only for treatment of influenza, whereas oseltamivir is used for both treatment and prophylaxis (Table 28.6).

Recall from Chapter 26 that virus-infected cells release small cytokine proteins called *interferons* that trigger a defensive response in neighboring host cells (Section 26.10). If produced and administered properly, interferons may have potential use as prescribed antimicrobial agents. The clinical utility of interferons depends on whether they can be delivered to specific

areas in the host to stimulate the production of antiviral proteins in uninfected host cells. Alternatively, appropriate interferon stimulators, such as viral nucleotides, nonvirulent viruses, or even synthetic nucleotides, if given to host cells prior to infection, may induce natural production of interferon.

Drugs That Target Eukaryotic Pathogens

Fungi cause a number of serious diseases (Sections 33.1 and 33.2), and their treatment is complicated by the fact that many antifungal agents act on metabolic pathways that are shared by fungi and their host cells, thus making the drugs toxic. As a result, many antifungal drugs can be used only for topical (surface) applications (Table 28.7).

A major group of antifungal compounds are *ergosterol inhibitors*, which work either by interacting directly with ergosterol or inhibiting its synthesis (Table 28.7). Ergosterol is present in fungal cytoplasmic membranes in place of the cholesterol found in animal cell cytoplasmic membranes. Important ergosterol inhibitors include the *polyene* antibiotics, which are produced by species of *Streptomyces* bacteria. Polyenes bind specifically to ergosterol, causing membrane permeability and cell death (Figure 28.32). By contrast, *azoles* and *allylamines* are broad-spectrum antifungal drugs that work by selectively inhibiting ergosterol biosynthesis. Treatment with these drugs causes membrane damage and alteration of critical membrane transport activities.

Echinocandins are cell wall inhibitors that block the activity of 1,3-β-D-glucan synthase, the enzyme that forms β-glucan polymers in the fungal cell wall (Figure 28.32 and Table 28.7). Because mammalian cells do not have 1,3-β-D-glucan synthase (or cell walls), the activity of these agents selectively kills fungal cells. Echinocandins are often used to treat *Candida* yeast infections, as well as some fungi that are resistant to other agents (Section 33.1).

Fungal cell walls also contain chitin, a polymer of *N*-acetylglucosamine found only in fungi and insects. Several *polyoxins* inhibit cell wall synthesis by interfering with chitin biosynthesis (Figure 28.32). Although not used clinically, polyoxins are widely used as agricultural fungicides. Other antifungal drugs inhibit folate biosynthesis, interfere with DNA topology during replication, or, in the case of drugs such as griseofulvin, disrupt microtubule aggregation during mitosis (Figure 28.32). Moreover, the nucleic acid analog 5-fluorocytosine (flucytosine) is an effective nucleic acid synthesis inhibitor in fungi.

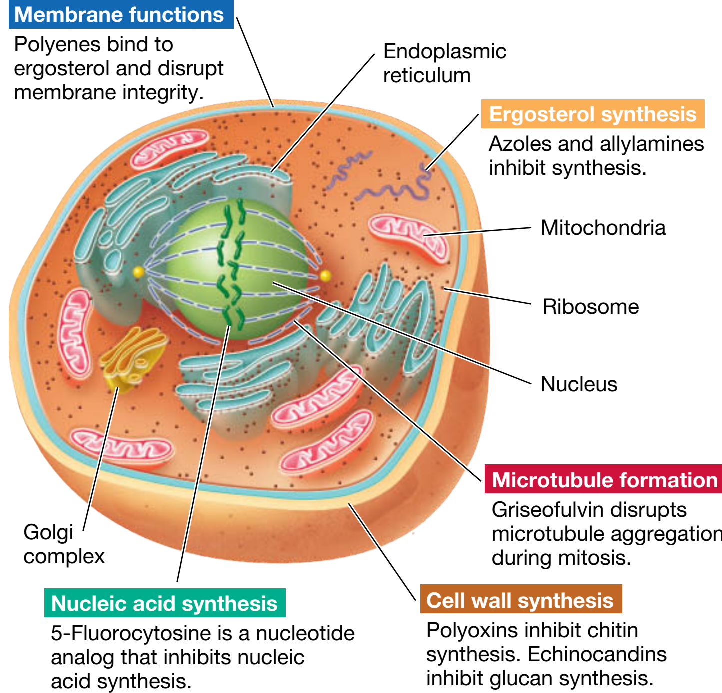


Figure 28.32 Targets of some antifungal agents. Traditional antibacterial agents are generally ineffective because fungi are eukaryotic cells. The cell wall and cell membrane ergosterol targets shown here are unique structures not present in vertebrate host cells.

