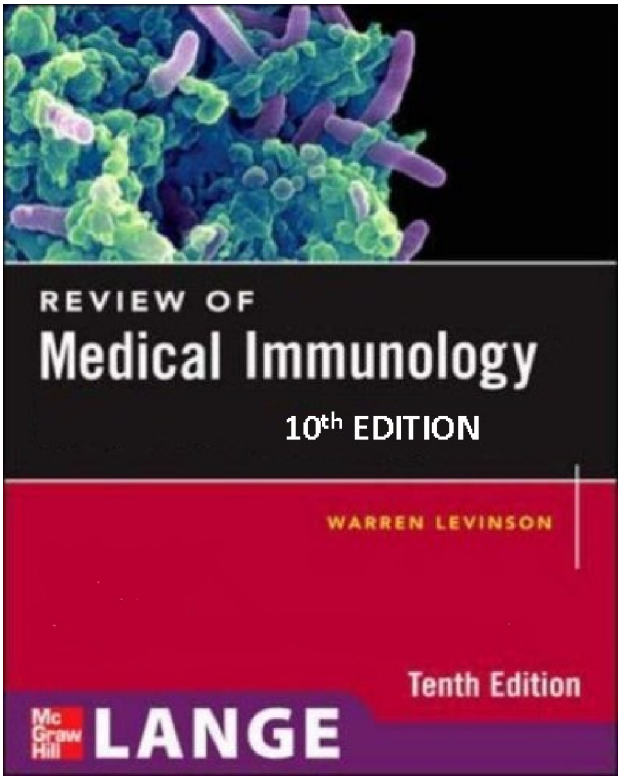


Part VII. Immunology

IMMUNITY: INTRODUCTION

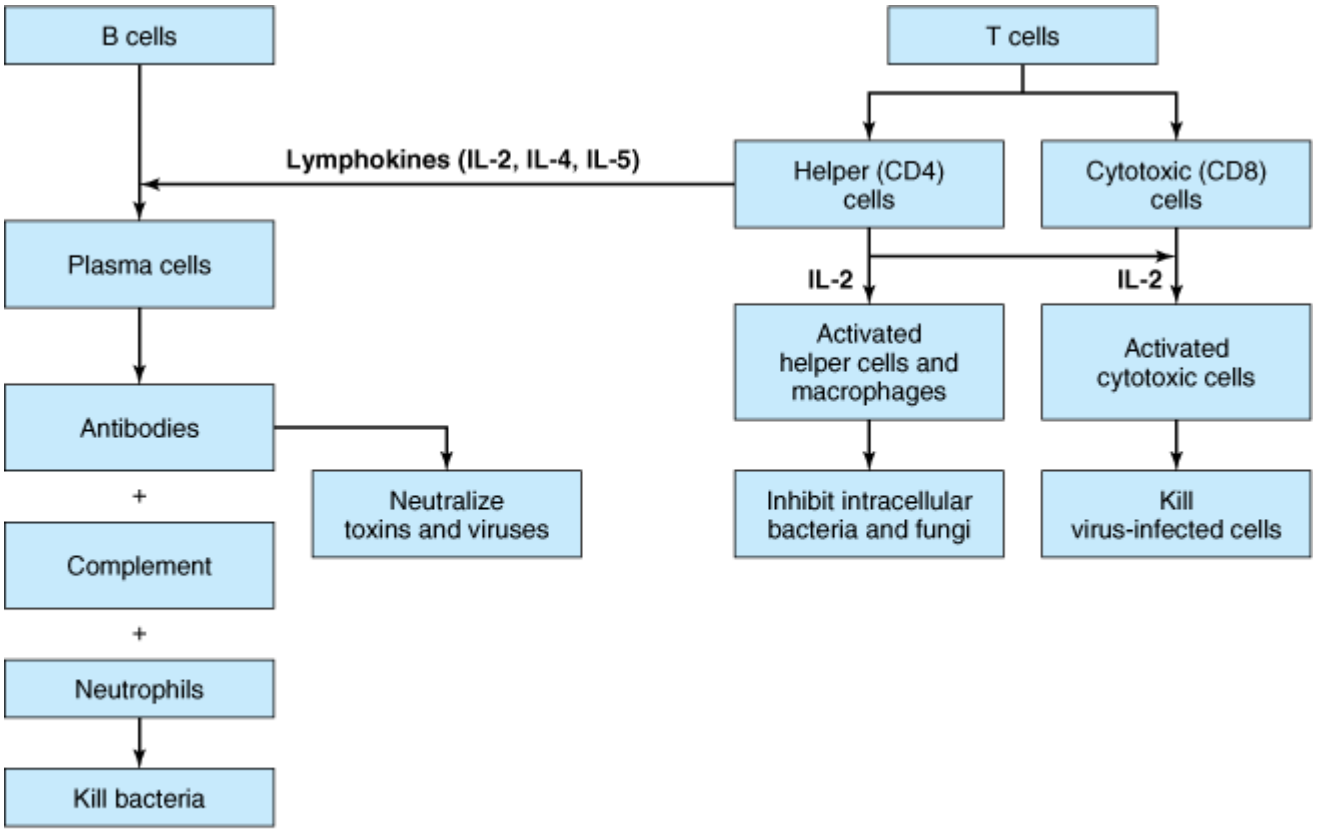
The main function of the immune system is to **prevent or limit infections** by microorganisms such as bacteria, viruses, fungi, and parasites. The first line of defense against microorganisms is the **intact skin and mucous membranes.** If microorganisms breach this line and enter the body, then the **innate arm** of the immune system (second line of defense) is available to destroy the invaders. Because the components of the innate arm (Table 57–1) are preformed and fully active, they can function immediately upon entry of the microorganisms. The ability of the innate arm to kill microorganisms is not specific. For example, a neutrophil can ingest and destroy many different kinds of bacteria.

Table 57–1. Main Components of Innate and Acquired Immunity that Contribute to Humoral (Antibody-Mediated) Immunity and Cell-Mediated Immunity.

	Humoral Immunity	Cell-Mediated Immunity
Innate	Complement	Macrophages
	Neutrophils	Natural killer cells
Acquired	B cells	Helper T cells
	Antibodies (made by plasma cells)	Cytotoxic T cells

Highly specific protection is provided by the **acquired (adaptive) arm** of the immune system (third line of defense), but it takes several days for this arm to become fully functional. The two components of the acquired arm are **cell-mediated immunity** and **antibody-mediated (humoral) immunity.** An overview of the functions and interactions between many of the important members of the innate and acquired arms of the immune response is provided in Figure 57–1. (The features of the innate and the acquired arms of the immune system are contrasted in Table 57–2.)

Figure 57–1.



Source: Levinson W: *Review of Medical Microbiology and Immunology*, 10th Edition: http://www.accessmedicine.com

Introduction to the interactions and functions of the major components of the immune system. **Left:** Antibody-mediated (humoral) immunity. This is our main defense against extracellular, encapsulated, pyogenic bacteria such as staphylococci and streptococci. Antibodies also neutralize toxins, such as tetanus toxin, as well as viruses, such as hepatitis B virus. **Right:** Cell-mediated immunity. There are two distinct components. (1) Helper T cells and macrophages are our main defense against intracellular bacteria, such as *Mycobacterium tuberculosis,* and fungi, such as *Histoplasma capsulatum.* (2) Cytotoxic T cells are an important defense against viruses and act by destroying virus-infected cells.

Table 57–2. Important Features of Innate and Acquired Immunity.

Type of Immunity	Specificity	Effective Immediately After Exposure to Microbe	Improves After Exposure	Has Memory
Innate	Nonspecific	Yes—acts within minutes	No	No
Acquired	Highly specific	No—requires several days before becoming effective	Yes	Yes

The cell-mediated arm consists primarily of **T lymphocytes** (e.g., helper T cells and cytotoxic T cells), whereas the antibody-mediated arm consists of antibodies (immunoglobulins) and **B lymphocytes** (and plasma cells). Some of the major functions of T cells and B cells are shown in Table 57–3. The main functions of antibodies are (1) to **neutralize toxins and viruses** and (2) to **opsonize bacteria,** making them easier to phagocytize. Opsonization is the process by which immunoglobulin G (IgG) antibody and the C3b component of complement enhance phagocytosis (see Figure 8–3). Cell-mediated immunity, on the other hand, inhibits organisms such as fungi, parasites, and certain intracellular bacteria such as *Mycobacterium tuberculosis;* it also kills **virus-infected cells** and **tumor cells.**

Table 57–3. Major Functions of T Cells and B Cells.

Antibody-Mediated Immunity (B Cells)	Cell-Mediated Immunity (T Cells)
1. Host defense against infection (opsonize bacteria, neutralize toxins and viruses)	1. Host defense against infection (especially *M. tuberculosis,* fungi, and virus-infected cells)
2. Allergy (hypersensitivity), e.g., hay fever, anaphylactic shock	2. Allergy (hypersensitivity), e.g., poison oak
3. Autoimmunity	3. Graft and tumor rejection
	4. Regulation of antibody response (help and suppression)

Both the cell-mediated and antibody-mediated responses are characterized by three important features: (1) they exhibit remarkable **diversity** (i.e., they can respond to millions of different antigens); (2) they have a long **memory** (i.e., they can respond many years after the initial exposure because memory T cells and memory B cells are produced); and (3) they exhibit exquisite **specificity** (i.e., their actions are specifically directed against the antigen that initiated the response).

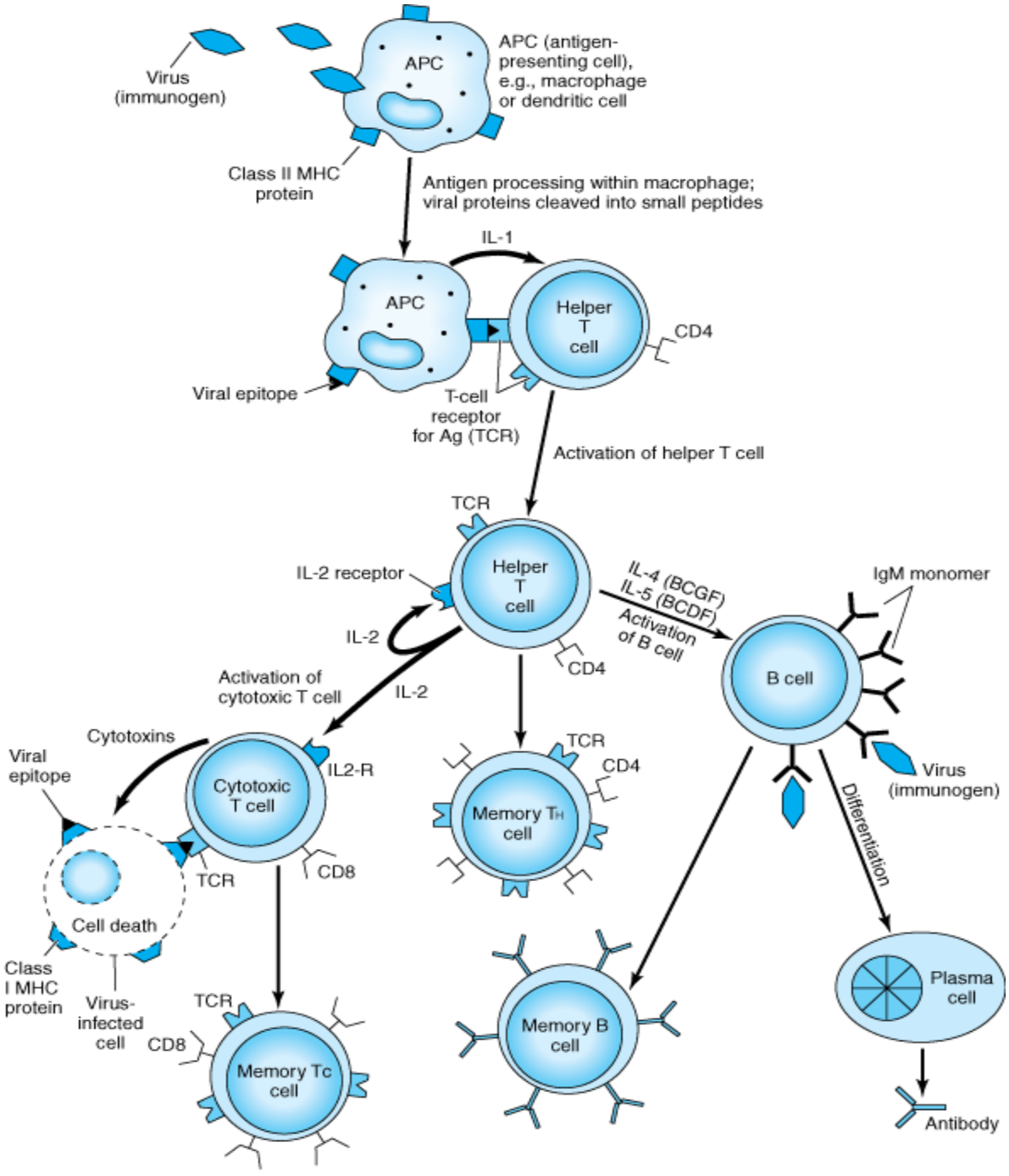
The combined effects of certain cells (e.g., T cells, B cells, macrophages, and neutrophils) and certain proteins (e.g., interleukins, antibodies, and complement) produce an **inflammatory response,** one of the body's main defense mechanisms. The process by which these components interact to cause inflammation is described in Chapter 8.

Macrophages and certain other phagocytic cells such as dendritic cells participate in both the innate and acquired arms of the immune response. They are, in effect, a bridge between the two arms. As part of the innate arm, they ingest and kill various microbes. They also present antigen to helper T cells, which is the essential first step in the activation of the acquired arm (see below). It is interesting to note that neutrophils, which are also phagocytes and have excellent microbicidal abilities, do *not* present antigen to helper T cells and therefore function in innate but not acquired immunity.

SPECIFICITY OF THE IMMUNE RESPONSE

Cell-mediated immunity and antibody are both highly specific for the invading organism. How do these specific protective mechanisms originate? The process by which these host defenses originate can be summarized by three actions: the **recognition** of the foreign organism by specific immune cells, the **activation** of these immune cells to produce a specific response (e.g., antibodies), and the **response** that specifically targets the organism for destruction. The following examples briefly describe how specific immunity to microorganisms occurs. An overview of these processes with a viral infection as the model is shown in Figure 57–2. A detailed description is presented in Chapter 58.

Figure 57–2.



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Overview of the process by which cell-mediated immunity and antibody-mediated immunity are induced by exposure to a virus. (Modified and reproduced, with permission, from Stites D, Terr A, Parslow T [editors]: Basic & Clinical Immunology, 9th ed. Originally published by Appleton & Lange. Copyright © 1997 by The McGraw-Hill Companies, Inc.)

Cell-Mediated Immunity

In the following example, a bacterium, e.g., *Mycobacterium tuberculosis,* enters the body and is ingested by a macrophage. The bacterium is broken down, and fragments of it called **antigens** or **epitopes** appear on the surface of the macrophage in association with **class II major histocompatibility complex (MHC)** proteins. The antigen–class II MHC protein complex interacts with an antigen-specific receptor on the surface of a **helper T lymphocyte.** Activation and clonal proliferation of this antigen-specific helper T cell occur as a result of the production of **interleukins,** the most important of which are interleukin-1 (produced by macrophages) and interleukin-2 (produced by lymphocytes). These activated helper T cells, aided by activated macrophages, mediate one important component of cellular immunity, i.e., a **delayed hypersensitivity reaction** specifically against *M. tuberculosis.*

Cytotoxic (cytolytic) T lymphocytes are also specific effectors of the cellular immune response, particularly against virus-infected cells. In this example, a virus, e.g., influenza virus, is inhaled and infects a cell of the respiratory tract. Viral envelope glycoproteins appear on the surface of the infected cell in association with **class I MHC** proteins. A cytotoxic T cell binds via its antigen-specific receptor to the viral antigen–class I MHC protein complex and is stimulated to grow into a clone of cells by interleukin-2 produced by helper T cells. These cytotoxic T cells specifically kill influenza virus–infected cells (and not cells infected by other viruses) by recognizing viral antigen–class I MHC protein complexes on the cell surface and releasing perforins that destroy the membrane of the infected cell.

Antibody-Mediated Immunity

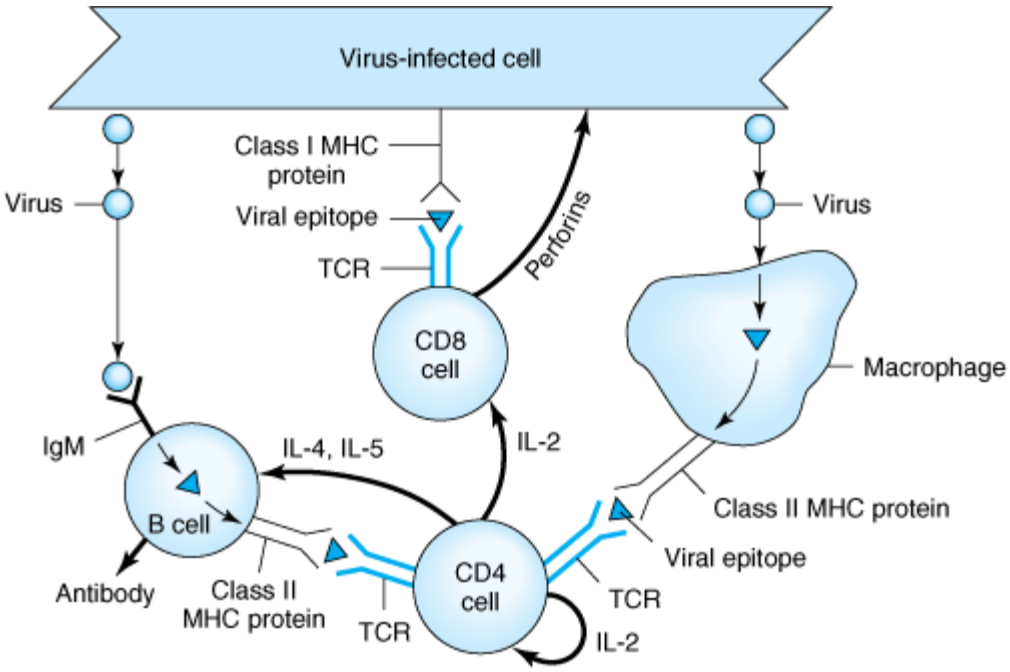
Antibody synthesis typically involves the cooperation of three cells: **macrophages, helper T cells,** and **B cells.** After processing by a macrophage, fragments of antigen appear on the surface of the macrophage in association with **class II MHC** proteins. The antigen–class II MHC protein complex binds to specific receptors on the surface of a helper T cell, which then produces interleukins such as interleukin-2 (T-cell growth factor), interleukin-4 (B-cell growth factor), and interleukin-5 (B-cell differentiation factor). These factors activate the B cell capable of producing antibodies specific for that antigen. (Note that the interleukins are nonspecific; the specificity lies in the T cells and B cells and is mediated by the antigen receptors on the surface of these cells.) The activated B cell proliferates and differentiates to form many plasma cells that secrete large amounts of **immunoglobulins** (antibodies).

Although antibody formation usually involves helper T cells, certain antigens, e.g., bacterial polysaccharides, can activate B cells directly, without the help of T cells, and are called **T-cell-independent antigens.** In this T-cell-independent response,

only IgM is produced by B cells because it requires interleukins 4 and 5 made by the helper T cell for the B cell to "class switch" to produce IgG, IgA, and IgE. See Chapter 59 for a discussion of "class switching," the process by which the B cell switches the antibody it produces from IgM to one of the other classes.

Figure 57–3 summarizes the human host defenses against virus-infected cells and illustrates the close interaction of various cells in mounting a coordinated attack against the pathogen. The specificity of the response is provided by the antigen receptor (T-cell receptor [TCR]) on the surface of both the CD4-positive T cell and the CD8-positive T cell and by the antigen receptor (IgM) on the surface of the B cell. The interleukins, on the other hand, are **not specific.**

Figure 57–3.



Source: Levinson W: *Review of Medical Microbiology and Immunology*, 10th Edition: http://www.accessmedicine.com

Induction of cell-mediated immunity and antibody against a viral infection. **Right:** Virus released by an infected cell is ingested and processed by an antigen-presenting cell (APC), e.g., a macrophage. The viral epitope is presented in association with a class II MHC protein to the virus-specific T-cell receptor (TCR) on the CD4 cell. The macrophage makes IL-1, which helps activate the CD4 cell. The activated CD4 cell makes interleukins (e.g., IL-2, which activates the CD8 cell to attack the virus-infected cell, and IL-4 and IL-5, which activate the B cell to produce antibody). The specificity of the cytotoxic response mounted by the CD8 cell is provided by its TCR, which recognizes the viral epitope presented by the virus-infected cell in association with a class I MHC protein. **Left:** Virus released by an infected cell interacts with the antigen receptor (IgM monomer) specific for that virus located on the surface of a B cell.

The virus is internalized, and the viral proteins are broken down into small peptides. B cells (as well as macrophages) can present viral epitopes in association with class II MHC proteins and activate CD4 cells. The CD4-positive helper cell produces IL-4 and IL-5, which induce the B cell to differentiate into a plasma cell that produces antibody specifically against this virus.

As depicted in Figure 57–3, B cells can perform two important functions during the induction process: (1) they **recognize antigens** with their surface IgM that acts as an antigen receptor and (2) they **present epitopes** to helper T cells in association with class II MHC proteins. Note that the IgM antigen receptor on the B cell can recognize not only foreign proteins but also carbohydrates, lipids, DNA, RNA, and other types of molecules. The class II MHC proteins of the B cell, however, can only present peptide fragments to the helper T cells. This distinction will become important when haptens are discussed later in this chapter. It is this remarkable ability of the IgM antigen receptor on the B cell to bind to an incredibly broad range of molecules that activates B cells to produce **antibodies against virtually every molecule known.** How the B cell generates such a diverse array of antibodies is described in Immunoglobulin Genes.

INNATE & ACQUIRED IMMUNITY

Our immune host defenses can be divided into two major categories: **innate (natural)** and **acquired (adaptive).** The features of these two important components of our host defenses are compared in Table 57–2.

Innate Immunity

Innate immunity is resistance that exists **prior to exposure** to the microbe (antigen). It is **nonspecific** and includes host defenses such as barriers to infectious agents (e.g., skin and mucous membranes), certain cells (e.g., natural killer cells), certain proteins (e.g., the complement cascade and interferons), and involves processes such as phagocytosis and inflammation (Table 57–4). Innate immunity **does not improve after exposure** to the organism, in contrast to acquired immunity, which does. In addition, **innate immune processes have no memory,** whereas acquired immunity is characterized by long-term memory.

Table 57–4. Important Components of Innate Immunity.

Factor	Mode of Action
I. Factors that limit entry of microorganisms into the body	
Keratin layer of intact skin	Acts as mechanical barrier

Lysozyme in tears and other secretions	Degrades peptidoglycan in bacteria cell wall
Respiratory cilia	Elevate mucus-containing trapped organisms
Low pH in stomach and vagina; fatty acids in skin	Retards growth of microbes
Surface phagocytes (e.g., alveolar macrophages)	Ingest and destroy microbes
Defensins (cationic peptides)	Create pores in microbial membrane
Normal flora of throat, colon, and vagina	Occupy receptors, which prevents colonization by pathogens
II. Factors that limit growth of microorganisms within the body	
Natural killer cells	Kill virus-infected cells
Neutrophils	Ingest and destroy microbes
Macrophages and dendritic cells	Ingest and destroy microbes, and present antigen to helper T cells
Interferons	Inhibit viral replication
Complement	C3b is an opsonin; membrane attack complex creates holes in bacterial membranes
Transferrin and lactoferrin	Sequester iron required for bacterial growth
Fever	Elevated temperature retards bacterial growth
Inflammatory response	Limits spread of microbes
APOBEC3G (apolipoprotein B RNA-editing enzyme)	Causes hypermutation in retroviral DNA and mRNA

Note that the innate arm of our host defenses performs two major functions: **killing invading microbes and activating acquired (adaptive) immune processes.** Some components of the innate arm, such as neutrophils, only kill microbes, whereas others, such as macrophages and dendritic cells, perform both functions,

i.e., they kill microbes and present antigen to helper T cells, which activates acquired immune processes.

Although innate immunity is often successful in eliminating microbes and preventing infectious diseases, it is, in the long run, *not* sufficient for human survival. This conclusion is based on the observation that children with severe combined immunodeficiency disease (SCID), who have intact innate immunity but no acquired immunity, suffer from repeated, life-threatening infections.

Several components of the innate arm recognize what is foreign by detecting certain carbohydrates or lipids on the surface of microorganisms that are different from those on human cells. Components of the innate arm have receptors called **pattern-recognition receptors** that recognize a molecular pattern present on the surface of many microbes and—very importantly—that is not present on human cells. By using this strategy, these components of the innate arm do not have to have a highly specific receptor for every different microbe but can still distinguish between what is foreign and what is self.

Note that the type of host defense mounted by the body differs depending on the type of organism. For example, a humoral (antibody-mediated) response is produced against one type of bacteria, but a cell-mediated response occurs in response to a different type of bacteria. The process that determines the type of response depends on the cytokines produced by the macrophages, and this in turn depends on which "pattern-recognition receptor" is activated by the organism, as described in the next paragraph.

Two important examples of this pattern recognition are:

1. Endotoxin is a lipopolysaccharide (LPS) found on the surface of most gram-negative bacteria (but not on human cells). The lipid A portion of LPS is the most important cause of septic shock and death in hospitalized patients. When released from the bacterial surface, LPS combines with LPS-binding protein, a normal component of plasma. This binding protein transfers LPS to a receptor on the surface of macrophages called CD14. LPS stimulates a pattern-recognition receptor called **toll-like receptor 4 (TLR4),** which transmits a signal, via several intermediates, to the nucleus of the cell. This induces the production of cytokines, such as IL-1, IL-6, IL-8, and tumor necrosis factor (TNF), and induces the costimulator protein, B7, which is required to activate helper T cells and to produce antibodies. Note that a different toll-like receptor, TLR2, signals the presence of gram-positive bacteria and yeasts because they have a different molecular pattern on their surface. Drugs that modify the action of these toll-like receptors may become important in preventing endotoxin-mediated septic shock, a leading cause of death in hospitalized patients.

2. Many bacteria and yeasts have a polysaccharide called mannan on their surface that is not present on human cells. (Mannan is a polymer of the sugar, mannose.) A pattern-recognition receptor called **mannan-binding lectin (MBL)** (also known as mannose-binding protein) binds to the mannan on the surface of the microbes, which then activates complement (see Chapter 63), resulting in death of the microbe. MBL also enhances phagocytosis (acts as an opsonin) via receptors to which it binds on the surface of phagocytes, such as macrophages. MBL is a normal serum protein whose concentration in the plasma is greatly increased during the acute-phase response.

The **acute-phase response,** which consists of an increase in the levels of various plasma proteins, e.g., C-reactive protein and mannose-binding protein, is also part of innate immunity. These proteins are synthesized by the liver and are nonspecific responses to microorganisms and other forms of tissue injury. The liver synthesizes these proteins in response to certain cytokines, namely, IL-1, IL-6, and TNF, produced by the macrophage after exposure to microorganisms. These cytokines, IL-1, IL-6, and TNF, are often called the **proinflammatory cytokines,** meaning that they enhance the inflammatory response.

Some acute-phase proteins bind to the surface of bacteria and activate complement, which can kill the bacteria. For example, C-reactive protein binds to a carbohydrate in the cell wall of *Streptococcus pneumoniae* and, as mentioned above, MBL binds to mannan (mannose) on the surface of many bacteria.

Defensins are another important component of innate immunity. Defensins are highly positively charged (i.e., cationic) peptides that create pores in the membranes of bacteria and thereby kill them. How they distinguish between microbes and our cells is unknown. Defensins are located primarily in the gastrointestinal and lower respiratory tracts. Neutrophils and Paneth cells in the intestinal crypts contain one type of defensin (α-defensins), whereas the respiratory tract produces different defensins called β-defensins.

α-Defensins also have antiviral activity. They interfere with human immunodeficiency virus (HIV) binding to the CXCR4 receptor and block entry of the virus into the cell. The production of α-defensins may explain why some HIV-infected individuals are long-term "nonprogressors."

APOBEC3G (apolipoprotein B RNA-editing enzyme) is an important member of the innate host defenses against retroviral infection, especially against HIV. APOBEC3G is an enzyme that causes hypermutation in retroviral DNA by deaminating cytosines in both mRNA and retroviral DNA, thereby inactivating these molecules and reducing infectivity. HIV defends itself against this innate host defense by producing Vif (viral

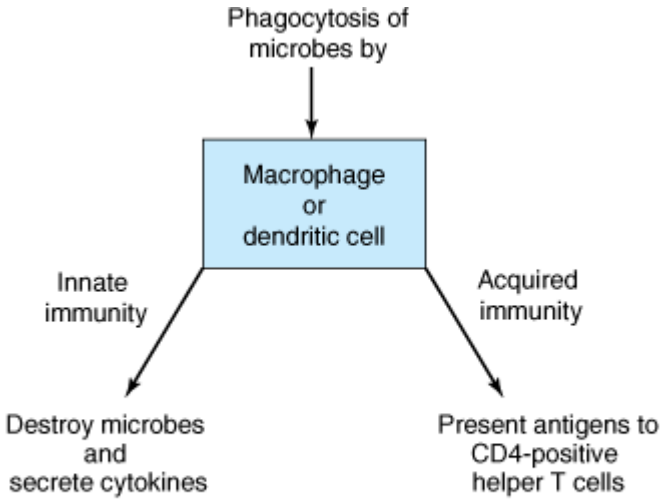
infectivity protein), which counteracts APOBEC3G, thereby preventing hypermutation from occurring.

Adaptive (Acquired) Immunity

Adaptive immunity occurs **after exposure** to an agent, **improves upon repeated exposure,** and is **specific.** It is mediated by antibody produced by B lymphocytes and by two types of T lymphocytes, namely, helper T cells and cytotoxic T cells. The cells responsible for acquired immunity have **long-term memory** for a specific antigen. Acquired immunity can be active or passive. Chapter 58 describes how the specificity and memory of acquired immunity is produced.

Macrophages and other antigen-presenting cells such as dendritic cells play an important role in both the innate and the acquired arms of the immune system (Figure 57–4). When they phagocytose and kill microbes, they function as part of the innate arm, but when they present antigen to a helper T lymphocyte, they activate the acquired arm that leads to the production of antibody and of cells such as cytotoxic T lymphocytes. Note that the acquired arm can be activated only after the innate arm has recognized the microbe.

Figure 57–4.



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Macrophages and other antigen-presenting cells, such as dendritic cells, participate in both the innate arm and the acquired arm of the immune system. These cells are considered part of the innate arm because they phagocytose and kill many types of microbes and also produce cytokines that cause inflammation. They are also part of the acquired arm because they present antigen in association with class II MHC proteins to CD4-positive helper T cells. (In common with all other nucleated cells, they also can present antigen in association with class I MHC proteins to CD8-positive cytotoxic T cells.)

ACTIVE & PASSIVE IMMUNITY

Active immunity is resistance induced after **contact** with foreign antigens, e.g., microorganisms. This contact may consist of clinical or subclinical infection, immunization with live or killed infectious agents or their antigens, or exposure to microbial products (e.g., toxins and toxoids). In all these instances, the host actively produces an immune response consisting of antibodies and activated helper and cytotoxic T lymphocytes.

The main advantage of active immunity is that resistance is **long-term** (Table 57–5). Its major disadvantage is its **slow onset,** especially the primary response (see Chapter 60).

Table 57–5. Characteristics of Active and Passive Immunity.

	Mediators	Advantages	Disadvantages
Active immunity	Antibody and T cells	Long duration (years)	Slow onset
Passive immunity	Antibody only	Immediate availability	Short duration (months)

Passive immunity is resistance based on antibodies **preformed** in another host. Administration of antibody against diphtheria, tetanus, botulism, etc., makes large amounts of antitoxin immediately available to neutralize the toxins. Likewise, preformed antibodies to certain viruses (e.g., rabies and hepatitis A and B viruses) can be injected during the incubation period to limit viral multiplication. Other forms of passive immunity are IgG passed from mother to fetus during pregnancy and IgA passed from mother to newborn during breast feeding.

The main advantage of passive immunization is the **prompt availability** of large amounts of antibody; disadvantages are the **short life span** of these antibodies and possible hypersensitivity reactions if globulins from another species are used. (See serum sickness in Chapter 65.)

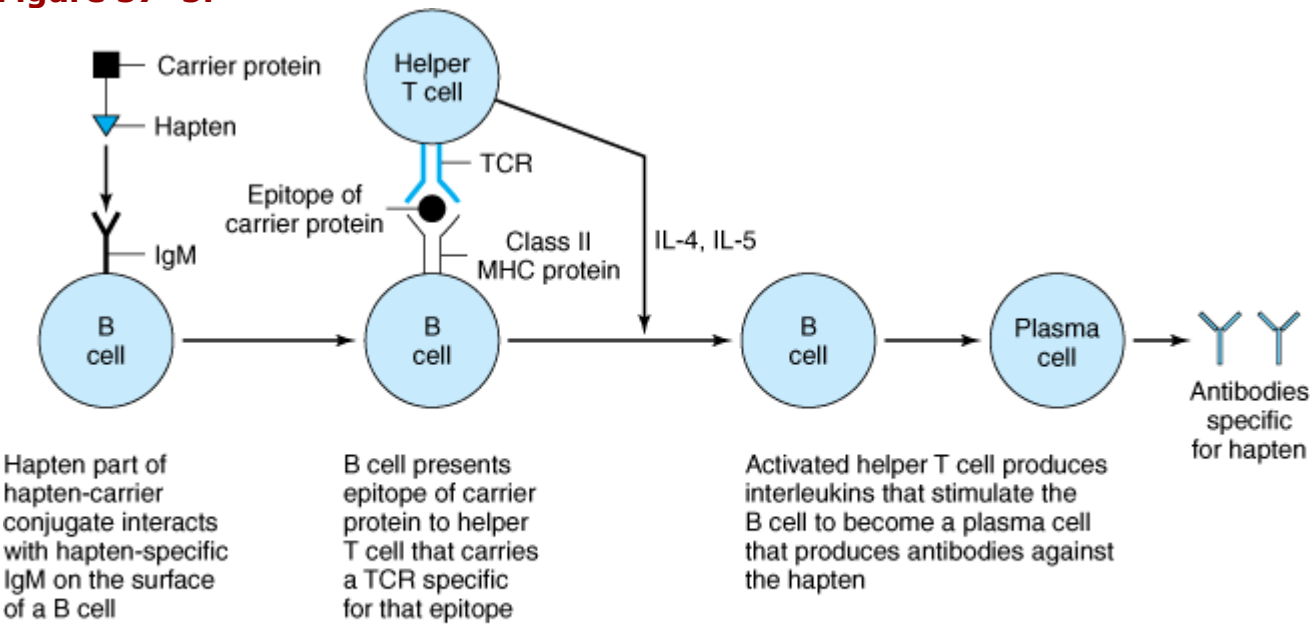
Passive-active immunity involves giving both preformed antibodies (immune globulins) to provide immediate protection and a vaccine to provide long-term protection. These preparations should be given at different sites in the body to prevent the antibodies from neutralizing the immunogens in the vaccine. This approach is used in the prevention of tetanus (see Chapters 12 and 17), rabies (see Chapters 36 and 39), and hepatitis B (see Chapters 36 and 41).

ANTIGENS

Antigens are molecules that react with antibodies, whereas immunogens are molecules that induce an immune response. In most cases, antigens are immunogens, and the terms are used interchangeably. However, there are certain important exceptions, e.g., haptens. A **hapten** is a molecule that is not immunogenic by itself but can react with specific antibody. Haptens are usually small molecules, but some high-molecular-weight nucleic acids are haptens as well. Many drugs, e.g., penicillins, are haptens, and the catechol in the plant oil that causes poison oak and poison ivy is a hapten.

Haptens are not immunogenic because they cannot activate helper T cells. The failure of haptens to activate is due to their inability to bind to MHC proteins; they cannot bind because they are not polypeptides and only polypeptides can be presented by MHC proteins. Furthermore, haptens are univalent and therefore cannot activate B cells by themselves. (Compare to the T-independent response of multivalent antigens in Chapter 58.) Although haptens cannot stimulate a primary or secondary response by themselves, they can do so when covalently bound to a "carrier" protein (Figure 57–5). In this process, the hapten interacts with an IgM receptor on the B cell and the hapten-carrier protein complex is internalized. A peptide of the carrier protein is presented in association with class II MHC protein to the helper T cells. The activated helper T cell then produces interleukins, which stimulate the B cells to produce antibody to the hapten (see Activation of T Cells, for additional information).

Figure 57–5.



Source: Levinson W: *Review of Medical Microbiology and Immunology*, 10th Edition: http://www.accessmedicine.com

Hapten-carrier conjugate induces antibody against the hapten. A hapten covalently bound to a carrier protein can induce antibody to a hapten by the mechanism depicted in the figure. A hapten alone cannot induce antibody, because the helper T cells are not activated by the hapten. Although the hapten alone (without the carrier protein) can bind to the IgM receptor on the B-cell surface, the interleukins essential for the B cell to become a plasma cell are not made.

Two additional ideas are needed to understand how haptens interact with our immune system. The first is that many haptens, such as drugs (e.g., penicillin) and poison oak oil, bind to our normal proteins, to which we are tolerant. The hapten-protein combination now becomes immunogenic; i.e., the hapten modifies the protein sufficiently such that when the hapten-peptide combination is presented by the MHC protein, it is recognized as foreign.

The second idea is that although most haptens are univalent, type I hypersensitivity reactions such as anaphylaxis (see Chapter 65) require cross-linking of adjacent IgEs to trigger the release of the mediators. By itself, a univalent hapten cannot cross-link, but when many hapten molecules are bound to the carrier protein, they are arranged in such a way that cross-linking can occur. This is how a univalent hapten, such as penicillin, causes anaphylaxis. Sufficient penicillin binds to one of our proteins to cross-link IgE. An excellent example of this is penicilloyl polylysine, which is used in skin tests to determine whether a patient is allergic to penicillin. Each lysine in the polylysine has a penicillin molecule attached to it. These univalent

penicillin molecules form a "multivalent" array and can cross-link adjacent IgEs on the surface of mast cells. The consequent release of mediators causes a "wheal and flare" reaction in the skin of the penicillin-allergic patient.

The interaction of antigen and antibody is highly specific, and this characteristic is frequently used in the diagnostic laboratory to identify microorganisms. Antigen and antibody bind by **weak forces** such as hydrogen bonds and van der Waals' forces rather than by covalent bonds. The strength of the binding (the affinity) is proportionate to the fit of the antigen with its antibody-combining site, i.e., its ability to form more of these bonds. The affinity of antibodies increases with successive exposures to the specific antigen (see Chapter 60). Another term, avidity, is also used to express certain aspects of binding. It need not concern us here.

The features of molecules that determine **immunogenicity** are as follows.

FOREIGNNESS

In general, molecules recognized as "self" are not immunogenic; i.e., we are tolerant to those self-molecules (see Chapter 66). To be immunogenic, molecules must be recognized as "nonself," i.e., foreign.

MOLECULAR SIZE

The most potent immunogens are proteins with high molecular weights, i.e., above 100,000. Generally, molecules with molecular weight below 10,000 are weakly immunogenic, and very small ones, e.g., an amino acid, are nonimmunogenic. Certain small molecules, e.g., haptens, become immunogenic only when linked to a carrier protein.

CHEMICAL-STRUCTURAL COMPLEXITY

A certain amount of chemical complexity is required; e.g., amino acid homopolymers are less immunogenic than heteropolymers containing two or three different amino acids.