Historically, diseases caused by protozoans, especially malaria, have been treated with *quinine* or, more recently, quinine derivatives, such as chloroquine and mefloquine. However, extensive resistance to these drugs among the *Plasmodium* spp. that cause malaria has led to the development of artemisinin-based alternatives. Artemisinin is produced in low amounts by the Chinese

TABLE 28.7 Antifungal agents

Category	Target	Examples	Use
Allylamines	Ergosterol synthesis	Terbinafine	Oral, topical
Aromatic antibiotic	Mitosis inhibitor	Griseofulvin	Oral
Azoles	Ergosterol synthesis	Clotrimazole	Topical
		Fluconazole	Oral
		Miconazole	Topical
Chitin synthesis inhibitor	Chitin synthesis	Nikkomycin Z	Experimental
Echinocandins	Cell wall synthesis	Caspofungin	Intravenous
Nucleic acid analogs	DNA synthesis	5-Fluorocytosine	Oral
Polyenes	Ergosterol synthesis	Amphotericin B	Oral, intravenous
		Nystatin	Oral, topical
Polyoxins	Chitin synthesis	Polyoxin A and B	Agricultural

wormwood plant, which has been used traditionally to control the cyclic fever associated with malaria. A more recent discovery has shown artemisinin to also be an effective treatment for certain helminth diseases, such as schistosomiasis (Section 33.7). Using the techniques of synthetic biology, a genetically engineered yeast strain is now being used to produce artemisinin, making the drug more widely available (Section 12.11 and Figure 12.33).

The drug of choice for many parasitic protozoans is *metronidazole* (or the related drug *tinidazole*), which targets anaerobic pathogens and is therefore also useful against infections caused by clostridia. Infections with *Giardia intestinalis* (giardiasis), *Trichomonas vaginalis* (trichomoniasis), and especially *Entamoeba histolytica* (amebic dysentery) have all been treated successfully with these drugs (Sections 33.3 and 33.4). The related protist *Cryptosporidium parvum* (cryptosporidiosis) is often treated with *nitazoxanide*, a drug that has also been effective against helminths. However, perhaps the most widely used antihelminthic drugs are *praziquantel*, for treating schistosomiasis and tapeworm infections, and *mebendazole* (and similar drugs), for treating a variety of helminth infections, including tapeworms, pinworms, hookworms, trichinosis, and ascariasis (Section 33.7).

MINIQUIZ

- What steps in the viral maturation process are inhibited by nucleoside analogs? By protease inhibitors? By interferons?
- Why are there fewer clinically effective antifungal and antiparasitic agents than antibacterial agents?

28.12 Antimicrobial Drug Resistance and New Treatment Strategies

Antimicrobial drug resistance is the acquired ability of a microorganism to resist the effects of an antimicrobial agent to which it was formerly susceptible. As we have discussed, many microorganisms produce antibiotics, and genes encoding antibiotic resistance are present in virtually all of them. Horizontal gene transfer between and among microorganisms disseminates antimicrobial drug resistance.

Antimicrobial Drug Resistance

Common mechanisms of bacterial resistance to antibiotics were discussed in the context of microbial growth in Section 7.10 and depicted in Figure 7.21*b*, and some examples are listed in **Table 28.8**. Antibiotic resistance can be genetically encoded on the bacterial chromosome, but more often, antibiotic-resistant bacteria isolated from patients contain drug-resistance genes located on horizontally transmitted *R* (*resistance*) *plasmids* (Section 4.2). Enzymes encoded by genes on R plasmids confer resistance by any of three classes of mechanisms: modifying and inactivating the drug, preventing uptake of the drug, or actively pumping the antibiotic out of the cell, a process called *efflux* (Table 28.8).