ANTIGENIC DETERMINANTS (EPITOPES)

Epitopes are small chemical groups on the antigen molecule that can elicit and react with antibody. An antigen can have one or more determinants (epitopes). Most antigens have many determinants; i.e., they are multivalent. In general, a determinant is roughly five amino acids or sugars in size. The overall three-dimensional structure is the main criterion of antigenic specificity.

DOSAGE, ROUTE, AND TIMING OF ANTIGEN ADMINISTRATION

These factors also affect immunogenicity. In addition, the genetic constitution of the host (HLA genes) determines whether a molecule is immunogenic. Different strains of the same species of animal may respond differently to the same antigen.

ADJUVANTS

Adjuvants enhance the immune response to an immunogen. They are chemically unrelated to the immunogen and differ from a carrier protein because the adjuvant is not covalently bound to the immunogen, whereas the carrier protein is. Adjuvants can act in a variety of ways: cause slow release of immunogen, thereby prolonging the stimulus; enhance uptake of immunogen by antigen-presenting cells; and induce costimulatory molecules ("second signals"). (See Chapter 58 regarding costimulators.) Another important mechanism of action of some adjuvants is to stimulate Toll-like receptors (see Innate Immunity and CD4 & CD8 Types of T Cells) on the surface of macrophages, which results in cytokine production that enhances the response of T cells and B cells to the immunogen (antigen). Some human vaccines contain adjuvants such as aluminum hydroxide or lipids.

AGE & THE IMMUNE RESPONSE

Immunity is **less than optimal** at both ends of life, i.e., in the **newborn** and the **elderly.** The reason for the relatively poor immune response in newborns is unclear, but newborns appear to have less effective T-cell function than do adults. In newborns, antibodies are provided primarily by the transfer of maternal IgG across the placenta. Because maternal antibody decays over time (little remains by 3–6 months of age), the risk of infection in the child is high. Colostrum also contains antibodies, especially secretory IgA, which can protect the newborn against various respiratory and intestinal infections.

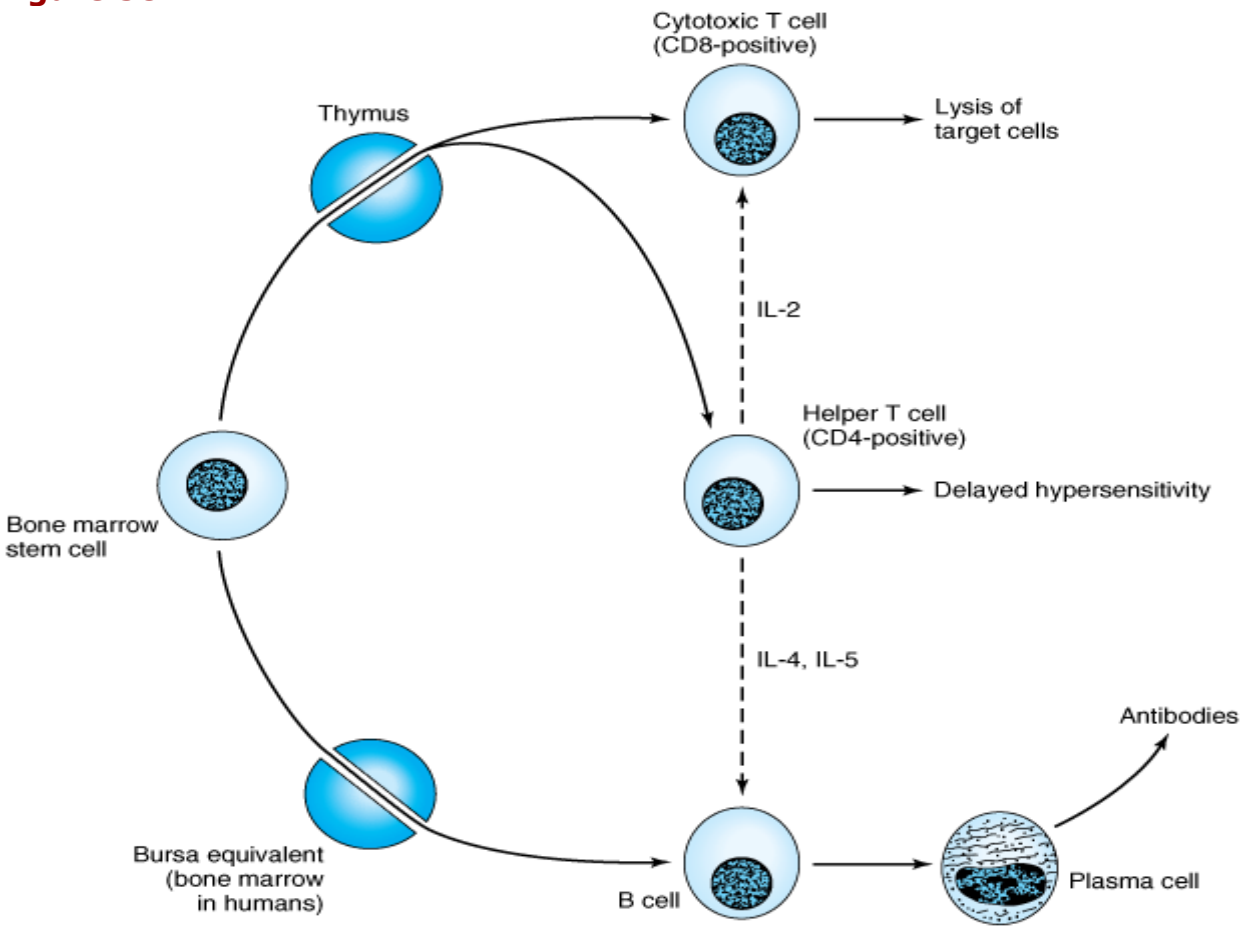
The fetus can mount an IgM response to certain (probably T-cell-independent) antigens, e.g., to *Treponema pallidum,* the cause of syphilis, which can be acquired congenitally. IgG and IgA begin to be made shortly after birth. The response to protein antigens is usually good; hence hepatitis B vaccine can be given at birth and poliovirus immunization can begin at 2 months of age. However, young children respond poorly to polysaccharide antigens unless they are conjugated to a carrier protein. For example, the pneumococcal vaccine containing the unconjugated polysaccharides does not induce protective immunity when given prior to 18 months of age, but the pneumococcal vaccine containing the polysaccharides conjugated to a carrier protein is effective when given as early as 2 months of age.

In the elderly, immunity generally declines. There is a reduced IgG response to certain antigens, fewer T cells, and a reduced delayed hypersensitivity response. As in the very young, the frequency and severity of infections are high. The frequency of autoimmune diseases is also high in the elderly, possibly because of a decline in the number of regulatory T cells, which allows autoreactive T cells to proliferate and cause disease.

ORIGIN OF IMMUNE CELLS

The capability of responding to immunologic stimuli rests mainly with lymphoid cells. During embryonic development, blood cell precursors originate mainly in the fetal liver and yolk sac; in postnatal life, the stem cells reside in the bone marrow. Stem cells differentiate into cells of the erythroid, myeloid, or lymphoid series. The latter evolve into two main lymphocyte populations: **T cells** and **B cells** (Figure 58–1 and Table 58–1). The ratio of T cells to B cells is approximately 3:1. Figure 58–1 describes the origin of B cells and the two types of T cells: helper T cells and cytotoxic T cells. Table 58–1 compares various important features of B cells and T cells. These features will be described in detail later in the chapter.

Figure 58–1.



Source: Levinson W: *Review of Medical Microbiology and Immunology*, 10th Edition: http://www.accessmedicine.com

Origin of T and B cells. Stem cells in the bone marrow (or fetal liver) are the precursors of both T and B lymphocytes. Stem cells differentiate into T cells in the thymus, whereas they differentiate into B cells in the bone marrow. Within the thymus, T cells become either CD4-positive (helper) cells or CD8-positive (cytotoxic) cells. B cells can differentiate into plasma cells that produce large amounts of antibodies (immunoglobulins). Dotted lines indicate interactions mediated by interleukins. (Modified and reproduced, with permission, from Brooks GF et al: *Medical Microbiology,* 20th ed. Originally published by Appleton & Lange.

Table 58–1. Comparison of T Cells and B Cells.

Feature	T Cells	B Cells
Antigen receptors on surface	Yes	Yes
Antigen receptor recognizes only processed peptides in association with MHC protein	Yes	No
Antigen receptor recognizes whole, unprocessed proteins and has no requirement for presentation by MHC protein	No	Yes
IgM on surface	No	Yes
CD3 proteins on surface	Yes	No
Clonal expansion after contact with specific antigen	Yes	Yes
Immunoglobulin synthesis	No	Yes
Regulator of antibody synthesis	Yes	No
IL-2, IL-4, IL-5, and gamma interferon synthesis	Yes	No
Effector of cell-mediated immunity	Yes	No
Maturation in thymus	Yes	No
Maturation in bursa or its equivalent	No	Yes

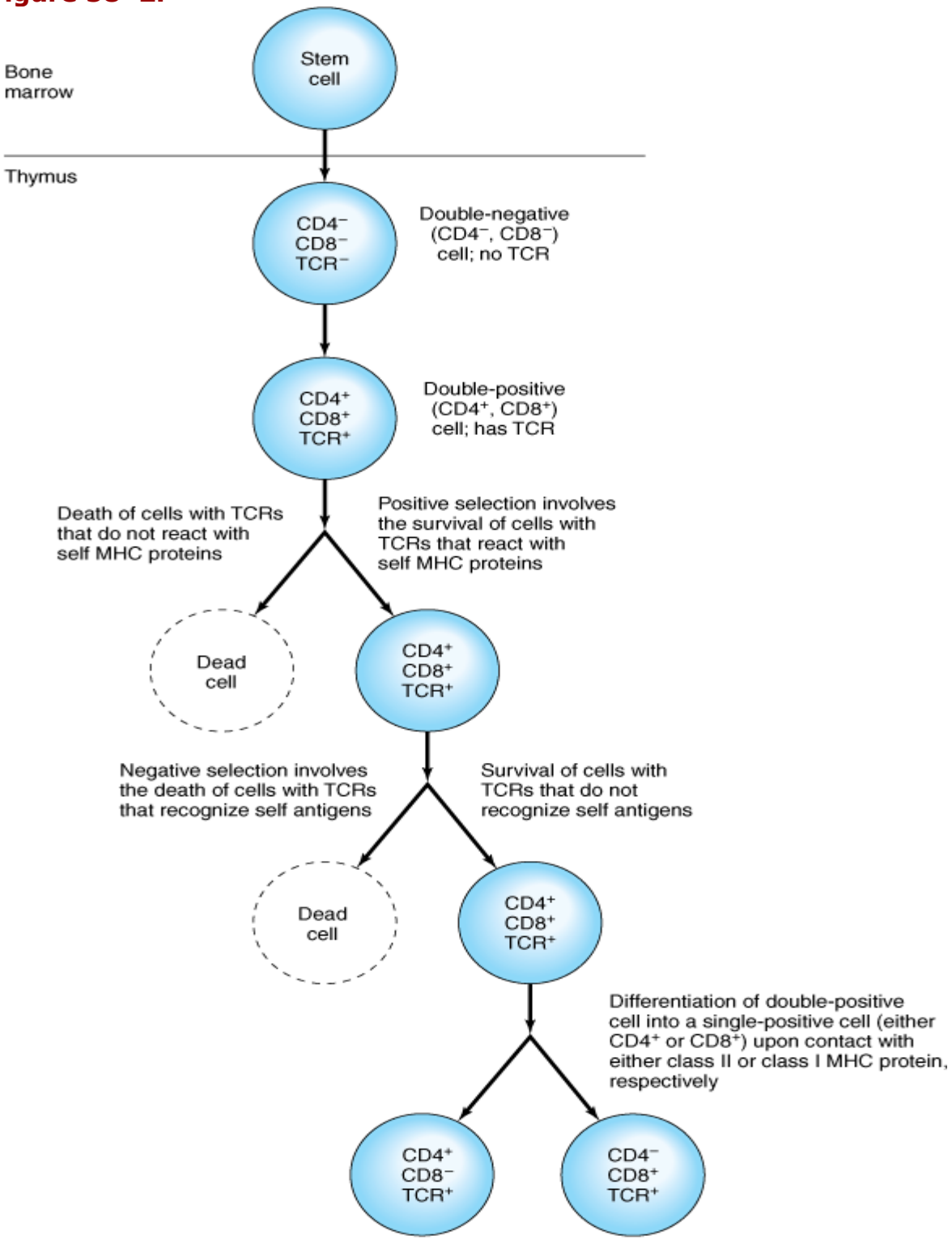
T-cell precursors differentiate into immunocompetent T cells within the thymus. Prior to entering the thymus, stem cells lack antigen receptors and lack CD3, CD4, and CD8 proteins on their surface. During passage through the thymus they differentiate

into T cells that can express both antigen receptors and the various CD proteins. The stem cells, which initially express neither CD4 nor CD8 (double-negatives), first differentiate to express both CD4 and CD8 (double-positives) and then proceed to express **either** CD4 or CD8. A double-positive cell will differentiate into a CD4-positive cell if it contacts a cell bearing class II MHC proteins but will differentiate into a CD8-positive cell if it contacts a cell bearing class I MHC proteins. (Mutant mice that do not make class II MHC proteins will not make CD4-positive cells, indicating that this interaction is required for differentiation into single-positive cells to occur.) The double-negative cells and the double-positive cells are located in the cortex of the thymus, whereas the single-positive cells are located in the medulla, from which they migrate out of the thymus into the blood and extrathymic tissue.

Within the thymus, two very important processes called **thymic education** occur.

1. CD4-positive, CD8-positive cells bearing antigen receptors for "self" proteins are killed (**clonal deletion**) by a process of "programmed cell death" called **apoptosis** (Figure 58–2). The removal of these self-reactive cells, a process called **negative selection,** results in **tolerance** to our own proteins, i.e., self-tolerance, and prevents autoimmune reactions (see Chapter 66).
2. CD4-positive, CD8-positive cells bearing antigen receptors that do not react with self MHC proteins (see the section on activation, Activation of T Cells) are also killed. This results in a **positive selection** for T cells that react well with self MHC proteins.

Figure 58–2.



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Development of T cells. Note the positive and negative selection that occurs in the thymus.

These two processes produce T cells that are selected for their ability to react both with foreign antigens via their antigen receptors and with self MHC proteins. Both of these features are required for an effective immune response by T cells.

Note that MHC proteins perform two essential functions in the immune response; one is the **positive selection** of T cells in the thymus, as just mentioned, and the other, which is described below, is the **presentation of antigens** to T cells, the initial step required to activate those cells. MHC proteins are also the most important antigens recognized in the graft rejection process (see Chapter 62).

During their passage through the thymus, each double-positive T cell synthesizes a different, highly specific antigen receptor called the **T-cell receptor (TCR).** The rearrangement of the variable, diversity, and joining genes (see Chapter 59) that encode the receptor occurs early in T-cell differentiation and accounts for the remarkable ability of T cells to recognize millions of different antigens.

Some T lymphocytes, perhaps as much as 40% of the total, do not develop in the thymus but rather in the "gut-associated lymphoid tissue" (GALT). These intraepithelial lymphocytes (IELs) are thought to provide protection against intestinal pathogens. Their antigen receptors and surface proteins are different from those of thymus-derived lymphocytes. IELs cannot substitute for thymus-derived lymphocytes because patients with DiGeorge's syndrome who lack a thymus (see Chapter 68) are profoundly immunodeficient and have multiple infections.

The thymus involutes in adults, yet T cells continue to be made. Two explanations have been offered for this apparent paradox. One is that a remnant of the thymus remains functional throughout life and the other is that an extrathymic site takes over for the involuted thymus. Individuals who have had their thymus removed still make T cells, which supports the latter explanation.

B-cell precursors differentiate into immunocompetent B cells in the bone marrow; they do not pass through the thymus. B cells also undergo clonal deletion of those cells bearing antigen receptors for self proteins, a process that induces tolerance and reduces the occurrence of autoimmune diseases (see Chapter 66). The site of clonal deletion of B cells is uncertain, but it is not the thymus.

Natural killer (NK) cells are large granular lymphocytes that do not pass through the thymus, do not have an antigen receptor, and do not bear CD4 or CD8 proteins. They recognize and kill target cells, such as virus-infected cells and tumor cells, without the requirement that the antigens be presented in association with class I or class II MHC proteins. Rather, NK cells target those cells to be killed by detecting that they do *not* display class I MHC proteins on the cell surface. This detection process is effective because many cells lose their ability to synthesize class I MHC proteins after they have been infected by a virus (see Natural Killer Cells).

In contrast to T cells, B cells, and NK cells, which differentiate from lymphoid stem cells, macrophages arise from myeloid precursors. Macrophages have two important functions, namely, phagocytosis and antigen presentation. They do not pass through the thymus and do not have an antigen receptor. On their surface, they display class II MHC proteins, which play an essential role in antigen presentation to helper T cells. Macrophages also display class I MHC proteins, as do all nucleated cells. The cell surface proteins that play an important role in the immune response are listed in Table 58–2.

Table 58–2. Cell Surface Proteins that Play an Important Role in the Immune Response.[1]

Type of Cells	Surface Proteins
Helper T cells	CD4, TCR,[2] CD28
Cytotoxic T cells	CD8, TCR
B cells	IgM, B7
Macrophages[3]	Class II MHC
Natural killer cells	Receptors for class I MHC
All cells other than mature red cells[4]	Class I MHC

[1]There are many other cell surface proteins that play a role in the immune response, but the proteins listed in this table are the most important for understanding the fundamental aspects of this response.

[2]TCR, T-cell antigen receptor.

[3]Macrophages and other "antigen-presenting cells."

[4]Mature red blood cells do not synthesize class I MHC proteins because they do not have a functioning nucleus.

T CELLS

T cells perform several important functions, which can be divided into two main categories, namely, **regulatory** and **effector.** The regulatory functions are mediated primarily by **helper** (CD4-positive) T cells, which produce **interleukins** (Table 58–3). For example, helper T cells make (1) interleukin-4 (IL-4) and IL-5, which help B cells produce antibodies; (2) IL-2, which activates CD4 and CD8 cells; and (3) gamma interferon, which activates macrophages, the main mediators of delayed hypersensitivity against intracellular organisms such as *Mycobacterium tuberculosis.* (Suppressor T cells are postulated to downregulate the immune response, but the precise action of these cells is uncertain.) The effector functions are carried out primarily by cytotoxic (CD8-positive) T cells, which kill virus-infected cells, tumor cells, and allografts.

Table 58–3. Main Functions of Helper T Cells.

Main Functions	Cytokine That Mediates That Function
Activates the antigen-specific helper T cell to produce a clone of these cells	IL-2
Activates cytotoxic T cells	IL-2
Activates B cells	IL-4 and IL-5
Activates macrophages	Gamma interferon

CD4 & CD8 Types of T Cells

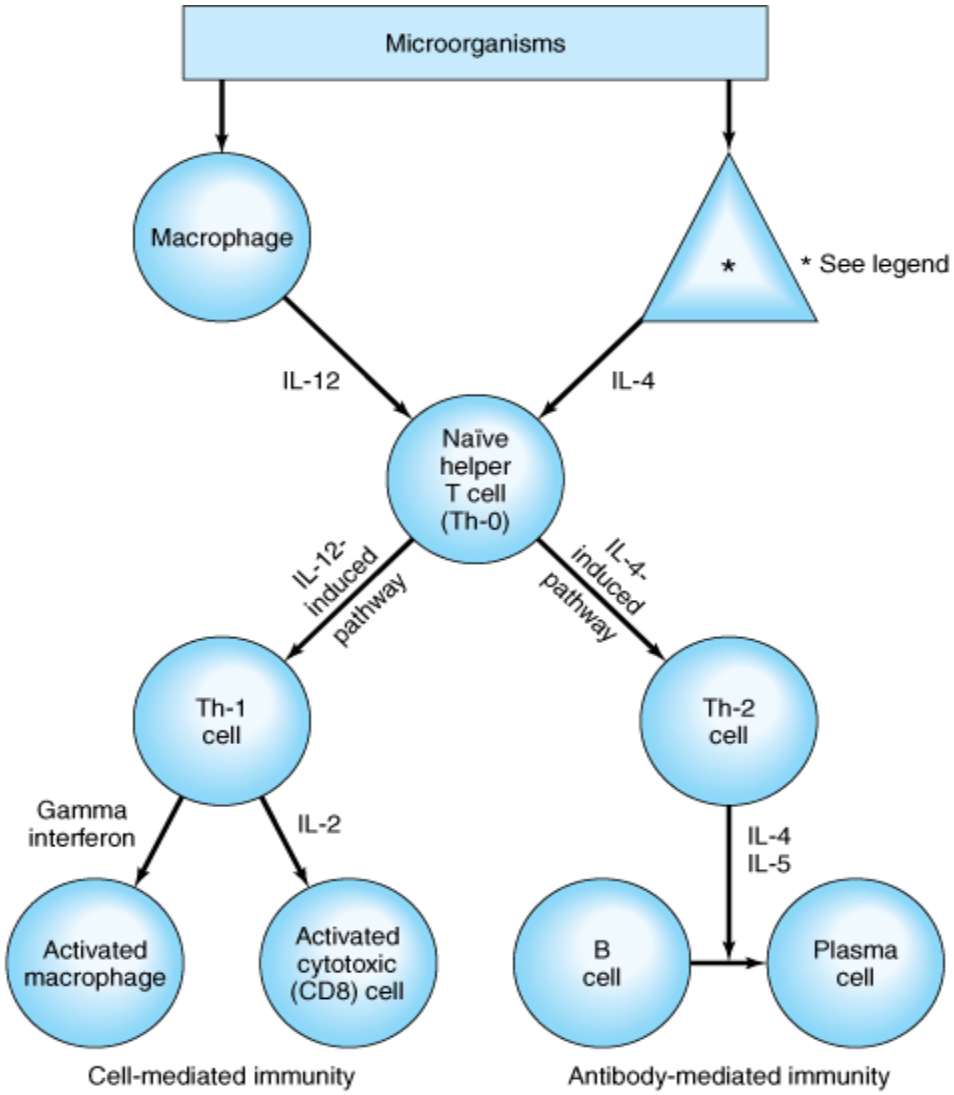
Within the thymus, perhaps within the outer cortical epithelial cells (nurse cells), T-cell progenitors differentiate under the influence of thymic hormones (thymosins and thymopoietins) into T-cell subpopulations. These cells are characterized by certain surface glycoproteins, e.g., CD3, CD4, and CD8. **All T cells have CD3** proteins on their surface in association with antigen receptors (T-cell receptor, TCR [see below]). The CD3 complex of five transmembrane proteins is involved with transmitting, from the outside of the cell to the inside, the information that the **antigen receptor is occupied.** One of the CD3 transmembrane proteins, the zeta chain, is linked to a tyrosine kinase called *fyn,* which is involved with signal transduction. The signal is transmitted via several second messengers, which are described in the section on activation (see below). CD4 is a single transmembrane polypeptide, whereas CD8

consists of two transmembrane polypeptides. They may signal via tyrosine kinase (the *lck* kinase) also.

T cells are subdivided into two major categories on the basis of whether they have CD4 or CD8 proteins on their surface. Mature T cells have either CD4 or CD8 proteins but not both.

CD4 lymphocytes perform the following **helper** functions: (1) they help B cells develop into antibody-producing plasma cells; (2) they help CD8 T cells to become activated cytotoxic T cells; and (3) they help macrophages effect delayed hypersensitivity (e.g., limit infection by *M. tuberculosis*). These functions are performed by two subpopulations of CD4 cells: **Th-1 cells** help activate cytotoxic T cells by producing IL-2 and help initiate the delayed hypersensitivity response by producing primarily IL-2 and gamma interferon, whereas **Th-2 cells** perform the B-cell helper function by producing primarily IL-4 and IL-5 (Figure 58–3). One important regulator of the balance between Th-1 cells and Th-2 cells is interleukin-12 (IL-12), which is produced by macrophages. IL-12 increases the number of Th-1 cells, thereby enhancing host defenses against organisms that are controlled by a delayed hypersensitivity response (Table 58–4). Another important regulator is gamma interferon, which inhibits the production of Th-2 cells. CD4 cells make up about 65% of peripheral T cells and predominate in the thymic medulla, tonsils, and blood.

Figure 58–3.



Source: Levinson W: *Review of Medical Microbiology and Immunology*, 10th Edition: http://www.accessmedicine.com

The origin of Th-1 and Th-2 cells. On the **left** side, the origin of Th-1 cells is depicted. Microorganisms are ingested by macrophages and IL-12 is produced. IL-12 induces naïve Th-0 cells to become Th-1 cells that produce gamma interferon and IL-2. These interleukins activate macrophages and cytotoxic T cells, respectively, and cell-mediated immunity occurs. On the **right** side, the origin of Th-2 cells is depicted. Microorganisms are ingested by an unknown type of cell (see footnote below) and IL-4 is produced. IL-4 induces naïve Th-0 cells to become Th-2 cells that produce IL-4 and IL-5. These interleukins activate B cells to become plasma cells and antibodies are produced. Not shown in the figure is an important regulatory step, namely, that IL-10 produced by Th-2 cells inhibits IL-12 production by macrophages and drives the system toward an antibody response and away from a cell-mediated response.

* The human cell that produces the IL-4, which induces naïve helper T cells to become Th-2 cells, has not been identified.

Table 58–4. Comparison of Th-1 Cells and Th-2 Cells.

Property	Th-1 Cells	Th-2 Cells
Produces IL-2 and gamma interferon	Yes	No
Produces IL-4, IL-5, IL-6, and IL-10	No	Yes
Enhances cell-mediated immunity and delayed hypersensitivity primarily	Yes	No
Enhances antibody production primarily	No	Yes
Stimulated by IL-12	Yes	No
Stimulated by IL-4	No	Yes

To mount a protective immune response against a specific microbe requires that the appropriate subpopulation, i.e., either Th-1 or Th-2 cells, play a dominant role in the response. For example, if an individual is infected with *M. tuberculosis* and Th-2 cells are the major responders, then humoral immunity will be stimulated rather than cell-mediated immunity. Humoral immunity is not protective against *M. tuberculosis* and the patient will suffer severe tuberculosis. Similarly, if an individual is infected with *Streptococcus pneumoniae* and Th-1 cells are the major responders, then humoral immunity will be not be stimulated and the patient will have severe pneumococcal disease. Precisely what component of a microbe activates either Th-1 or Th-2 cells is unknown.

How the appropriate response is stimulated is known for one medically important organism, namely, *M. tuberculosis.* A lipoprotein of that bacterium interacts with a specific "toll-like receptor" on the surface of the macrophage, which induces the production of IL-12 by the macrophage. IL-12 drives the differentiation of naïve helper T cells to form the Th-1 type of helper T cells that are required to mount a cell-mediated (delayed hypersensitivity) response against the organism.

CD8 lymphocytes perform cytotoxic functions; that is, they kill virus-infected, tumor, and allograft cells. They kill by either of two mechanisms, namely, the release of perforins, which destroy cell membranes, or the induction of programmed cell death (apoptosis). CD8 cells predominate in human bone marrow and gut lymphoid tissue and constitute about 35% of peripheral T cells.

Activation of T Cells

The activation of **helper T cells** requires that their TCR recognize a complex on the surface of antigen-presenting cells (APCs), e.g., macrophages and dendritic cells,[1] consisting of **both the antigen and a class II MHC protein.**

What follows is a description of this activation process beginning with the ingestion of the foreign protein (or microbe) into the APC. Within the cytoplasm of the macrophage, the foreign protein is cleaved into small peptides that associate with the class II MHC proteins. The complex is transported to the surface of the macrophage, where the antigen, in association with a class II MHC protein, is presented to the receptor on the CD4-positive helper cell. Similar events occur within a virus-infected cell, except that the cleaved viral peptide associates with a **class I** rather than a class II MHC protein. The complex is transported to the surface, where the viral antigen is presented to the receptor on a **CD8-positive cytotoxic cell.** Remember the rule of eight: CD4 cells interact with class II ($4 \times 2 = 8$), and CD8 cells interact with class I ($8 \times 1 = 8$).

There are many different alleles within the class I and class II MHC genes; hence, there are many different MHC proteins. These various MHC proteins bind to different peptide fragments. The polymorphism of the MHC genes and the proteins they encode are a means of presenting many different antigens to the T-cell receptor. Note that class I and class II MHC proteins can *only* present peptides; other types of molecules do not bind and therefore cannot be presented. Note also that MHC proteins present peptides derived from self proteins as well as from foreign proteins; therefore whether an immune response occurs is determined by whether a T cell bearing a receptor specific for that peptide has survived the positive and negative selection processes in the thymus.

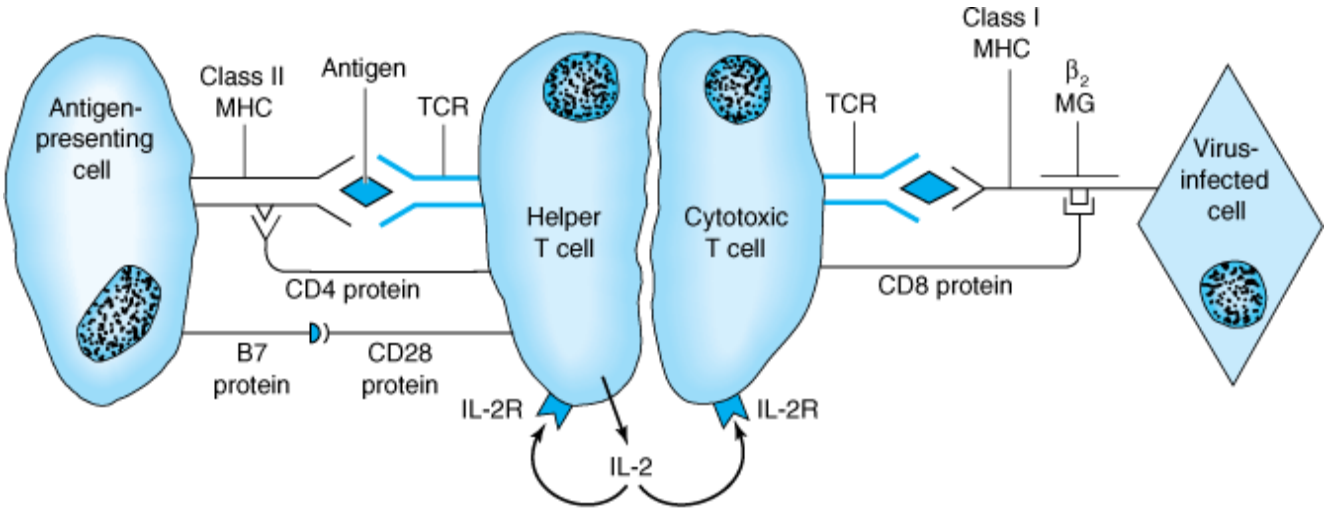
[1]Macrophages and dendritic cells are the most important antigen-presenting cells, but B cells and Langerhans' cells on the skin also present antigen, i.e., have class II proteins on their surface. An essential first step for certain antigen-presenting cells, e.g., Langerhans' cells in the skin, is migration from the site of the skin infection to the local lymphoid tissue, where helper T cells are encountered.

COSTIMULATION IS REQUIRED TO ACTIVATE T CELLS

Two signals are required to activate T cells. The first signal in the activation process is the interaction of the antigen and the MHC protein with the T-cell receptor specific for that antigen (Figure 58–4). **IL-1** produced by the macrophage is also necessary for efficient helper T-cell activation. Note that when the T-cell receptor interacts with the antigen-MHC protein complex, the CD4 protein on the surface of the helper T cell also interacts with the class II MHC protein. In addition to the binding of the CD4 protein with the MHC class II protein, other proteins interact to

help stabilize the contact between the T cell and the APC; e.g., LFA-1 protein[2] on T cells (both CD4-positive and CD8-positive) binds to ICAM-1 protein[2] on APCs.

Figure 58–4.



Source: Levinson W: *Review of Medical Microbiology and Immunology*, 10th Edition: http://www.accessmedicine.com

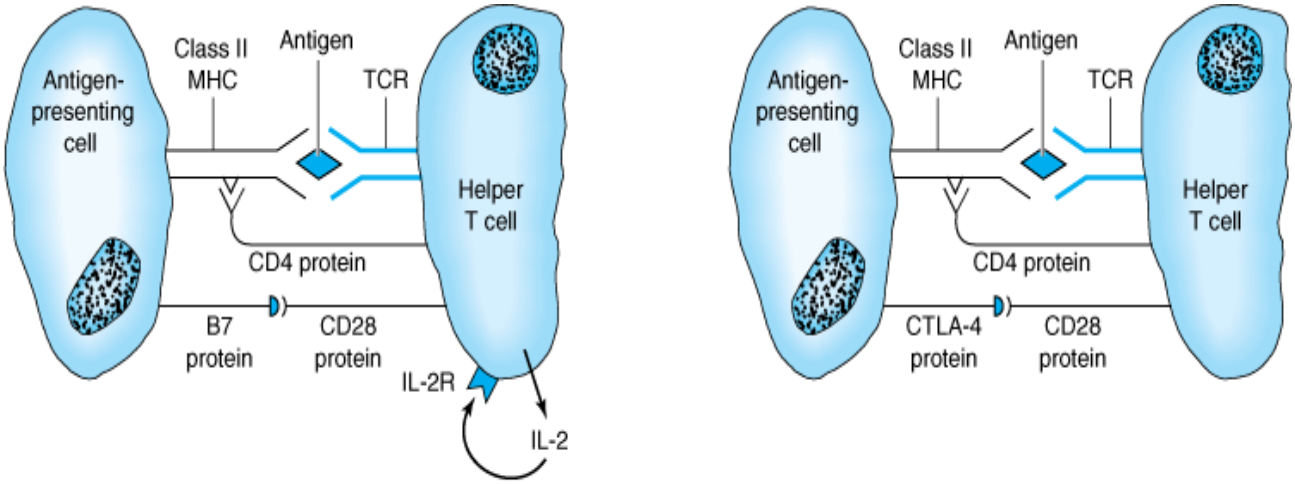
Activation of T cells. **Left:** An antigen-presenting cell (APC) presents processed antigen in association with a class II MHC protein. The antigen is recognized by the T-cell receptor (TCR) specific for that antigen, and the helper T cell is activated to produce interleukin-2 (IL-2). IL-2 binds to its receptor on the helper T cell and further activates it. Note that CD4 protein on the helper T cell binds to the MHC class II protein on the APC, which stabilizes the interaction between the two cells, and that B7 on the APC must interact with CD28 on the helper T cell for full activation of helper T cells to occur. **Right:** A virus-infected cell presents viral antigen in association with class I MHC protein. The viral antigen is recognized by the TCR specific for that antigen and, in conjunction with IL-2 produced by the helper T cell, the cytotoxic T cell is activated to kill the virus-infected cell. The CD8 protein on the cytotoxic T cell binds to the class I protein on the virus-infected cell, which stabilizes the interaction between the two cells. Note that the class II MHC protein consists of two polypeptides, both of which are encoded by genes in the HLA locus. The class I protein, in contrast, consists of one polypeptide encoded by the HLA locus and β_2-microglobulin (β_2 MG), which is encoded elsewhere.

A second **costimulatory signal** is also required; that is, B7 protein on the APC must interact with CD28 protein on the helper T cell (Figure 58–4). If the costimulatory signal occurs, IL-2 is made by the helper T cell, and it is this step that is crucial to producing a helper T cell capable of performing its regulatory, effector, and memory functions. If, on the other hand, the T-cell receptor interacts with its antigen (epitope) and the costimulatory signal does not occur, a state of unresponsiveness called **anergy** ensues (see Chapter 66). The anergic state is specific for that epitope.

Other helper T cells specific for other epitopes are not affected. Production of the costimulatory protein depends on activation of the "toll-like receptor" on the APC surface. Foreign antigens, such as bacterial proteins, induce B7 protein, whereas self antigens do not.

After the T cell has been activated, a different protein called CTLA-4 (cytotoxic T lymphocyte antigen-4) appears on the T-cell surface and binds to B7 by displacing CD28. The **interaction of B7 with CTLA-4 inhibits T-cell activation** by blocking IL-2 synthesis (Figure 58–5). This restores the activated T cell to a quiescent state and thereby plays an important role in T-cell homeostasis. Mutant T cells that lack CTLA-4 and therefore cannot be deactivated participate with increased frequency in autoimmune reactions. Furthermore, administration of CTLA-4 reduced the rejection of organ transplants in experimental animals.

Figure 58–5.



Source: Levinson W: *Review of Medical Microbiology and Immunology*, 10th Edition: http://www.accessmedicine.com

Inhibition of activated helper T cells. When the activated helper T cells are no longer needed, a return to a quiescent state occurs when an inhibitory protein called CTL-4 is displayed on the surface of the antigen-presenting cell. CTL-4 binds more strongly to CD28 than does B7 and so displaces B7 from its interaction with CD28. This inhibits the synthesis of IL-2 and the T cell enters a resting state. **Left:** Activation of the helper T cells occurs because B7 protein is displayed on the surface of the antigen-presenting cell and interacts with CD28 on the helper T cell. (This is the same process as that depicted on the left side of Figure 58-4.) **Right:** CTL-4 protein is displayed on the surface of the antigen-presenting cell and interacts with CD28 on the helper T cell. As a result, IL-2 is no longer synthesized. (Modified and reproduced, with permission, from Pantaleo G et al: Mechanisms of Disease: The Immunopathogenesis of Human Immunodeficiency Virus Infection. *NEJM* 1993;328:327. Copyright © 1993 Massachusetts Medical Society. All rights reserved.)

The clinical importance of CTLA-4 is dramatically illustrated by the effectiveness of abatacept (Orencia) in rheumatoid arthritis. Abatacept is CTLA-4-IG, a fusion protein composed of CTLA-4 and a fragment of the Fc domain of human IgG. The Fc fragment provides resistance against degradation, resulting in increased plasma levels of CTLA-4 for a longer duration than CTLA-4 alone. The mechanism of action of abatacept is the binding of CTLA-4 to B7 thereby displacing CD-28 from its binding to B7. This results in a reduction of the helper T cell activity and a reduction in the inflammatory response.

[2]LFA proteins belong to a family of cell surface proteins called integrins, which mediate adhesion to other cells. Integrin proteins are embedded in the surface membrane and have both extracellular and intracellular domains. Hence they interact with other cells externally and with the cytoplasm internally. Abbreviations: LFA, lymphocyte function-associated antigen; ICAM, intercellular adhesion molecule.

T CELLS RECOGNIZE ONLY PEPTIDES

T cells recognize*only*polypeptide antigens. Furthermore, they recognize those polypeptides only when they are presented in association with MHC proteins. Helper T cells recognize antigen in association with class II MHC proteins, whereas cytotoxic T cells recognize antigen in association with class I MHC proteins. This is called **MHC restriction;** i.e., the two types of T cells (CD4 helper and CD8 cytotoxic) are "restricted" because they are able to recognize antigen *only* when the antigen is presented with the proper class of MHC protein. This restriction is mediated by specific binding sites primarily on the T-cell receptor, but also on the CD4 and CD8 proteins that bind to specific regions on the class II and class I MHC proteins, respectively.

Generally speaking, class I MHC proteins present **endogenously synthesized** antigens, e.g., viral proteins, whereas class II MHC proteins present the antigens of **extracellular** microorganisms that have been phagocytized, e.g., bacterial proteins. One important consequence of these observations is that killed viral vaccines do not activate the cytotoxic (CD8-positive) T cells, because the virus does not replicate within cells and therefore viral epitopes are not presented in association with class I MHC proteins. Class I and class II proteins are described in more detail in Chapter 62.