The widespread use of antibiotics (Figure 28.28*a*) provides favorable conditions for the spread of R plasmids because they carry genes that confer an immediate selective advantage. The ubiquity of resistance genes limits the long-term use of any single antibiotic as an effective antimicrobial agent. A classic example is the development of multidrug resistance in the bacterium *Neisseria gonorrhoeae*. Penicillin, widely used to treat gonorrhea into the 1980s, became ineffective and was replaced by ciprofloxacin, which, in turn, lost much of its efficacy after just 10 years of use (**Figure 28.33**). This prompted a switch in treatment to the β-lactamase-resistant ceftriaxone, and more recently, a combination of ceftriaxone and azithromycin. Combining two unrelated antimicrobial agents such as this often reduces resistance because it is less likely that a mutant strain resistant to one antibiotic will also be resistant to the second antibiotic. However, certain R plasmids confer multiple drug resistance and can thwart multiple antibiotic therapy as a clinical strategy.

TABLE 28.8 Bacterial resistance to antibiotics

Resistance mechanism	*Antibiotic example*	*Genetic basis of resistance*	*Mechanism present in*
Reduced permeability	Penicillins	Chromosomal	Gram-negative bacteria
Inactivation of antibiotic Examples: β-lactamases; modifying enzymes, such as methylases, acetylases, phosphorylases, and others	Penicillins Chloramphenicol Aminoglycosides	Plasmid and chromosomal Plasmid and chromosomal Plasmid	*Staphylococcus aureus* Enteric bacteria *Neisseria gonorrhoeae* *Staphylococcus aureus* Enteric bacteria
Alteration of target Examples: RNA polymerase, rifamycin; ribosome, erythromycin and streptomycin; DNA gyrase, quinolones	Erythromycin Rifamycin Streptomycin Norfloxacin	Chromosomal	*Staphylococcus aureus* Enteric bacteria Enteric bacteria Enteric bacteria *Staphylococcus aureus*
Development of resistant biochemical pathway	Sulfonamides	Chromosomal	Enteric bacteria *Staphylococcus aureus*
Efflux (pumping out of cell)	Tetracyclines Chloramphenicol Erythromycin	Plasmid Chromosomal Chromosomal	Enteric bacteria *Staphylococcus aureus* *Bacillus subtilis* *Staphylococcus*

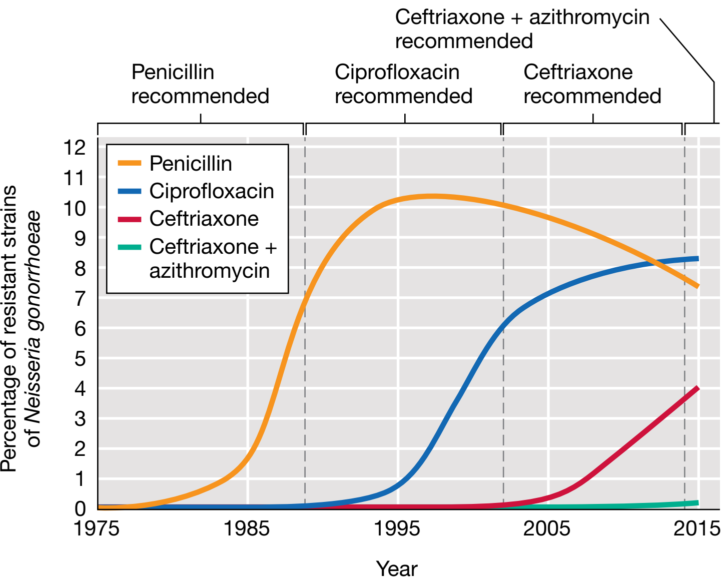


Figure 28.33 The emergence of multidrug resistance in *Neisseria gonorrhoeae*. Resistance to penicillin developed in the 1980s to the point that it could no longer be recommended for treatment of gonorrhea, at which point ciprofloxacin, a quinolone, became the drug of choice. By the early 2000s, resistance to ciprofloxacin prompted a change to ceftriaxone. The current recommendation is a combination of ceftriaxone plus azithromycin.

Overuse of antibiotics also accelerates resistance. In addition to their traditional use as a treatment for infections, antibiotics are widely used as supplements to farm animal feeds both as growth-promoting substances and as prophylactic additives to prevent the occurrence of disease. For example, broad-spectrum antibiotics widely used clinically, such as the fluoroquinolone ciprofloxacin, are used extensively as a feed additive, especially by the poultry industry. Although the trend is improving, antibiotics are also used in clinical practice far more often than necessary, and this problem is compounded by patient noncompliance—many patients stop taking antibiotics as soon as they feel better. Antibiotic resistance can be minimized if drugs are used only for treatment of susceptible pathogens and are given in sufficiently high doses and for sufficient lengths of time to eradicate the pathogen before resistant mutants can propagate.

To prevent the further emergence of multidrug-resistant pathogens, the Centers for Disease Control and Prevention (CDC) publishes guidelines stressing the importance of vaccination, rapidly and accurately diagnosing and treating infections, using antimicrobial agents prudently, and preventing pathogen transmission.

New Drugs and New Treatment Strategies

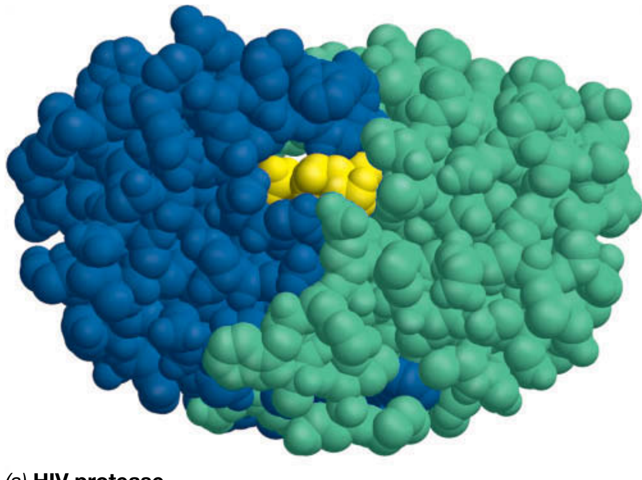
Conservative, appropriate administration of antibiotics can prolong or even resurrect the clinical usefulness of available drugs, but the long-term solution to antimicrobial drug resistance requires continuous development of new drugs through design or discovery. Developing new analogs of existing antimicrobial compounds is usually more cost effective than discovering new drugs, and analogs may actually have greater antimicrobial activity than the parent compound. For example, using natural penicillin as the starting compound, systematic chemical substitution of the *N*-acyl group can generate hundreds of penicillin derivatives, many with broad-spectrum activity (Figure 28.29). Using this basic strategy, semisynthetic analogs of β-lactam antibiotics, tetracycline, and vancomycin (**Figure 28.34**) have been synthesized.

Novel antimicrobial compounds are more difficult to identify than analogs of existing drugs because new antimicrobial compounds must work at unique sites in metabolism or be structurally dissimilar to existing compounds to avoid resistance mechanisms. Computer-based methods accelerate novel compound design by maximizing molecular binding in a virtual environment. A dramatic success in computer-directed drug design was the development of *saquinavir*, a protease inhibitor that impedes the multiplication of HIV in infected individuals by binding the active site of the HIV protease enzyme (**Figure 28.35**). As an analog of the HIV precursor protein, saquinavir displaces the authentic protease substrate and inhibits virus maturation. Several other computer-designed protease inhibitors, including *indinavir* (Figure 28.35*b*), are currently in use for the treatment of HIV/AIDS (Section 30.15).

Another strategy to overcome antimicrobial resistance is to select for antibiotics that interact with unexploited targets. Platensimycin is the first antimicrobial drug targeted to disrupt bacterial lipid biosynthesis (Table 28.5 and Figure 28.27). Platensimycin is especially active against gram-positive pathogens, including drug-resistant staphylococci and enterococci. To select an agent for a defined target, in this case an enzyme in the bacterial lipid synthesis pathway, scientists used antisense RNA (Section 6.11) to limit the amount of accessible mRNA required

Figure 28.34 Vancomycin. Many pathogenic strains now show intermediate drug resistance to the parent structure of vancomycin, but chemically substituting the carbonyl oxygen at the position shown in red with a methylene (=CH_2) group restores much of the lost activity. Like penicillin, vancomycin prevents cross-linking of peptidoglycan and is most effective against gram-positive pathogens.