This distinction between endogenously synthesized and extracellularly acquired proteins is achieved by processing the proteins in different compartments within the cytoplasm. The endogenously synthesized proteins, e.g., viral proteins, are cleaved by a proteasome, and the peptide fragments associate with a "TAP transporter" that transports the fragment into the rough endoplasmic reticulum, where it associates with the class I MHC protein. The complex of peptide fragment and class I MHC

protein then migrates via the Golgi apparatus to the cell surface. In contrast, the extracellularly acquired proteins are cleaved to peptide fragments within an endosome, where the fragment associates with class II MHC proteins. This complex then migrates to the cell surface.

An additional protection that prevents endogenously synthesized proteins from associating with class II MHC proteins is the presence of an "invariant chain" that is attached to the class II MHC proteins when these proteins are outside of the endosome. The invariant chain is degraded by proteases within the endosome, allowing the peptide fragment to attach to the class II MHC proteins only within that compartment.

B cells, on the other hand, can interact directly with antigens via their surface immunoglobulins (IgM and IgD). Antigens do not have to be presented to B cells in association with class II MHC proteins, unlike T cells. Note that B cells can then present the antigen, after internalization and processing, to helper T cells in association with class II MHC proteins located on the surface of the B cells (see the section on B Cells, below). Unlike the antigen receptor on T cells, which recognizes only peptides, the antigen receptors on B cells (IgM and IgD) recognize many different types of molecules, such as peptides, polysaccharides, nucleic acids, and small molecules, e.g., drugs such as penicillin.

These differences between T cells and B cells explain the hapten-carrier relationship described in Chapter 57. To stimulate hapten-specific antibody, the hapten must be covalently bound to the carrier protein. The hapten binds to the IgM receptor on the B-cell surface. That IgM is specific for the hapten, not the carrier protein. The hapten-carrier conjugate is internalized and the carrier protein processed into small peptides that are presented in association with class II MHC proteins to a helper T cell bearing a receptor for that peptide. The helper T cell then secretes lymphokines that activate the B cell to produce antibodies to the hapten.

When the antigen-MHC protein complex on the APC interacts with the T-cell receptor, a signal is transmitted by the CD3 protein complex through several pathways that eventually lead to a large influx of calcium into the cell. (The details of the signal transduction pathway are beyond the scope of this book, but it is known that stimulation of the T-cell receptor activates a series of phosphokinases, which then activate phospholipase C, which cleaves phosphoinositide to produce inositol triphosphate, which opens the calcium channels.) Calcium activates calcineurin, a serine phosphatase. Calcineurin moves to the nucleus and is involved in the activation of the genes for IL-2 and the IL-2 receptor. (Calcineurin function is blocked by cyclosporine, one of the most effective drugs used to prevent rejection of organ transplants [see Chapter 62].)

The end result of this series of events is the activation of the helper T cell to produce various lymphokines, e.g., **IL-2,** as well as the **IL-2 receptor.** IL-2, also known as T-cell growth factor, stimulates the helper T cell to multiply into a clone of antigen-specific helper T cells. Most cells of this clone perform effector and regulatory functions, but some become **memory** cells (see below), which are capable of being rapidly activated upon exposure to antigen at a later time. (Cytotoxic T cells and B cells also form memory cells.) Note that IL-2 stimulates CD8 cytotoxic T cells as well as CD4 helper T cells. Activated CD4-positive T cells also produce another lymphokine called **gamma interferon,** which increases the expression of class II MHC proteins on APCs. This enhances the ability of APCs to present antigen to T cells and upregulates the immune response. (Gamma interferon also enhances the microbicidal activity of macrophages.)

The process of activating T cells does not function as a simple "on–off" switch. The binding of an epitope to the T-cell receptor can result in either full activation, partial activation in which only certain lymphokines are made, or no activation, depending on which of the signal transduction pathways is stimulated by that particular epitope. This important observation may have profound implications for our understanding of how helper T cells shape our response to infectious agents.

There are three genes at the class I locus (A, B, and C) and three genes at the class II locus (DP, DQ, and DR). We inherit one set of class I and one set of class II genes from each parent. Therefore, our cells can express as many as six different class I and six different class II proteins (see Chapter 62). Furthermore, there are multiple alleles at each gene locus. Each of these MHC proteins can present peptides with a different amino acid sequence. This explains, in part, our ability to respond to many different antigens.

Memory T Cells

Memory T (and B) cells, as the name implies, endow our host defenses with the ability to respond rapidly and vigorously for many years after the initial exposure to a microbe or other foreign material. This memory response to a specific antigen is due to several features: (1) many memory cells are produced, so that the secondary response is greater than the primary response, in which very few cells respond; (2) memory cells live for many years or have the capacity to reproduce themselves; (3) memory cells are activated by smaller amounts of antigen and require less costimulation than do naïve, unactivated T cells; and (4) activated memory cells produce greater amounts of interleukins than do naïve T cells when they are first activated.

T-Cell Receptor

The T-cell receptor (TCR) for antigen consists of two polypeptides, alpha and beta,[3] which are associated with CD3 proteins.

TCR polypeptides are similar to immunoglobulin heavy chains in that (1) the genes that code for them are formed by rearrangement of multiple regions of DNA (see Chapter 59); (2) there are V (variable), D (diversity), J (joining), and C (constant) segments that rearrange to provide diversity, giving rise to an estimated number of more than 10^7 different receptor proteins; (3) the variable regions have hypervariable domains; and (4) the two genes (RAG-1 and RAG-2) that encode the recombinase enzymes that catalyze these gene rearrangements are similar in T cells and B cells.

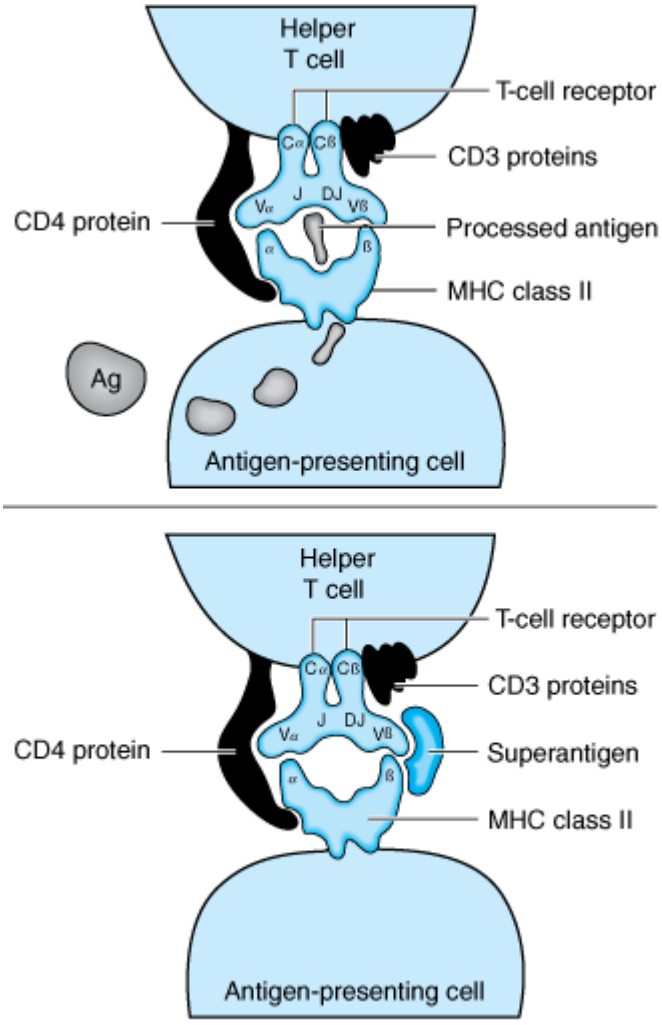
Note that each T cell has a unique T-cell receptor on its surface, which means that hundreds of millions of different T cells exist in each person. Activated T cells, like activated B cells, clonally expand to yield large numbers of cells specific for that antigen.

Although TCRs and immunoglobulins (antibodies) are analogous in that they both interact with antigen in a highly specific manner, the T-cell receptor is different in two important ways: (1) it has two chains rather than four and (2) it recognizes antigen only in conjunction with MHC proteins, whereas immunoglobulins recognize free antigen. Note that the receptor on the surface of B cells (either IgM or IgG) recognizes antigen directly without the need for presentation by MHC proteins. Also TCR proteins are always anchored into the outer membrane of T cells. There is no circulating form as there is with certain antibodies (e.g., monomeric IgM is in the B-cell membrane, but pentameric IgM circulates in the plasma).

Effect of Superantigens on T Cells

Certain proteins, particularly staphylococcal enterotoxins and toxic shock syndrome toxin, act as "superantigens" (Figure 58–6). In contrast to the typical (nonsuper) antigen, which activates one (or a few) helper T cell, superantigens are "super" because they activate a large number of helper T cells. For example, toxic shock syndrome toxin binds directly to class II MHC proteins without internal processing of the toxin. This complex interacts with the variable portion of the beta chain (Vβ) of the T-cell receptor of many T cells.[4]

Figure 58–6.



Source: Levinson W: *Review of Medical Microbiology and Immunology*, 10th Edition: http://www.accessmedicine.com

Activation of helper T cells by superantigen. **Top:** The helper T cell is activated by the presentation of processed antigen in association with class II MHC protein to the antigen-specific portion of the T-cell receptor. Note that superantigen is not involved and that only one or a small number of helper T cells specific for the antigen are activated. **Bottom:** The helper T cell is activated by the binding of superantigen to the Vβ portion of the T-cell receptor outside of its antigen-specific site without being processed by the antigen-presenting cell. Because it bypasses the antigen-specific site, superantigen can activate many helper T cells. (Modified and reproduced, with permission, from Pantaleo G et al: *N Engl J Med* 1993;328:327.)

This activates the T cells, causing the release of IL-2 from the T cells and IL-1 and TNF from macrophages. These interleukins account for many of the findings seen in

toxin-mediated staphylococcal diseases. Certain viral proteins, e.g., those of mouse mammary tumor virus (a retrovirus), also possess superantigen activity.

Features of T Cells

T cells constitute 65–80% of the recirculating pool of small lymphocytes. Within lymph nodes, they are located in the inner, subcortical region, not in the germinal centers. (B cells make up most of the remainder of the pool of small lymphocytes and are found primarily in the germinal centers of lymph nodes.) The life span of T cells is long: months or years. They can be stimulated to divide when exposed to certain mitogens, e.g., phytohemagglutinin or concanavalin A (endotoxin, a lipopolysaccharide found on the surface of gram-negative bacteria, is a mitogen for B cells but not T cells). Most human T cells have receptors for sheep erythrocytes on their surface and can form "rosettes" with them; this finding serves as a means of identifying T cells in a mixed population of cells.

Effector Functions of T Cells

There are two important components of host defenses mediated by T cells: delayed hypersensitivity and cytotoxicity.

DELAYED HYPERSENSITIVITY

Delayed hypersensitivity reactions are produced particularly against antigens of **intracellular microorganisms** including certain fungi, e.g., *Histoplasma* and *Coccidioides,* and certain intracellular bacteria, e.g., *M. tuberculosis.* Delayed hypersensitivity is mediated by **macrophages** and **CD4 cells,** in particular by the Th-1 subset of CD4 cells. Important interleukins for these reactions include gamma interferon, macrophage activation factor, and macrophage migration inhibition factor. CD4 cells produce the interleukins, and macrophages are the ultimate effectors of delayed hypersensitivity. A deficiency of cell-mediated immunity manifests itself as a marked susceptibility to infection by such microorganisms.

In the case of *M. tuberculosis,* a lipoprotein of the bacterium stimulates a specific "toll-like receptor" on the macrophage, which signals the cell to synthesize IL-12. IL-12 then induces naïve helper T cells to differentiate into the Th-1 type of helper T cells that participates in the cell-mediated (delayed hypersensitivity) response.

Th-1 cells produced gamma interferon, which activates macrophages, thereby enhancing their ability to kill *M. tuberculosis.* This "IL-12–gamma interferon axis" is very important in the ability of our host defenses to control infections by intracellular pathogens, such as *M. tuberculosis* and *Listeria monocytogenes.*

CYTOTOXICITY

The **cytotoxic response** is concerned primarily with destroying **virus-infected cells** and **tumor cells** but also plays an important role in **graft rejection.** In

response to virus-infected cells, the CD8 lymphocytes must recognize both viral antigens and class I molecules on the surface of infected cells. To kill the virus-infected cell, the cytotoxic T cell must be activated by IL-2 produced by a helper (CD-4-positive) T cell. To become activated to produce IL-2, helper T cells recognize viral antigens bound to class II molecules on an APC, e.g., a macrophage. The activated helper T cells secrete cytokines such as IL-2, which stimulates the virus-specific cytotoxic T cell to form a clone of activated cytotoxic T cells. These cytotoxic T cells kill the virus-infected cells by inserting **perforins** and degradative enzymes called **granzymes** into the infected cell. Perforins form a channel through the membrane, the cell contents are lost, and the cell dies. Granzymes are proteases that degrade proteins in the cell membrane, which also leads to the loss of cell contents. They also activate caspases that initiate apoptosis, resulting in cell death. After killing the virus-infected cell, the cytotoxic T cell itself is not damaged and can continue to kill other cells infected with the same virus. Cytotoxic T cells have no effect on free virus, only on virus-infected cells.

A third mechanism by which cytotoxic T cells kill target cells is the **Fas-Fas ligand (FasL)** interaction. Fas is a protein displayed on the surface of many cells. When a cytotoxic T-cell receptor recognizes an epitope on the surface of a target cell, FasL is induced in the cytotoxic T cell. When Fas and FasL interact, apoptosis (death) of the target cell occurs. NK cells can also kill target cells by Fas-FasL-induced apoptosis.

In addition to direct killing by cytotoxic T cells, virus-infected cells can be destroyed by a combination of IgG and phagocytic cells. In this process, called **antibody-dependent cellular cytotoxicity (ADCC),** antibody bound to the surface of the infected cell is recognized by IgG receptors on the surface of phagocytic cells, e.g., macrophages or NK cells, and the infected cell is killed. The ADCC process can also kill helminths (worms). In this case, IgE is the antibody involved and eosinophils are the effector cells. IgE binds to surface proteins on the worm, and the surface of eosinophils displays receptors for the epsilon heavy chain. The major basic protein located in the granules of the eosinophils is released and damages the surface of the worm.

Many tumor cells develop new antigens on their surface. These antigens bound to class I proteins are recognized by cytotoxic T cells, which are stimulated to proliferate by IL-2. The resultant clone of cytotoxic T cells can kill the tumor cells, a phenomenon called **immune surveillance.**

In response to allografts, cytotoxic (CD8) cells recognize the class I MHC molecules on the surface of the foreign cells. Helper (CD4) cells recognize the foreign class II molecules on certain cells in the graft, e.g., macrophages and lymphocytes. The activated helper cells secrete IL-2, which stimulates the cytotoxic cell to form a clone of cells. These cytotoxic cells kill the cells in the allograft.

Regulatory Functions of T Cells

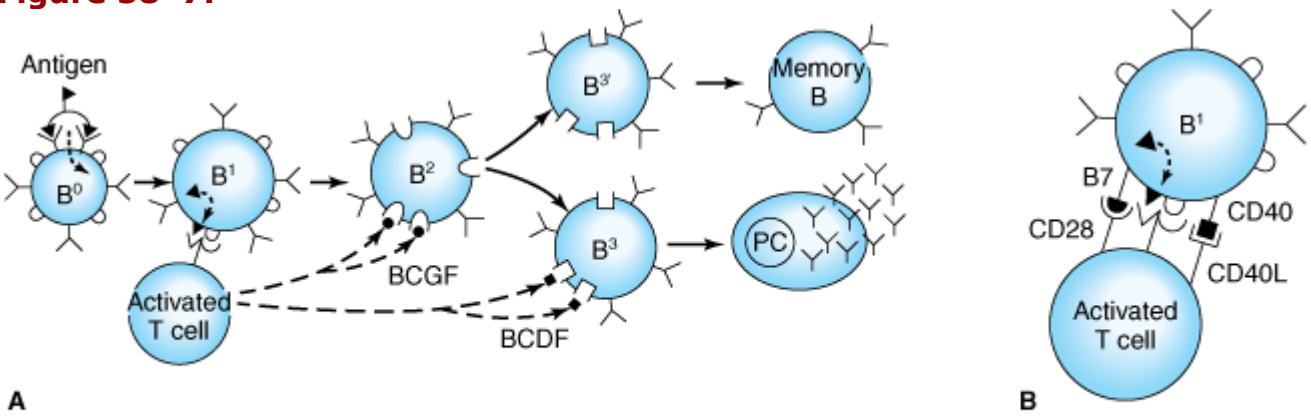
T cells play a central role in regulating both the humoral (antibody) and cell-mediated arms of the immune system.

ANTIBODY PRODUCTION

Antibody production by B cells usually requires the participation of helper T cells (**T-cell-dependent response**), but antibodies to some antigens, e.g., polymerized (multivalent) macromolecules such as bacterial capsular polysaccharide, are **T-cell-independent.** These polysaccharides are long chains consisting of repeated subunits of several sugars. The **repeated subunits act as a multivalent antigen** that cross-links the IgM antigen receptors on the B cell and activates it in the absence of help from CD4 cells. Other macromolecules, such as DNA, RNA, and many lipids, also elicit a T-cell-independent response.

In the following example illustrating the T-cell-dependent response, B cells are used as the APC, although macrophages commonly perform this function. In this instance, antigen binds to surface IgM or IgD, is internalized within the B cell, and is fragmented. Some of the fragments return to the surface in association with class II MHC molecules (Figure 58–7A).[5] These interact with the receptor on the helper T cell, and, if the costimulatory signal is given by the B7 protein on the B cell interacting with CD28 protein on the helper T cell, the helper T cell is then stimulated to produce lymphokines, e.g., IL-2, B-cell growth factor (IL-4), and B-cell differentiation factor (IL-5). IL-4 and IL-5 induce "class switching" from IgM, which is the first class of immunoglobulins produced, to other classes, namely, IgG, IgA, and IgE (see the end of Chapter 59). These factors stimulate the B cell to divide and differentiate into many antibody-producing plasma cells.

Figure 58–7.



Source: Levinson W: *Review of Medical Microbiology and Immunology*, 10th Edition: http://www.accessmedicine.com

A: B-cell activation by helper T cells. B^0 is a resting B cell to which a multivalent antigen ( ) is attaching to monomer IgM receptors (**Y**). The antigen is internalized, and a fragment ( ) is

returned to the surface in conjunction with a class II molecule (∩). A receptor on an activated T cell recognizes the complex on the B-cell surface, and the T cell produces B-cell growth factor (BCGF, IL-4; ●) and B-cell differentiation factor (BCDF, IL-5; ■). These factors cause the progression of the B^1 cell to form B^2 and B^3 cells, which differentiate into antibody-producing (e.g., pentamer IgM) plasma cells (PC). Memory B cells are also produced. **B:** Inducible protein B7 (◗) on the B cell must interact with CD28 protein on the helper T cell in order for the helper T cell to be fully activated, and CD40L (CD40 ligand) on the helper T cell must interact with CD40 on the B cell for the B cell to be activated and synthesize the full range of antibodies. (Modified and reproduced, with permission, from Stites DP, Terr A [editors]: *Basic & Clinical Immunology,* 7th ed. Originally published by Appleton & Lange.)

Note that interleukins alone are *not* sufficient to activate B cells. A membrane protein on activated helper T cells, called CD40 ligand (CD40L), must interact with a protein called CD40 on the surface of the resting B cells to stimulate the differentiation of B cells into antibody-producing plasma cells (Figure 58–7B). Furthermore, other proteins on the surface of these cells serve to strengthen the interaction between the helper T cell and the antigen-presenting B cell; e.g., CD28 on the T cell interacts with B7 on the B cell, and LFA-1 on the T cell interacts with ICAM-1 on the B cell. (There are also ICAM proteins on the T cell that interact with LFA proteins on the B cell.)