UNIT 6



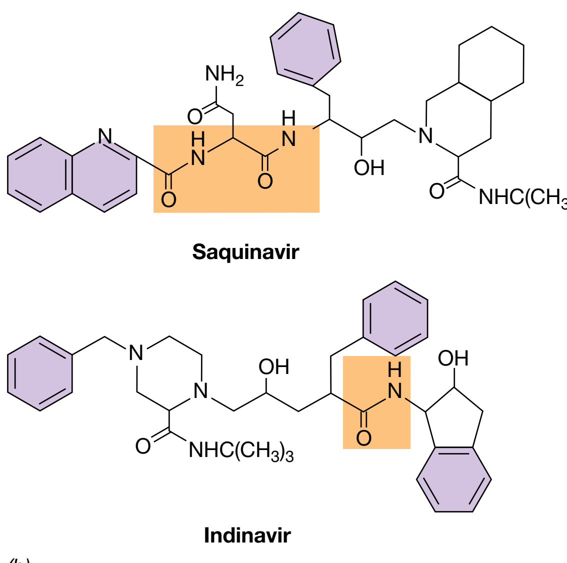


Figure 28.35 **Computer-generated anti-HIV drugs.** *(a)* The HIV protease homodimer. Individual polypeptide chains are shown in green and blue. A peptide (yellow) is bound in the active site. HIV protease cleaves an HIV precursor protein, a necessary step in virus maturation. Blocking of the protease site by the bound peptide inhibits precursor processing and HIV maturation. This structure is derived from information in the Protein Data Bank. *(b)* These anti-HIV drugs are peptide analogs called protease inhibitors that were designed by computer to block the active site of HIV protease. The areas highlighted in orange show the regions analogous to peptide bonds in proteins.

to produce a key lipid biosynthesis enzyme in *Staphylococcus aureus*. This reduced fatty acid synthesis in the cells and increased the sensitivity of the crippled *S. aureus* strain to antibiotics that inhibit fatty acid synthesis. After screening thousands of natural products from potential antibiotic producers, scientists isolated platensimycin from the soil bacterium *Streptomyces platensis*. This strategy identifies target-specific antibiotics present in low concentrations and is applicable to virtually any target for which the gene sequence (and, hence, the corresponding antisense RNA sequence) is known.

The efficacy of some antibiotics can be preserved if they are administered with compounds that thwart antibiotic resistance mechanisms. For example, several β-lactam *antibiotics* can be combined with β-lactamase *inhibitors* to preserve antibiotic activity in β-lactam-resistant microbes. For instance, ampicillin (Figure 28.29) can be mixed with the β-lactamase inhibitor clavulanic acid to produce the combination drug Augmentin. The inhibitor binds β-lactamase (produced by the resistant pathogen) irreversibly. This prevents ampicillin from being degraded and maintains the efficacy of the antibiotic.

Drug combination therapy has also revolutionized treatment of HIV infections. Currently, a combination therapy consisting of nucleoside analogs and a protease inhibitor is recommended. This drug treatment protocol is termed HAART, for *h*ighly *a*ctive *a*nti-*r*etroviral *t*herapy. As with antibacterial combination regimens, HAART is designed to target two independent viral functions: nucleoside analogs target virus replication, and protease inhibitors target virus maturation. Because the probability of a virus developing resistance to multiple drugs is much less than the probability of it becoming resistant to a single drug, HAART therapy has been a successful strategy in controlling HIV infections (Section 30.15).

MINIQUIZ

- Identify the basic mechanisms of antibiotic resistance in bacteria and describe what practices encourage their dissemination.
- What does vancomycin have in common with penicillin? How can native vancomycin be improved?
- Explain the advantages and disadvantages of developing new drugs based on existing drug analogs. What other methods exist for developing new drugs?