In the T-cell-dependent response, all classes of antibody are made (IgG, IgM, IgA, etc.), whereas in the **T-cell-independent response, primarily IgM is made.** This indicates that lymphokines produced by the helper T cell are needed for class switching. The T-cell-dependent response generates memory B cells, whereas the T-cell-independent response does not; therefore, a secondary antibody response (see Chapter 60) does not occur in the latter. The T-cell-independent response is the main response to bacterial capsular polysaccharides, because these molecules are not effectively processed and presented by APCs and hence do not activate helper T cells. The most likely reason for this is that polysaccharides do not bind to class II MHC proteins, whereas peptide antigens do.

CELL-MEDIATED IMMUNITY

In the cell-mediated response, the initial events are similar to those described above for antibody production. The antigen is processed by macrophages, is fragmented, and is presented in conjunction with class II MHC molecules on the surface. These interact with the receptor on the helper T cell, which is then stimulated to produce lymphokines such as IL-2 (T-cell growth factor), which stimulates the specific helper and cytotoxic T cells to grow.

SUPPRESSION OF CERTAIN IMMUNE RESPONSES

A subset of T cells called regulatory T cells (TR) has been shown to inhibit several immune-mediated diseases, especially autoimmune diseases, in animals. (These cells are also called suppressor T cells.) TR cells are 5–10% of the CD4-positive cells and are characterized by possessing the CD25 marker. Interleukin-6 produced by activated dendritic cells can block the inhibitory action of TR cells and allow effector T cells to function properly in defense against pathogenic microbes. It is not known how regulatory cells reduce/suppress the immune response.

When there is an imbalance in numbers or activity between CD4 and CD8 cells, cellular immune mechanisms are greatly impaired. For example, in lepromatous leprosy there is unrestrained multiplication of *Mycobacterium leprae,* a lack of delayed hypersensitivity to *M. leprae* antigens, a lack of cellular immunity to that organism, and an excess of CD8 cells in lesions. Removal of some CD8 cells can restore cellular immunity in such patients and limit *M. leprae* multiplication. In acquired immunodeficiency syndrome (AIDS), the normal ratio of CD4:CD8 cells (>1.5) is greatly reduced. Many CD4 cells are destroyed by the human immunodeficiency virus (HIV), and the number of CD8 cells increases. This imbalance, i.e., a loss of helper activity and an increase in suppressor activity, results in a susceptibility to opportunistic infections and certain tumors.

One important part of the host response to infection is the increased expression of class I and class II MHC proteins induced by various cytokines, especially interferons such as gamma interferon. The increased amount of MHC proteins leads to increased antigen presentation and a more vigorous immune response. However, certain viruses can suppress the increase in MHC protein expression, thereby enhancing their survival. For example, hepatitis B virus, adenovirus, and cytomegalovirus can prevent an increase in class I MHC protein expression, thereby reducing the cytotoxic T-cell response against cells infected by these viruses.

[3]Some TCRs have a different set of polypeptides called gamma and delta. These TCRs are unusual because they do not require that antigen be presented in association with MHC proteins. Gamma/delta T cells constitute approximately 10% of all T cells. Some of the T cells bearing these TCRs are involved in cell-mediated immunity against *M. tuberculosis.*

[4]Each superantigen, e.g., the different staphylococcal enterotoxins, interacts with different Vβ chains. This explains why many but not all helper T cells are activated by the various superantigens.

[5]Note that one important difference between B cells and T cells is that B cells recognize antigen itself, whereas T cells recognize antigen only in association with MHC proteins.

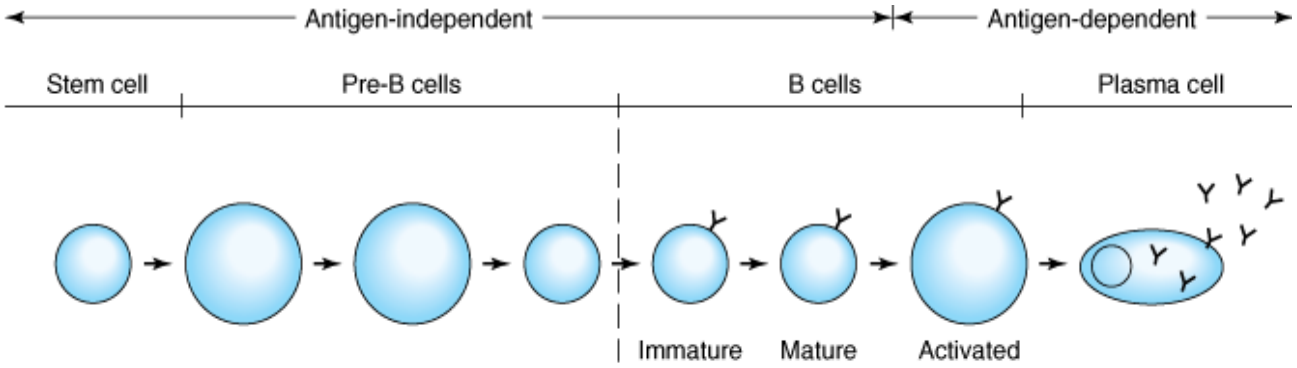
B CELLS

B cells perform two important functions: (1) They differentiate into plasma cells and produce antibodies and (2) they can present antigen to helper T cells.

Origin

During embryogenesis, B-cell precursors are recognized first in the fetal liver. From there they migrate to the **bone marrow,** which is their main location during adult life. Unlike T cells, they **do not require the thymus for maturation.** Pre-B cells lack surface immunoglobulins and light chains but do have μ-heavy chains in the cytoplasm. The maturation of B cells has two phases: the antigen-independent phase consists of stem cells, pre-B cells, and B cells, whereas the antigen-dependent phase consists of the cells that arise subsequent to the interaction of antigen with the B cells, e.g., activated B cells and plasma cells (Figure 58–8). B cells display surface IgM, which serves as a receptor for antigens. This surface IgM is a monomer, in contrast to circulating IgM, which is a pentamer. The monomeric IgM on the surface has an extra transmembrane domain that anchors the protein in the cell membrane that is not present in the circulating pentameric form of IgM. Surface IgD on some B cells may also be an antigen receptor. Pre-B cells are found in the bone marrow, whereas B cells circulate in the bloodstream.

Figure 58–8.



Source: Levinson W: *Review of Medical Microbiology and Immunology*, 10th Edition: http://www.accessmedicine.com

Maturation of B cells. B cells arise from stem cells and differentiate into pre-B cells expressing $^{\mu}$heavy chains in the cytoplasm and then into B cells expressing monomer IgM on the surface. This occurs independent of antigen. Activation of B cells and differentiation into plasma cells is dependent on antigen. Cells to the left of the vertical dotted line do not have IgM on their surface, whereas B cells, to the right of the vertical line, do. $^{\mu}$, mu heavy chains in cytoplasm; **Y**, IgM. (Modified and reproduced, with permission, from Stites DP, Terr A [editors]: *Basic & Clinical Immunology,* 7th ed. Originally published by Appleton & Lange. Copyright © 1991 by The McGraw-Hill Companies, Inc.)

B cells constitute about 30% of the recirculating pool of small lymphocytes, and their life span is short, i.e., days or weeks. Approximately 10^9 B cells are produced each day. Within lymph nodes, they are located in germinal centers; within the spleen, they are found in the white pulp. They are also found in the gut-associated lymphoid tissue, e.g., Peyer's patches.

Clonal Selection

How do antibodies arise? Does the antigen "instruct" the B cell to make an antibody, or does the antigen "select" a B cell endowed with the preexisting capacity to make the antibody?

It appears that the latter alternative, i.e., **clonal selection,** accounts for antibody formation. Each individual has a large pool of B lymphocytes (about 10^7). Each immunologically responsive B cell bears a surface receptor (either IgM or IgD) that can react with one antigen (or closely related group of antigens); i.e., there are about 10^7 different specificities. An antigen interacts with the B lymphocyte that shows the best "fit" with its immunoglobulin surface receptor. After the antigen binds, the B cell is stimulated to proliferate and form a clone of cells. These selected B cells soon become plasma cells and secrete antibody specific for the antigen. Plasma cells synthesize the immunoglobulins with the same antigenic specificity (i.e., they have the same heavy chain and the same light chain) as those carried by the selected B cell. Antigenic specificity does *not* change when heavy-chain class switching occurs (see Chapter 59).

Note that clonal selection also occurs with T cells. The antigen interacts with a specific receptor located on the surface of either a CD4-positive or a CD8-positive T cell. This "selects" this cell and activates it to expand into a clone of cells with the same specificity.

Activation of B Cells

In the following example, the B cell is the APC. Multivalent antigen binds to surface IgM (or IgD) and cross-links adjacent immunoglobulin molecules. The immunoglobulins aggregate to form "patches" and eventually migrate to one pole of the cell to form a cap. Endocytosis of the capped material follows, the antigen is processed, and epitopes appear on the surface in conjunction with class II MHC proteins. This complex is recognized by a helper T cell with a receptor for the antigen on its surface[6] The T cell now produces various interleukins (IL-2, IL-4, and IL-5) that stimulate the growth and differentiation of the B cell.

The activation of B cells to produce the full range of antibodies requires two other interactions in addition to recognition of the epitope by the T-cell antigen receptor and the production of IL-4 and IL-5 by the helper T cell. These costimulatory

interactions, which occur between surface proteins on the T and B cells, are as follows: (1) CD28 on the T cell must interact with B7 on the B cell and (2) CD40L on the T cell must interact with CD40 on the B cell. The CD28-B7 interaction is required for activation of the T cell to produce IL-2, and the CD40L-CD40 interaction is required for class switching from IgM to IgG and other immunoglobulin classes to occur.

Effector Functions of B Cells/Plasma Cells

The end result of the activation process is the production of many **plasma cells** that produce large amounts of immunoglobulins specific for the epitope. Plasma cells secrete thousands of antibody molecules per second for a few days and then die. Some activated B cells form **memory cells,** which can remain quiescent for long periods but are capable of being activated rapidly upon reexposure to antigen. Most memory B cells have surface IgG that serves as the antigen receptor, but some have IgM. Memory T cells secrete interleukins that enhance antibody production by the memory B cells. The presence of these cells explains the rapid appearance of antibody in the secondary response (see Chapter 60).

[6]Macrophages bearing antigen bound to class II MHC proteins can also present antigen to the T cell, resulting in antibody formation. In general, B cells are poor activators of "virgin" T cells in the primary response because B cells do not make IL-1. B cells are, however, very good activators of memory T cells because little, if any, IL-1 is needed.

ANTIGEN-PRESENTING CELLS

Macrophages

Macrophages have three main functions: phagocytosis, antigen presentation and cytokine production (Table 58–5).

Table 58–5. Important Features of Macrophages.

Functions	Mechanisms
Phagocytosis	Ingestion and killing of microbes in phagolysosomes. Killing caused by reactive oxygen intermediates such as superoxides, reactive nitrogen intermediates such as nitric oxide, and lysosomal enzymes such as proteases, nucleases, and lysozyme.
Antigen presentation	Presentation of antigen in association with class II MHC proteins to CD4-positive helper T cells. Also displays B7 protein, which acts as a costimulator of helper T cells.

Cytokine production	Synthesis and release of cytokines such as IL-1 and TNF, and chemokines such as IL-8.

Cytokine production Synthesis and release of cytokines such as IL-1 and TNF, and chemokines such as IL-8.

1. **Phagocytosis.** Macrophages ingest bacteria, viruses, and other foreign particles. They have surface Fc receptors that interact with the Fc portion of IgG, thereby enhancing the uptake of opsonized organisms. Macrophages also have receptors for C3b, another important opsonin. After ingestion, the phagosome containing the microbe fuses with a lysosome. The microbe is killed within this phagolysosome by reactive oxygen and reactive nitrogen compounds and by lysosomal enzymes.
2. **Antigen presentation.** Foreign material is ingested and degraded, and fragments of antigen are presented on the macrophage cell surface (in conjunction with class II MHC proteins) for interaction with the TCR of CD4-positive helper T cells. Degradation of the foreign protein stops when the fragment associates with the class II MHC protein in the cytoplasm. The complex is then transported to the cell surface by specialized "transporter" proteins.
3. **Cytokine production.** Macrophages produce several cytokines (macrokines, monokines), the most important of which are IL-1 and TNF. IL-1 (endogenous pyrogen) plays a role in the activation of helper T cells, and TNF is an important inflammatory mediator (see Mediators Produced by Macrophages That Affect Other Cells). In addition, macrophages produce interleukin-8 (IL-8), an important chemokine that attracts neutrophils and T cells to the site of infection.

These three functions are greatly enhanced when a process called **macrophage activation** occurs. Macrophages are activated initially by substances such as bacterial lipopolysaccharide (LPS, endotoxin), by bacterial peptidoglycan, and by bacterial DNA. (Human DNA is methylated, whereas bacterial DNA is unmethylated and therefore is perceived as foreign.) These substances interact with "toll-like receptors" on the macrophage surface and signal the cell to produce certain cytokines. Macrophages are also activated by gamma interferon produced by helper T cells. Gamma interferon increases the synthesis of class II MHC proteins, which enhances antigen presentation and increases the microbicidal activity of macrophages.

Macrophages are derived from bone marrow histiocytes and exist both free, e.g., monocytes, and fixed in tissues, e.g., Kupffer cells of the liver. Macrophages migrate

to the site of inflammation, attracted by certain mediators, especially C5a, an anaphylatoxin released in the complement cascade.

Dendritic Cells

Dendritic cells are a third type of cell that function as "professional" antigen-presenting cells (macrophages and B cells are the other two); i.e., they express class II MHC proteins and present antigen to CD4-positive T cells. They are particularly important because they are the **main inducers of the primary antibody response.** The name "dendritic" describes their many long, narrow processes (that resemble neuronal dendrites), which make them very efficient at making contact with foreign material. Dendritic cells are primarily located under the skin and the mucosa, e.g., Langerhans' cells in the skin. Dendritic cells migrate from their peripheral location under the skin and mucosa to local lymph nodes, where they present antigen to helper T cells.

SUMMARY OF THE INTERACTION OF ANTIGEN-PRESENTING CELLS, T CELLS, & B CELLS

The interactive process is initiated by the ingestion of a microbe by an antigen-presenting cell, for example, the ingestion of a bacterium by a dendritic cell in the skin. The dendritic cell migrates to the lymph node via lymph vessels, attracted there by chemokines. In the lymph node, the dendritic cell presents antigen to the T cell bearing a receptor specific for that antigen. While this process is occurring, fragments of the microbe circulate to the lymph node and bind directly to the B cell antigen receptor (membrane IgM). The antigen is internalized, processed, and presented to helper T cells with the correct receptor. Various chemokines and chemokine receptors (such as CCR7) facilitate the migration of these cells to a junctional area in the lymph node where they have a high probability of interacting with each other. The proximity of the B cell to the helper T cell allows interleukins produced by the helper T cell to efficiently activate antibody synthesis by the B cell.

FOLLICULAR DENDRITIC CELLS

These cells have a similar appearance to the dendritic cells mentioned above but are quite different from them in their location and function. Follicular dendritic cells (FDCs) are located in the B-cell-containing germinal centers of the follicles in the spleen and lymph nodes. They do not present antigen to helper T cells because they do not produce class II MHC proteins. Rather, they capture antigen–antibody complexes via Fc receptors located on their surface. The antigen–antibody complexes are then detected by activated B cells. The antibody produced by these B cells undergoes affinity maturation. (Affinity maturation is the improvement in the affinity of an antibody for the antigen that occurs upon repeated exposure to the antigen.)

Affinity maturation is described in Chapter 60. In addition, FDCs produce chemokines that attract B cells to the follicles in the spleen and lymph nodes.

NATURAL KILLER CELLS

NK cells play an important role in the innate host defenses (Table 58–6). They specialize in killing virus-infected cells and tumor cells by secreting cytotoxins (perforins and granzymes) similar to those of cytotoxic T lymphocytes and by participating in Fas-Fas ligand-mediated apoptosis. They are called "natural" killer cells because they are active without prior exposure to the virus, are not enhanced by exposure, and are not specific for any virus. They can kill without antibody, but antibody (IgG) enhances their effectiveness, a process called antibody-dependent cellular cytotoxicity (ADCC) (see the section on Effector Functions of T Cells [above]). IL-12 and gamma interferon are potent activators of NK cells. Approximately 5–10% of peripheral lymphocytes are NK cells.

Table 58–6. Important Features of Natural Killer (NK) Cells.

I. Nature of NK Cells
Large granular lymphocytes
Lack T-cell receptor, CD3 proteins, and surface IgM and IgD
Thymus not required for development
Normal numbers in Severe Combined Immunodeficiency Disease (SCID) patients
Activity not enhanced by prior exposure
II. Function of NK Cells
Kill virus-infected cells and cancer cells
Killing is nonspecific, i.e., not specific for viral or cancer antigens
Killing is not dependent on foreign antigen presentation by class I or II MHC proteins
Killing is activated by the failure of a cell to present self antigen in association with class I MHC proteins or by a reduction in the number of class I MHC proteins on the cell surface
Kill by producing perforins and granzymes, which cause apoptosis of target cell

NK cells are lymphocytes with some T-cell markers, but they do not have to pass through the thymus in order to mature. They have no immunologic memory and, unlike cytotoxic T cells, have no T-cell receptor; also, killing does not require recognition of MHC proteins. In fact, NK cells have receptors that detect the presence of class I MHC proteins on the cell surface. If a cell displays sufficient class I MHC proteins, that cell is *not* killed by the NK cell. Many virus-infected cells and tumor cells display a significantly reduced amount of class I MHC proteins, and it is those cells that are recognized and killed by the NK cells. Humans who lack NK cells are predisposed to life-threatening infections with varicella-zoster virus and cytomegalovirus.

NK cells detect the presence of cancer cells by recognizing a protein called MICA that is found on the surface of many cancer cells but not normal cells. Interaction of MICA with a receptor on NK cells triggers the production of cytotoxins by the NK cell and death of the tumor cell.

POLYMORPHONUCLEAR NEUTROPHILS

Neutrophils are a very important component of our innate host defenses, and severe bacterial infections occur if they are too few in number (neutropenia) or are deficient in function, as in chronic granulomatous disease. They have cytoplasmic granules that stain a pale pink (neutral) color with blood stains such as Wright stain, in contrast to eosinophils and basophils whose granules stain red and blue, respectively. These granules are lysosomes, which contain a variety of degradative enzymes that are important in the **bactericidal** action of these cells. The process of phagocytosis and the bactericidal action of neutrophils is described in detail in Chapter 8.

Neutrophils have receptors for IgG on their surface so IgG is the only immunoglobulin that opsonizes, i.e., makes bacteria more easily phagocytosed. Note that neutrophils do not display class II MHC proteins on their surface and therefore do not present antigen to helper T cells. This is in contrast to macrophages that are also phagocytes but do present antigen to helper T cells.

Neutrophils can be thought of as a "two-edged" sword. The positive edge of the sword is their powerful microbicidal activity, but the negative edge is the tissue damage caused by the release of degradative enzymes. An excellent example of the latter is the damage to the glomeruli in acute post-streptococcal glomerulonephritis. The damage is caused by enzymes released by neutrophils attracted to the glomeruli by C5a activated by the antigen–antibody complexes deposited on the glomerular membrane.

EOSINOPHILS

Eosinophils are white blood cells with cytoplasmic granules that appear red when stained with Wright stain. The red color is caused by the negatively charged eosin dye binding to the positively charged major basic protein in the granules. The eosinophil count is elevated in two medically important types of diseases: **parasitic diseases,** especially those caused by nematodes (see Chapter 56), and **hypersensitivity diseases,** such as asthma and serum sickness (see Chapter 65). Diseases caused by protozoa are typically not characterized by eosinophilia.

The function of eosinophils has not been clearly established. It seems likely that their main function is to defend against the migratory larvae of nematodes, such as *Strongyloides* and *Trichinella.* They attach to the surface of the larvae and discharge the contents of their granules, which in turn damages the cuticle of the larvae. Attachment to the larvae is mediated by receptors on the eosinophil surface for the Fc portion of the heavy chain of IgG and IgE.