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Chapter Review

I • The Clinical Microbiology Setting

28.1 Clinical laboratory safety requires training and planning to prevent contamination and possible infection of laboratory workers. Specific precautions and procedures proportional to the risk of infection by a given agent, designated by biosafety levels (BSL), must be in place to handle contaminated materials and patient specimens.

Q Which laboratories require an occupational exposure control plan for handling bloodborne pathogens? Why is this necessary?

28.2 Patients in healthcare facilities are unusually susceptible to infectious disease because of their compromised health and potential exposure to pathogens present in the facilities. Many healthcare-associated infections are caused by drug-resistant pathogens.

Q What are "healthcare-associated infections" (HAI) and how are they acquired? What problems can they cause?

II • Isolating and Characterizing Infectious Microorganisms

28.3 Appropriate sampling, observation, and culture techniques are necessary to isolate and identify potential pathogens. The selection of techniques requires knowledge of the ecology, physiology, and metabolism of suspected pathogens. Most pathogens exhibit unique metabolic patterns when grown on specialized selective and differential media. Growth-dependent patterns provide information helpful for unambiguous pathogen identification.

Q In order to ensure accurate diagnosis, what measures should be taken during the collection of specimens and while detecting and culturing pathogens?

28.4 Pathogens isolated from clinical samples are often tested for antibiotic susceptibility to ensure appropriate antibiotic therapy. Testing is based on the minimum inhibitory concentration of an agent necessary to completely inhibit growth of a pathogen.

Q How does the traditional method of determining minimum inhibitory concentration (MIC) compare to current practice?

III • Immunological and Molecular Tools for Disease Diagnosis

28.5 An immune response is a natural outcome of infection. Specific immune responses characterized by a rise in antibody titers and positive T cell–mediated skin tests can be used to provide evidence of infections and to monitor convalescence. Monoclonal antibodies reproducibly provide specificity for a wide range of diagnostic and therapeutic purposes.

Q Why does antibody titer rise after infection? Is a high antibody titer indicative of an ongoing infection? Explain. Why is it necessary to obtain an acute and a convalescent blood sample to monitor infections?

28.6 Precipitation and agglutination reactions produce visible results involving antigen–antibody interactions. Fluorescent antibodies are used for quick, accurate identification of pathogens and other antigenic substances in tissue samples, blood, and other complex mixtures. Fluorescent antibody–based methods can be used for identification of a variety of microbial cell types.

Q Why are agglutination tests so widely used in clinical diagnostics? How are fluorescent antibodies used to diagnose diseases? What advantages do immunofluorescent techniques have over traditional culturing?

28.7 Enzyme immunoassays (EIAs), rapid tests, and immunoblots are sensitive and specific immunological assays. These tests can be engineered to detect either antibody or antigen for diagnosis of infections by any of a large number of pathogens.

Q EIAs are extremely sensitive diagnostic tools. Why, then, is the immunoblot (Western blot) procedure used to confirm screening tests that are positive for human immunodeficiency virus (HIV)?

28.8 Nucleic acid amplification (PCR) methods are applied as extremely specific diagnostic tools used for a large number of pathogens. Quantitative PCR (qPCR) and qualitative PCR techniques provide quantification and identification of pathogens, and reverse transcription PCR (RT-PCR) is especially useful for detecting RNA viruses.

Q Distinguish between quantitative and qualitative PCR. How does qualitative PCR expand upon the qPCR technique?

IV • Prevention and Treatment of Infectious Diseases

28.9 Immunization induces artificial active immunity and is widely used to prevent infectious diseases. Vaccines are either attenuated or inactivated pathogens or pathogen products or are genetically engineered antigens. The latter eliminate exposure to pathogens and, in some cases, even to protein antigen. Application of this strategy is providing safer vaccines targeted to individual pathogen antigens.

Q List the immunizations recommended for children in the United States. For which of these have you been immunized? List the diseases for which you may have acquired immunity naturally.

28.10 Antibiotics are chemically diverse antimicrobial compounds produced by microorganisms. Each antibiotic works by inhibiting a specific cellular process in the target microorganisms. The β-lactam antibiotics, including penicillins and cephalosporins, target bacterial cell wall synthesis and are the most important class of clinical antibiotics. The aminoglycosides, macrolides, and tetracycline antibiotics selectively interfere with protein synthesis in *Bacteria*. The quinolones are an important class of synthetic antibacterial drugs that inhibit DNA synthesis. Daptomycin and platensimycin are structurally novel antibiotics that target cytoplasmic membrane functions and lipid biosynthesis, respectively.

Q What are the common sources for natural antibiotics? How do these antimicrobial drugs differ from growth factor analogs, such as the sulfa drugs? Why are β-lactam antibiotics generally more effective against gram-positive bacteria than against gram-negative bacteria?

28.11 Antiviral agents selectively target virus-specific enzymes and processes. Useful agents include analogs and compounds that inhibit nucleic acid polymerases and viral genome replication. Protease inhibitors interfere with viral maturation steps. Because fungi are *Eukarya*, antifungal agents exhibiting selective toxicity are difficult to find. Nevertheless, some effective antifungal agents are available, and they primarily target fungi-specific structures and biosynthetic processes.

Q Why is host toxicity a common problem with antiviral and antifungal drugs? Identify the targets that allow for selective toxicity of antifungal agents.

28.12 The use of antimicrobial drugs inevitably leads to resistance in the targeted microorganisms. The development of resistance can be accelerated by the indiscriminate use of antimicrobial drugs, and many pathogens have developed resistance. New antimicrobial compounds must continually be discovered and developed to deal with drug-resistant pathogens and to enhance our ability to treat infectious diseases. Computer-based modeling and other novel strategies are helping to address this challenge.

Q What practices contribute to the spread of antibiotic resistance? Explain how antisense RNA strategies can extend traditional methods of natural product selection for antibiotic discovery.

Application Questions

1. Define the procedures you would use to isolate and identify a new pathogen. Keep in mind Koch's postulates (Figure 1.29) as you form your answer. Be sure to include growth-dependent assays, immunoassays, and molecular assays. Which of your assays could be adapted to be used as a routine, high-throughput test for rapid clinical diagnosis?
2. Viruses and fungi present special problems for drug therapy. Explain the issues inherent in drug treatment of both groups, and explain whether you agree with the preceding statement. Give specific examples, and suggest at least one group of agents that might target both types of infectious agents.
3. Describe three important reasons why semisynthetic penicillins were first developed. Which clinical challenges does each of these reasons address? What key part of the penicillin molecule must be retained for any semisynthetic penicillin to be active?
4. Imagine yourself as a clinical microbiologist with all of the diagnostic tools described in this chapter available for your analyses. Which tool(s) would you use (and why) if (1) A patient had a life-threatening infection caused by a difficult-to-culture bacterium and where treatment of the infection was absolutely dependent on an extremely rapid identification of the pathogen? (2) A patient had a less severe bacterial infection where the pathogen was easily culturable and treatable?

Chapter Glossary

Agglutination a reaction between antibody and particle-bound antigen resulting in visible clumping of the particles

Aminoglycoside an antibiotic, such as streptomycin, containing amino sugars linked by glycosidic bonds

Antibiotic a chemical substance produced by a microorganism that kills or inhibits the growth of another microorganism

Antimicrobial drug resistance the acquired ability of a microorganism to resist the effects of an antimicrobial agent to which it was formerly susceptible

β-lactam antibiotic penicillin, cephalosporin, or a related antibiotic that contains the four-membered heterocyclic β-lactam ring

Differential media growth media that allow identification of microorganisms based on phenotypic properties

Enzyme immunoassay (EIA) a test that uses antibodies or antigens linked to enzymes to detect antigens or antibodies in body fluids

Fluorescent antibody an antibody molecule covalently modified with a fluorescent dye that makes the antibody visible under fluorescent light

Fusion inhibitor a peptide that blocks the fusion of viral and target cell cytoplasmic membranes

Growth factor analog a chemical agent that has a similar structure to and blocks the uptake or utilization of a growth factor

Healthcare-associated infection (HAI) an infection acquired by a patient in a healthcare facility, particularly during a stay in the facility. Also called a *nosocomial infection*.