Another function of eosinophils may be to mitigate the effects of immediate hypersensitivity reactions because the granules of eosinophils contain histaminase, an enzyme that degrades histamine, which is an important mediator of immediate reactions. However, the granules of the eosinophils also contain leukotrienes and peroxidases, which can damage tissue and cause inflammation. The granules also contain major basic protein that damages respiratory epithelium and contributes to the pathogenesis of asthma.

Eosinophils can phagocytose bacteria but they do so weakly and are not sufficient to protect against pyogenic bacterial infections in neutropenic patients. Although they can phagocytose, they do not present antigen to helper T cells. The growth and differentiation of eosinophils is stimulated by interleukin-5.

BASOPHILS & MAST CELLS

Basophils are white blood cells with cytoplasmic granules that appear blue when stained with Wright stain. The blue color is caused by the positively charged methylene blue dye binding to several negatively charged molecules in the granules. Basophils circulate in the bloodstream, whereas mast cells, which are similar to basophils in many ways, are fixed in tissue, especially under the skin and in the mucosa of the respiratory and gastrointestinal tracts.

Basophils and mast cells have receptors on their surface for the Fc portion of the heavy chain of IgE. When adjacent IgE molecules are cross-linked by antigen, immunologically active mediators, such as histamine, and enzymes, such as peroxidases and hydrolases, are released. These cause inflammation and, when

produced in large amounts, cause **severe immediate hypersensitivity reactions such as systemic anaphylaxis.**

Mast cells also play an important role in the innate response to bacteria and viruses. The surface of mast cells contain Toll-like receptors that recognize bacteria and viruses. The mast cells respond by releasing cytokines and enzymes from their granules that mediate inflammation and attract neutrophils and dendritic cells to the site of infection. Dendritic cells are important antigen-presenting cells that initiate the adaptive response. The role of mast cells in inflammation has been demonstrated in rheumatoid arthritis. These cells produce both inflammatory cytokines and the enzymes that degrade the cartilage in the joints.

IMPORTANT CYTOKINES

The important functions of the main cytokines are described in Table 58–7.

Table 58–7. Important Functions of the Main Cytokines.

Cytokine	Major Source	Important Functions
Interleukin-1	Macrophages	Proinflammatory cytokine. Activates helper T cells and endothelial cells. Induces fever.
Interleukin-2	Th-1 subset of helper T cells	Activates helper and cytotoxic T cells. Also activates B cells.
Interleukin-4	Th-2 subset of helper T cells	Stimulates B-cell growth. Increases isotype switching and IgE. Increases Th-2 subset of helper T cells.
Interleukin-5	Th-2 subset of helper T cells	Stimulates B-cell differentiation. Increases eosinophils and IgA.
Gamma interferon	Th-1 subset of helper T cells	Stimulates phagocytosis and killing by macrophages and NK cells. Increases class I and II MHC protein expression.
Tumor necrosis factor	Macrophages	Proinflammatory cytokine. Low concentration: activates neutrophils and increases their adhesion to endothelial cells. High concentration: mediates septic shock, acts as cachectin, causes necrosis of tumors.

Mediators Affecting Lymphocytes

1. **IL-1** is a protein produced mainly by macrophages. It activates a wide variety of target cells, e.g., T and B lymphocytes, neutrophils, and endothelial cells. It is a proinflammatory cytokine, i.e., plays an important role, along with tumor necrosis factor (TNF), in inducing inflammation. In addition, IL-1 is **endogenous pyrogen,** which acts on the hypothalamus to cause the fever associated with infections and other inflammatory reactions. (Exogenous pyrogen is endotoxin, a lipopolysaccharide found in the cell wall of gram-negative bacteria [see Chapter 7].)

2. **IL-2** is a protein produced mainly by helper T cells that **stimulates both helper and cytotoxic T cells** to grow. **IL-2 is T-cell growth factor.** Resting T cells are stimulated by antigen (or other stimulators) both to produce IL-2 and to form IL-2 receptors on their surface, thereby acquiring the capacity to respond to IL-2. Interaction of IL-2 with its receptor stimulates DNA synthesis.

3. IL-4 is a protein produced by the Th-2 class of helper T cells that induces class switching to IgE. IL-4 is the most characteristic cytokine produced by Th-2 cells (Figure 58–3).

4. IL-5 is a protein produced by the Th-2 class of helper T cells that induces class switching to IgA and activates eosinophils. Eosinophils are an important host defense against many helminths (worms), e.g., *Strongyloides* (see Chapter 56), and are increased in immediate hypersensitivity (allergic) reactions (see Chapter 65).

5. IL-6 is produced by helper T cells and macrophages. It stimulates B cells to differentiate, induces fever by affecting the hypothalamus, and induces the production of acute-phase proteins by the liver. Acute-phase proteins are described in Innate Immunity.

6. IL-10 and IL-12 regulate the production of Th-1 cells, the cells that mediate delayed hypersensitivity (Figure 58–3). IL-12 is produced by macrophages and promotes the development of Th-1 cells, whereas IL-10 is produced by Th-2 cells and inhibits the development of Th-1 cells by limiting gamma interferon production. (Gamma interferon is described below.) The relative amounts of IL-4, IL-10, and IL-12 drive the differentiation of Th-1 and Th-2 cells and therefore enhance either cell-mediated or humoral immunity, respectively. This is likely to have important medical consequences because the main host defense against certain infections is either cell-mediated or humoral immunity. For example, *Leishmania* infections in mice are lethal if a humoral response predominates but are controlled if a vigorous cell-mediated response occurs.

   The **IL-12–gamma interferon axis** is very important in the ability of our host defenses to control infections by intracellular pathogens, such as *M.*

tuberculosis and *L. monocytogenes.* IL-12 increases the number of Th-1 cells, and Th-1 cells produce the gamma interferon that activates the macrophages that phagocytose and kill the intracellular bacterial pathogens mentioned above.

7. IL-13 is implicated as the mediator of allergic airway disease (asthma). IL-13 is made by Th-2 cells and binds to a receptor that shares a chain with the IL-4 receptor. In animals, IL-13 was shown to be necessary and sufficient to cause asthma. IL-13 is involved in producing the airway hyperresponsiveness seen in asthma but not in increasing the amount of IgE.
8. The main function of transforming growth factor-β (TGF-β) is to **inhibit** the growth and activities of T cells. It is viewed as an "anti-cytokine" because, in addition to its action on T cells, it can inhibit many functions of macrophages, B cells, neutrophils, and natural killer cells by counteracting the action of other activating factors. Although it is a "negative regulator" of the immune response, it stimulates wound healing by enhancing the synthesis of collagen. It is produced by many types of cells, including T cells, B cells, and macrophages. In summary, the role of TGF-β is to dampen or suppress the immune response when it is no longer needed after an infection and to promote the healing process.

Mediators Affecting Macrophages & Monocytes

Chemokines are a group of cytokines that can attract either macrophages or neutrophils to the site of infection. The term "chemokine" is a contraction of **chemo**tactic and cyto**kine.** Chemokines are produced by various cells in the infected area, such as endothelial cells and resident macrophages. The circulating neutrophils and macrophages (monocytes) are attracted to the site by an increasing gradient of chemokines, then bind to selectins on the endothelial cell surface. Chemokines also activate integrins on the surface of the neutrophils and macrophages that bind to ICAM proteins on the endothelial cell surface. The interaction between integrin and ICAM facilitates the movement of the white cells into the tissue to reach the infected area.

Approximately 50 chemokines have been identified; they are small polypeptides ranging in size from 68 to 120 amino acids. The alpha-chemokines have two adjacent cysteines separated by another amino acid (Cys-X-Cys), whereas the beta-chemokines have two adjacent cysteines (Cys-Cys) (Table 58–8). The alpha-chemokines attract neutrophils and are produced by activated mononuclear cells. IL-8 is a very important member of this group. The beta-chemokines attract macrophages and monocytes and are produced by activated T cells. RANTES and MCAF are important beta-chemokines.

Table 58–8. Chemokines of Medical Importance.

Class	Chemistry	Attracts	Produced by	Examples
Alpha	C-X-C	Neutrophils	Activated mononuclear cells	Interleukin-8
Beta	C-C	Monocytes	Activated T cells	RANTES,[1] MCAF[2]

[1]RANTES is an abbreviation for *r*egulated upon *a*ctivation, *n*ormal *T e*xpressed and *s*ecreted.

[2]MCAF is an abbreviation for *m*acrophage *c*hemoattractant and *a*ctivating *f*actor.

There are specific receptors for chemokines on the surface of cells, such as neutrophils and monocytes. Interaction of the chemokine with its receptor results in changes in cell surface proteins that allow the cell to adhere to and migrate through the endothelium to the site of infection.

Mediators Affecting Polymorphonuclear Leukocytes

1. TNF activates the phagocytic and killing activities of neutrophils and increases the synthesis of adhesion molecules by endothelial cells. The adhesion molecules mediate the attachment of neutrophils at the site of infection.
2. Chemotactic factors for neutrophils, basophils, and eosinophils selectively attract each cell type. Interleukin-8 and complement component C5a are important attractants for neutrophils. (See the discussion of chemokines [above] and Table 58–8.)
3. Leukocyte-inhibitory factor inhibits migration of neutrophils, analogous to migration-inhibitory factor (below). Its function is to retain the cells at the site of infection.

Mediators Affecting Stem Cells

IL-3 is made by activated helper T cells and supports the growth and differentiation of bone marrow stem cells. Granulocyte-macrophage colony-stimulating factor (GM-CSF, sargramostim) is made by T lymphocytes and macrophages. It stimulates the growth of granulocytes and macrophages and enhances the antimicrobial activity of macrophages. It is used clinically to improve regeneration of these cells after bone marrow transplantation. Granulocyte colony-stimulating factor (G-CSF, filgrastim) is made by various cells, e.g., macrophages, fibroblasts, and endothelial cells. It enhances the development of neutrophils from stem cells and is used clinically to prevent infections in patients who have received cancer chemotherapy. The

stimulation of neutrophil production by G-CSF and GM-CSF results in the increased number of these cells in the peripheral blood after infection.

Mediators Produced by Macrophages that Affect Other Cells

1. TNF-α is an **inflammatory mediator** released primarily by macrophages. It has many important effects that differ depending on the concentration. At low concentrations, it increases the synthesis of adhesion molecules by endothelial cells, which allows neutrophils to adhere to blood vessel walls at the site of infection. It also activates the respiratory burst within neutrophils, thereby enhancing the killing power of these phagocytes. It increases lymphokine synthesis by helper T cells and stimulates the growth of B cells. At high concentrations, it is an important mediator of **endotoxin-induced septic shock;** antibody to TNF-α prevents the action of endotoxin. (The action of endotoxin is described in Chapter 7.) TNF-α is also known as **cachectin** because it inhibits lipoprotein lipase in adipose tissue, thereby reducing the utilization of fatty acids. This results in cachexia. TNF-α, as its name implies, causes the **death and necrosis of certain tumors** in experimental animals. It may do this by promoting intravascular coagulation that causes infarction of the tumor tissue. Note the similarity of this intravascular coagulation with the DIC of septic shock, both of which are caused by TNF-α.

2. **Nitric oxide** (NO) is an important mediator made by macrophages in response to the presence of endotoxin, a lipopolysaccharide found in the cell wall of gram-negative bacteria. NO causes vasodilation, which contributes to the hypotension seen in septic shock. Inhibitors of NO synthase, the enzyme that catalyzes the synthesis of NO from arginine, can prevent the hypotension associated with septic shock.

3. **Macrophage migration inhibitory factor** (MIF) is another important mediator made by macrophages in response to endotoxin. The function of MIF is to retain the macrophages at the site of infection. Recent studies have shown that MIF plays a major role in the induction of septic shock. Antibody against MIF can prevent septic shock in animals genetically incapable of producing TNF. The mechanism of action of MIF in septic shock is unclear at this time.

Mediators with Other Effects

1. **Interferons** are glycoproteins that block virus replication and exert many immunomodulating functions. Alpha interferon (from leukocytes) and beta interferon (from fibroblasts) are induced by viruses (or double-stranded RNA) and have antiviral activity (see Chapter 33). **Gamma interferon** is a lymphokine produced primarily by the Th-1 subset of helper T cells. It is one of the most potent activators of the phagocytic activity of macrophages, NK cells, and neutrophils, thereby enhancing their ability to kill microorganisms and tumor cells. For example, it greatly increases the killing of intracellular

bacteria, such as *M. tuberculosis,* by macrophages. It also increases the synthesis of class I and II MHC proteins in a variety of cell types. This enhances antigen presentation by these cells.

2. **Lymphotoxin** (also known as TNF-β) is made by activated T lymphocytes and causes effects similar to those of TNF-α. It binds to the same receptor as TNF-α and hence has the same effects as TNF-α.

Chapter 59. Antibodies

ANTIBODIES: INTRODUCTION

Antibodies are globulin proteins (immunoglobulins) that react specifically with the antigen that stimulated their production. They make up about 20% of the protein in blood plasma. Blood contains three types of globulins, alpha, beta, and gamma, based on their electrophoretic migration rate. Antibodies are gamma globulins. There are five classes of antibodies: IgG, IgM, IgA, IgD, and IgE. Antibodies are subdivided into these five classes based on differences in their heavy chains.

The most important functions of antibodies are to **neutralize toxins and viruses,** to **opsonize microbes** so they are more easily phagocytosed, to **activate complement,** and to **prevent the attachment** of microbes to mucosal surfaces. The specific antibody classes that mediate these functions are described in Table 59–1. In addition to these functions, antibodies have a **catalytic (enzymatic) capability** that is described in a separate section at the end of this chapter.

Table 59–1. Properties of Human Immunoglobulins.

Property	IgG	IgA	IgM	IgD	IgE
Percentage of total immunoglobulin in serum (approx)	75	15	9	0.2	0.004
Serum concentration (mg/dL) (approx)	1000	200	120	3	0.05
Sedimentation coefficient	7S	7S or 11S[1]	19S	7S	8S
Molecular weight (x1000)	150	170 or 400[1]	900	180	190
Structure	Monomer	Monomer or dimer	Monomer or pentamer	Monomer	Monomer
H-chain symbol	γ	α	μ	δ	ε
Complement fixation	+	–	+	–	–
Transplacental passage	+	–	–	–	–
Mediation of allergic responses	–	–	–	–	+

Found in secretions	–	+	–	–	–
Opsonization	+	–	–[2]	–	–
Antigen receptor on B cell	–	–	+	?	–
Polymeric form contains J chain	–	+	+	–	–

[1]The 11S form is found in secretions (e.g., saliva, milk, and tears) and fluids of the respiratory, intestinal, and genital tracts.

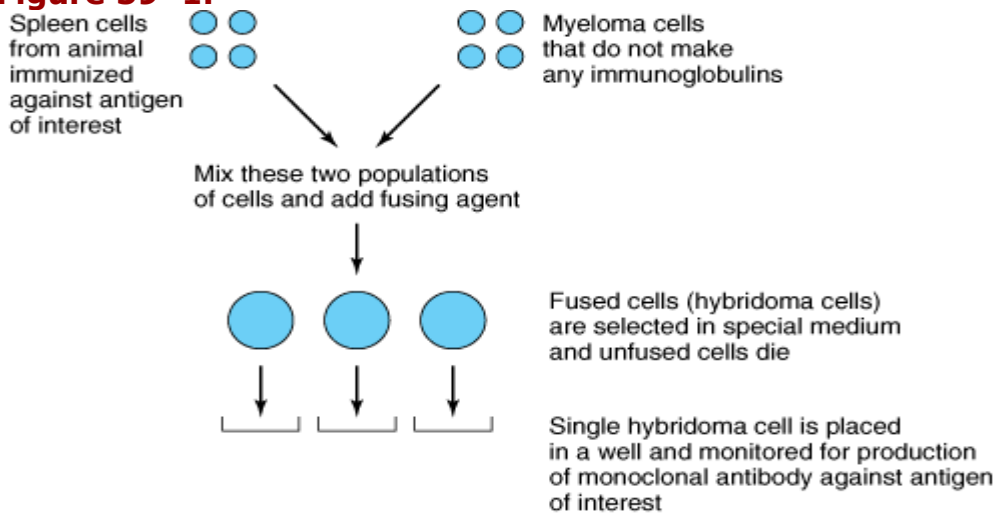
[2] IgM opsonizes indirectly by activating complement. This produces C3b, which is an opsonin.

MONOCLONAL ANTIBODIES

Antibodies that arise in an animal in response to typical antigens are heterogeneous, because they are formed by several different clones of plasma cells; i.e., they are **polyclonal.** Antibodies that arise from a single clone of cells, e.g., in a plasma cell tumor (myeloma),[1] are homogeneous; i.e., they are **monoclonal.**

Monoclonal antibodies also can be made in the laboratory by fusing a myeloma cell with an antibody-producing cell (Figure 59–1; also see Hybridomas & Monoclonal Antibodies). Such **hybridomas** produce virtually unlimited quantities of monoclonal antibodies that are useful in diagnostic tests and in research (see Hybridomas & Monoclonal Antibodies).

Figure 59–1.



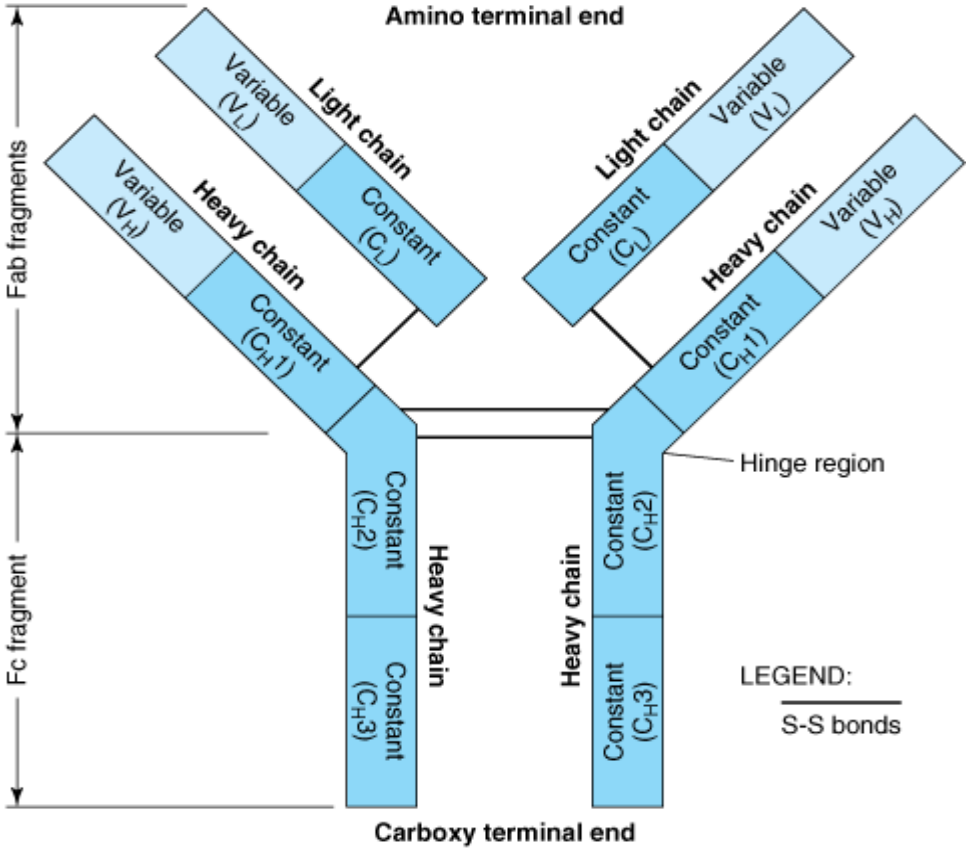
Production of monoclonal antibodies.

[1]Multiple myeloma is a malignant disease characterized by an overproduction of plasma cells (B cells). All the myeloma cells in a patient produce the same type of immunoglobulin molecule, which indicates that all the cells arose from a single progenitor. Excess κor λL chains are synthesized and appear as dimers in the urine. These are known as Bence Jones proteins and have the unusual attribute of precipitating at 50–60°C but dissolving when the temperature is raised to the boiling point.