Immunoblot (Western blot) the use of labeled antibodies to detect specific proteins after separation by electrophoresis and transfer to a membrane

Macrolide erythromycin or a related antibiotic that contains a lactone ring bonded to sugars

Minimum inhibitory concentration (MIC) the smallest amount of an agent needed to completely inhibit the growth of an organism in vitro

Monoclonal antibody (mAb) a single type of antibody made by a single B cell hybridoma clone

Nonnucleoside reverse transcriptase inhibitor (NNRTI) a nonnucleoside analog used to inhibit viral reverse transcriptase

Nucleoside reverse transcriptase inhibitor (NRTI) a nucleoside analog used to inhibit viral reverse transcriptase

Precipitation a reaction between antibody and a soluble antigen resulting in a visible, insoluble complex

Protease inhibitor a class of drug designed to inhibit viral protease

Quinolone a synthetic antibacterial compound that inhibits DNA gyrase and prevents supercoiling of bacterial DNA

Selective media culture media that allow the growth of certain organisms while inhibiting the growth of others through one or more added media components

Selective toxicity the ability of a compound to inhibit or kill a pathogen without adversely affecting the host

Sensitivity the lowest amount of antigen that can be detected by a diagnostic test

Serology the study of antigen–antibody reactions in vitro

Specificity the ability of an antibody or a lymphocyte to recognize a single antigen, or of a diagnostic test to identify a specific pathogen

Sulfa drugs synthetic growth factor analogs that inhibit folic acid biosynthesis in *Bacteria*

Tetracycline an antibiotic characterized by the four-member naphthacene ring structure

Titer the quantity of a substance, such as antibody, in a solution

Vaccination (immunization) the inoculation of a host with inactive or weakened pathogens or pathogen products to stimulate protective active immunity

Vaccine an inactivated or attenuated pathogen, or a harmless pathogen product, used to induce artificial active immunity

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A Mysterious New Disease Outbreak

Although epidemiologists pay particular attention to large-scale disease outbreaks—cholera and the like—a disease tracker's job often entails investigating a very restricted outbreak. Such was the case with the recent *Elizabethkingia* outbreak in southern Wisconsin (USA).

Elizabethkingia anophelis is a gram-negative bacterium (main photo) of the phylum *Bacteroidetes* that forms translucent colonies on blood agar media (inset photo). The genus name *Elizabethkingia* honors American medical bacteriologist Elizabeth King, and the species epithet *anophelis* reflects the fact that this organism is the dominant bacterium in the gut of *Anopheles gambiae*, the mosquito that carries the malarial parasite. *Elizabethkingia* inhabits soil and water and is rarely linked to disease. However, in the spring of 2016, the Wisconsin Department of Public Health received reports of at least 63 confirmed cases of *E. anophelis* infection, and two additional cases, one each in Illinois and Michigan, were also reported. Of these 65 cases, 20 deaths (~30% mortality) occurred, showing that *E. anophelis* infections were indeed a serious threat.

E. anophelis is an opportunistic pathogen that can cause meningitis or bloodstream infections and can also colonize the respiratory tract. In the 2016 outbreak, the majority of infections and deaths were in patients over the age of 65 who had serious underlying health issues such as cancer, diabetes, recent surgery, or the like. This epidemiological observation suggested the possibility of a common source of infection. However, because *E. anophelis* is so widespread in the environment, many potential sources had to be checked. Common disease vehicles such as contaminated food or water were almost completely ruled out, as was person-to-person transmission. Contaminated medical equipment and a few other sources were suggested as possible explanations for the infections. However, pinpointing the primary source of the Wisconsin *E. anophelis* outbreak has thus far proven elusive.

Nevertheless, epidemiologists remain busy using their well-honed analytical skills to systematically eliminate some explanations for the Wisconsin *E. anophelis* outbreak while grouping together the most likely possibilities for further analysis. Using epidemiological methods, the source and the mode(s) of transmission of this pathogen will eventually be revealed and this should reduce *Elizabethkingia* infections to the usual number (5–10) typically reported from the entire United States each year.

Source: Multistate outbreak of infections caused by *Elizabethkingia anophelis*. 2016. Centers for Disease Control and Prevention, Atlanta, Georgia (USA). June 16, 2016.