IMMUNOGLOBULIN STRUCTURE

Immunoglobulins are glycoproteins made up of **light** (L) and **heavy** (H) polypeptide chains. The terms "light" and "heavy" refer to molecular weight; light chains have a molecular weight of about 25,000, whereas heavy chains have a molecular weight of 50,000–70,000. The simplest antibody molecule has a **Y** shape (Figure 59–2) and consists of four polypeptide chains: two H chains and two L chains. The four chains are linked by disulfide bonds. An individual antibody molecule always consists of **identical** H chains and **identical** L chains. This is primarily the result of two phenomena: allelic exclusion (see Allelic Exclusion) and regulation within the B cell, which ensure the synthesis of either kappa (κ) or lambda (λ) L chains but not both.

Figure 59–2.



Source: Levinson W: *Review of Medical Microbiology and Immunology*, 10th Edition: http://www.accessmedicine.com

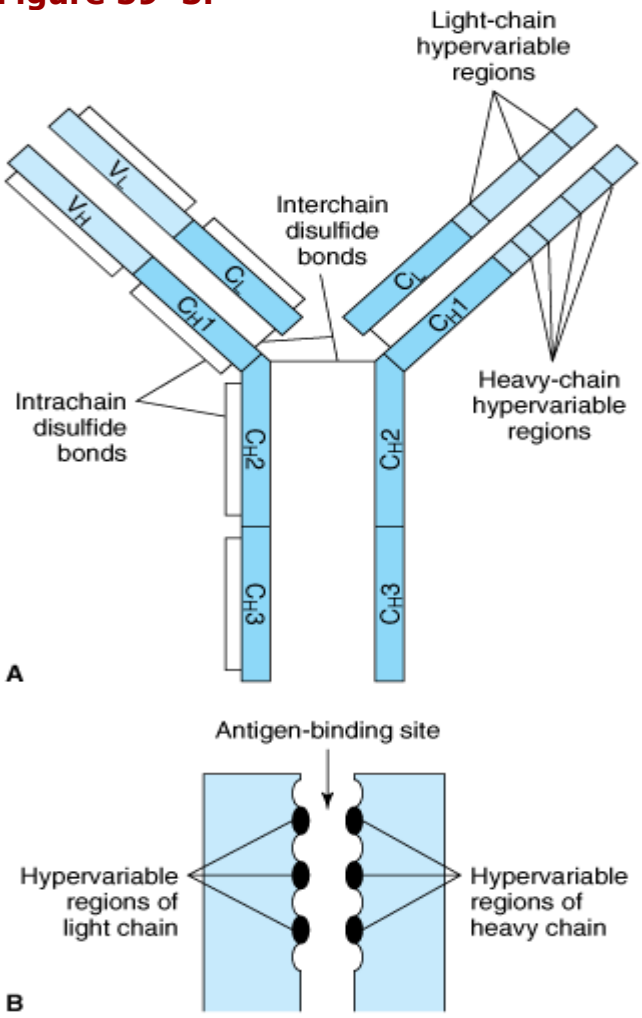
Structure of IgG. The **Y**-shaped IgG molecule consists of two light chains and two heavy chains. Each light chain consists of a variable region and a constant region. Each heavy chain consists of a variable region and a constant region that is divided into three domains: C_H1, C_H2, and C_H3. The C_H2 domain contains the complement-binding site, and the C_H3 domain is the site of attachment of IgG to receptors on neutrophils and macrophages. The antigen-binding site is formed by the variable regions of both the light and heavy chains. The specificity of the antigen-binding site is a function of the amino acid sequence of the hypervariable regions (see Figure 59–3). (Modified and reproduced, with permission, from Brooks GF et al: *Medical Microbiology,* 20th ed. Originally published by Appleton & Lange. Copyright © 1995 by The McGraw-Hill Companies, Inc.)

L and H chains are subdivided into **variable** and **constant** regions. The regions are composed of three-dimensionally folded, repeating segments called domains. An L chain consists of one variable (V_L) and one constant (C_L) domain. Most H chains consist of one variable (V_H) and three constant (C_H) domains. (IgG and IgA have three C_H domains, whereas IgM and IgE have four.) Each domain is approximately

110 amino acids long. The **variable** regions of both the light and heavy chain are responsible for **antigen-binding,** whereas the **constant** region of the heavy chain is responsible for **various biologic functions,** e.g., complement activation and binding to cell surface receptors. The complement binding site is in the C_H2 domain. The constant region of the light chain has no known biologic function.

The variable regions of both L and H chains have three extremely variable (**hypervariable**) amino acid sequences at the amino-terminal end that form the antigen-binding site (Figure 59–3). Only 5–10 amino acids in each hypervariable region form the antigen-binding site. Antigen–antibody binding involves electrostatic and van der Waals' forces and hydrogen and hydrophobic bonds rather than covalent bonds. The remarkable specificity of antibodies is due to these hypervariable regions (see the discussion of idiotypes in Isotypes, Allotypes, & Idiotypes).

Figure 59–3.



Source: Levinson W: *Review of Medical Microbiology and Immunology*, 10th Edition: http://www.accessmedicine.com

The antigen-binding site is formed by the hypervariable regions. **A:** Hypervariable regions on IgG. **B:** Magnified view of antigen-binding site. (Modified and reproduced, with permission, from Stites DP, Terr A, Parslow T [editors]: *Basic & Clinical Immunology,* 8th ed. Originally published by Appleton & Lange. Copyright © 1994 by The McGraw-Hill Companies, Inc.)

L chains belong to one of two types, κ(**kappa**) or λ(**lambda**), on the basis of amino acid differences in their constant regions. Both types occur in all classes of immunoglobulins (IgG, IgM, etc.), but any one immunoglobulin molecule contains only one type of L chain.[2]

The amino-terminal portion of each L chain participates in the antigen-binding site. H chains are distinct for each of the five immunoglobulin classes and are designated γ, α, μ, ε, and δ (Table 59–2). The amino-terminal portion of each H chain participates in the antigen-binding site; the carboxy terminal forms the Fc fragment, which has the biologic activities described above and in Table 59–2.

Table 59–2. Important Functions of Immunoglobulins.

Immunoglobulin	Major Functions
IgG	Main antibody in the secondary response. Opsonizes bacteria, making them easier to phagocytize. Fixes complement, which enhances bacterial killing. Neutralizes bacterial toxins and viruses. Crosses the placenta.
IgA	Secretory IgA prevents attachment of bacteria and viruses to mucous membranes. Does not fix complement.
IgM	Produced in the primary response to an antigen. Fixes complement. Does not cross the placenta. Antigen receptor on the surface of B cells.
IgD	Uncertain. Found on the surface of many B cells as well as in serum.
IgE	Mediates immediate hypersensitivity by causing release of mediators from mast cells and basophils upon exposure to antigen (allergen). Defends against worm infections by causing release of enzymes from eosinophils. Does not fix complement. Main host defense against helminth infections.

If an antibody molecule is treated with a proteolytic enzyme such as papain, peptide bonds in the "hinge" region are broken, producing two identical **Fab fragments,** which carry the antigen-binding sites, and one **Fc fragment,** which is involved in placental transfer, complement fixation, attachment site for various cells, and other biologic activities (Figure 59–2).

[2]In humans, the ratio of immunoglobulins containing κchains to those containing λ chains is approximately 2:1.

HYBRIDOMAS & MONOCLONAL ANTIBODIES

Hybridoma cells have the remarkable ability to produce large quantities of a single molecular species of immunoglobulin. These immunoglobulins, which are known as monoclonal antibodies, are called "monoclonal" because they are made by a clone of cells that arose from a single cell. Note, however, that this single cell is, in fact, formed by the fusion of two different cells; i.e., it is a hybrid, hence the term "hybridoma."

Hybridoma cells are made in the following manner: (1) An animal, e.g., a mouse, is immunized with the antigen of interest. (2) Spleen cells from this animal are grown in a culture dish in the presence of mouse myeloma cells. The myeloma cells have two important attributes: they grow indefinitely in culture, and they do not produce immunoglobulins. (3) Fusion of the cells is encouraged by adding certain chemicals, e.g., polyethylene glycol. (4) The cells are grown in a special culture medium (HAT medium) that supports the growth of the fused, hybrid cells but not of the "parental" cells. (5) The resulting clones of cells are screened for the production of antibody to the antigen of interest.

Chimeric monoclonal antibodies consisting of mouse variable regions and human constant regions are being made for use in treating human diseases such as leukemia. The advantages of the human constant chain are that human complement is activated (whereas it is not if the constant region is mouse-derived) and that antibodies against the monoclonal antibody are not formed (whereas antibodies are formed if the constant region is mouse-derived). The advantage of the mouse variable region is that it is much easier to obtain monoclonal antibodies against, for example, a human tumor antigen by inoculating a mouse with the tumor cells. Chimeric antibodies can kill tumor cells either by complement-mediated cytotoxicity or by delivering toxins, e.g., diphtheria toxin, specifically to the tumor cell.

Monoclonal antibodies are now used in a variety of clinical situations, such as immunosuppression related to organ transplants, treatment of autoimmune disease, treatment of cancer, and the prevention of infectious disease. Table 62–2 describes these monoclonal antibodies, their cellular targets, and their clinical use.

IMMUNOGLOBULIN CLASSES

IgG

Each IgG molecule consists of two L chains and two H chains linked by disulfide bonds (molecular formula H2L2). Because it has two identical antigen-binding sites, it is said to be **divalent.** There are four subclasses, IgG1–IgG4, based on antigenic differences in the H chains and on the number and location of disulfide bonds. IgG1 makes up most (65%) of the total IgG. IgG2 antibody is directed against polysaccharide antigens and is an important host defense against encapsulated bacteria.

IgG is the predominant antibody in the **secondary response** and constitutes an important defense against bacteria and viruses (Table 59–1). IgG is the only antibody to **cross the placenta;** only its Fc portion binds to receptors on the surface of placental cells. It is therefore the **most abundant immunoglobulin in newborns.** IgG is one of the two immunoglobulins that can activate complement; IgM is the other (see Chapter 63).

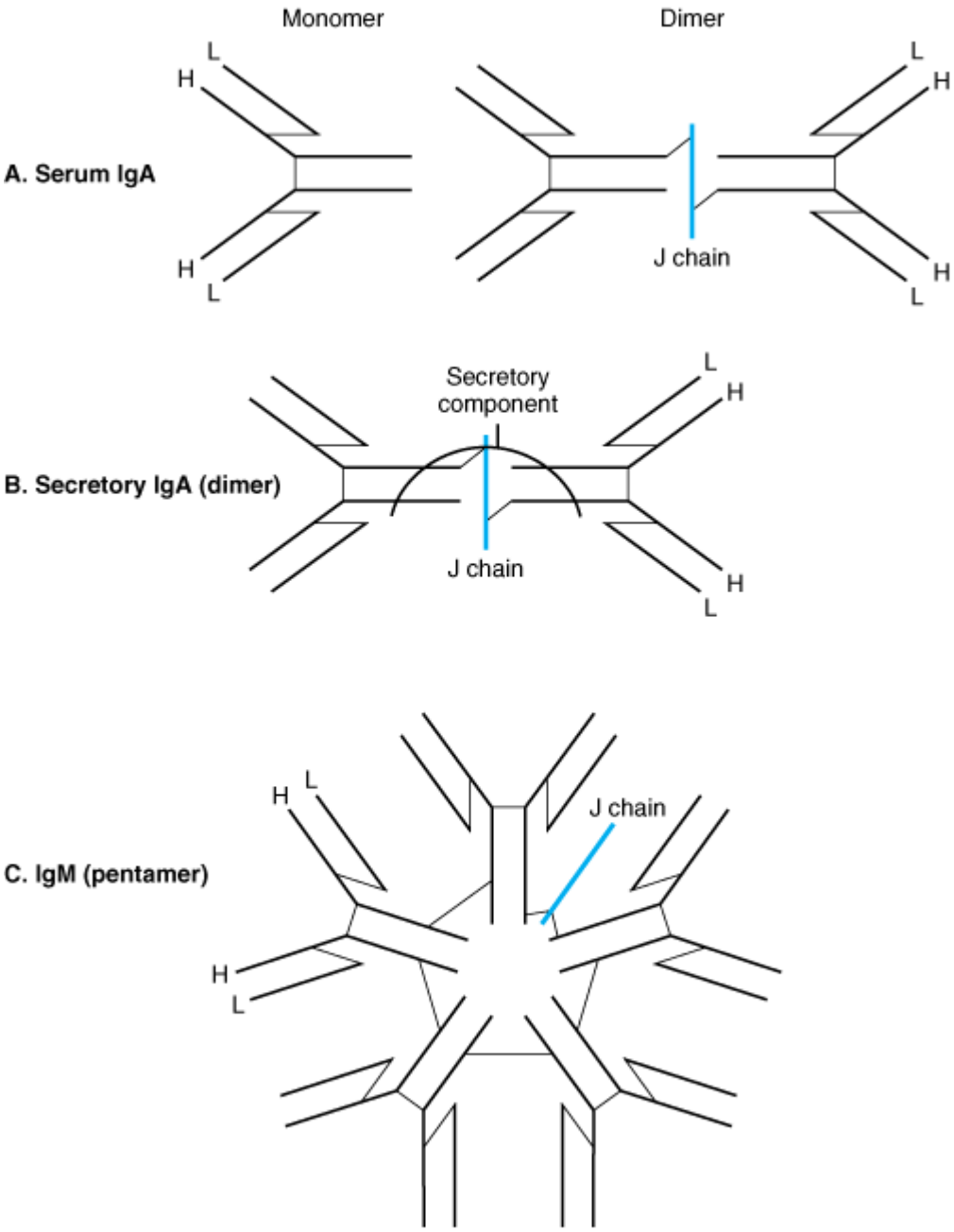
IgG is the immunoglobulin that **opsonizes.** It can opsonize, i.e., enhance phagocytosis, because there are receptors for the γH chain on the surface of phagocytes. IgM does not opsonize directly, because there are no receptors on the phagocyte surface for the μH chain. However, IgM activates complement, and the resulting C3b can opsonize because there are binding sites for C3b on the surface of phagocytes.

IgG has various sugars attached to the heavy chains, especially in the C2 domain. The medical importance of these sugars is that they determine whether IgG will have a proinflammatory or antiinflammatory effect. For example, if the IgG molecule has a terminal *N*-acetyl glucosamine, it is proinflammatory because it will bind to mannose-binding ligand and activate complement (see Chapter 63 and Figure 63–1). In contrast, if the IgG has a sialic acid side chain, then it will not bind and becomes antiinflammatory. Thus, IgG proteins specific for a single antigen that are made by a single plasma cell can, at various times, possess different properties depending on these sugar modifications.

IgA

IgA is the main immunoglobulin in **secretions** such as colostrum, saliva, tears, and respiratory, intestinal, and genital tract secretions. It prevents attachment of microorganisms, e.g., bacteria and viruses, to mucous membranes. Each secretory IgA molecule consists of two H2L2 units plus one molecule each of J (joining) chain[3] and secretory component (Figure 59–4). The two heavy chains in IgA are αheavy chains.

Figure 59–4.



Source: Levinson W: *Review of Medical Microbiology and Immunology*, 10th Edition: http://www.accessmedicine.com

Structure of serum IgA (**A**), secretory IgA (**B**), and IgM (**C**). Note that both IgA and IgM have a J chain but that only secretory IgA has a secretory component. (Reproduced, with permission, from Stites D, Terr A, Parslow T [editors]: *Basic & Clinical Immunology,* 8th ed. Originally published by Appleton & Lange. Copyright © 1994 by The McGraw-Hill Companies, Inc.)

The secretory component is a polypeptide synthesized by epithelial cells that provides for IgA passage to the mucosal surface. It also protects IgA from being degraded in the intestinal tract. In serum, some IgA exists as monomeric H2L2.

IgM

IgM is the main immunoglobulin produced early in the **primary response.** It is present as a monomer on the surface of virtually all B cells, where it functions as an antigen-binding receptor.[4] In serum, it is a **pentamer** composed of five H2L2 units plus one molecule of J (joining) chain (Figure 59–4). IgM has a μ-heavy chain. Because the pentamer has 10 antigen-binding sites, it is the **most efficient** immunoglobulin in agglutination, complement fixation (activation), and other antibody reactions and is important in defense against bacteria and viruses. It can be produced by the fetus in certain infections. It has the **highest avidity** of the immunoglobulins; its interaction with antigen can involve all 10 of its binding sites.

IgD

This immunoglobulin has no known antibody function but may function as an antigen receptor; it is present on the surface of many B lymphocytes. It is present in small amounts in serum.

IgE

IgE is medically important for two reasons: (1) it mediates immediate (anaphylactic) hypersensitivity (see Chapter 65), and (2) it participates in host defenses against certain parasites, e.g., helminths (worms) (see Chapter 56). The Fc region of IgE binds to the surface of mast cells and basophils. Bound IgE serves as a receptor for antigen (allergen). When the antigen-binding sites of adjacent IgEs are cross-linked by allergens, several mediators are released by the cells and immediate (anaphylactic) hypersensitivity reactions occur (see Figure 65–1). Although IgE is present in **trace** amounts in normal serum (approximately 0.004%), persons with allergic reactivity have greatly increased amounts, and IgE may appear in external secretions. IgE does not fix complement and does not cross the placenta.

IgE is the main host defense against certain important helminth (worm) infections, such as *Strongyloides, Trichinella, Ascaris,* and the hookworms *Necator* and *Ancylostoma.* The serum IgE level is usually increased in these infections. Because worms are too large to be ingested by phagocytes, they are killed by eosinophils that release worm-destroying enzymes. IgE specific for worm proteins binds to receptors on eosinophils, triggering the antibody-dependent cellular cytotoxicity (ADCC) response.

[3]Only IgA and IgM have J chains. Only these immunoglobulins exist as multimers (dimers and pentamers, respectively). The J chain initiates the polymerization process, and the multimers are held together by disulfide bonds between their Fc regions.

[4]The surface monomer IgM and the serum IgM both have μ-heavy chains, but the heavy chain of the surface IgM has a hydrophobic sequence that mediates binding within the cell membrane, whereas the serum IgM does not.

ISOTYPES, ALLOTYPES, & IDIOTYPES

Because immunoglobulins are proteins, they are antigenic, and that property allows them to be subdivided into isotypes, allotypes, and idiotypes.

1. **Isotypes** are defined by antigenic (amino acid) differences in their constant regions. Although different antigenically, all isotypes are found in all normal humans. For example, IgG and IgM are different isotypes; the constant region of their H chains (γ and μ) is different antigenically (the five immunoglobulin classes—IgG, IgM, IgA, IgD, and IgE—are different isotypes; their H chains are antigenically different). The IgG isotype is subdivided into four subtypes, IgG1, IgG2, IgG3, and IgG4, based on antigenic differences of their heavy chains. Similarly, IgA1 and IgA2 are different isotypes (the antigenicity of the constant region of their H chains is different), and κ and λ chains are different isotypes (their constant regions also differ antigenically).
2. **Allotypes,** on the other hand, are additional antigenic features of immunoglobulins that vary among individuals. They vary because the genes that code for the L and H chains are polymorphic, and individuals can have different alleles. For example, the λH chain contains an allotype called Gm, which is due to a one- or two-amino-acid difference that provides a different antigenicity to the molecule. Each individual inherits different allelic genes that code for one or another amino acid at the Gm site.[5]
3. **Idiotypes** are the antigenic determinants formed by the specific amino acids in the hypervariable region.[6] Each idiotype is unique for the immunoglobulin produced by a specific clone of antibody-producing cells. Anti-idiotype antibody reacts only with the hypervariable region of the specific immunoglobulin molecule that induced it.

[5]Allotypes related to λH chains are called Gm (an abbreviation of gamma); allotypes related to κL chains are called Inv (an abbreviation of a patient's name).

[6]Any one of these antigen determinants is called an idiotope.

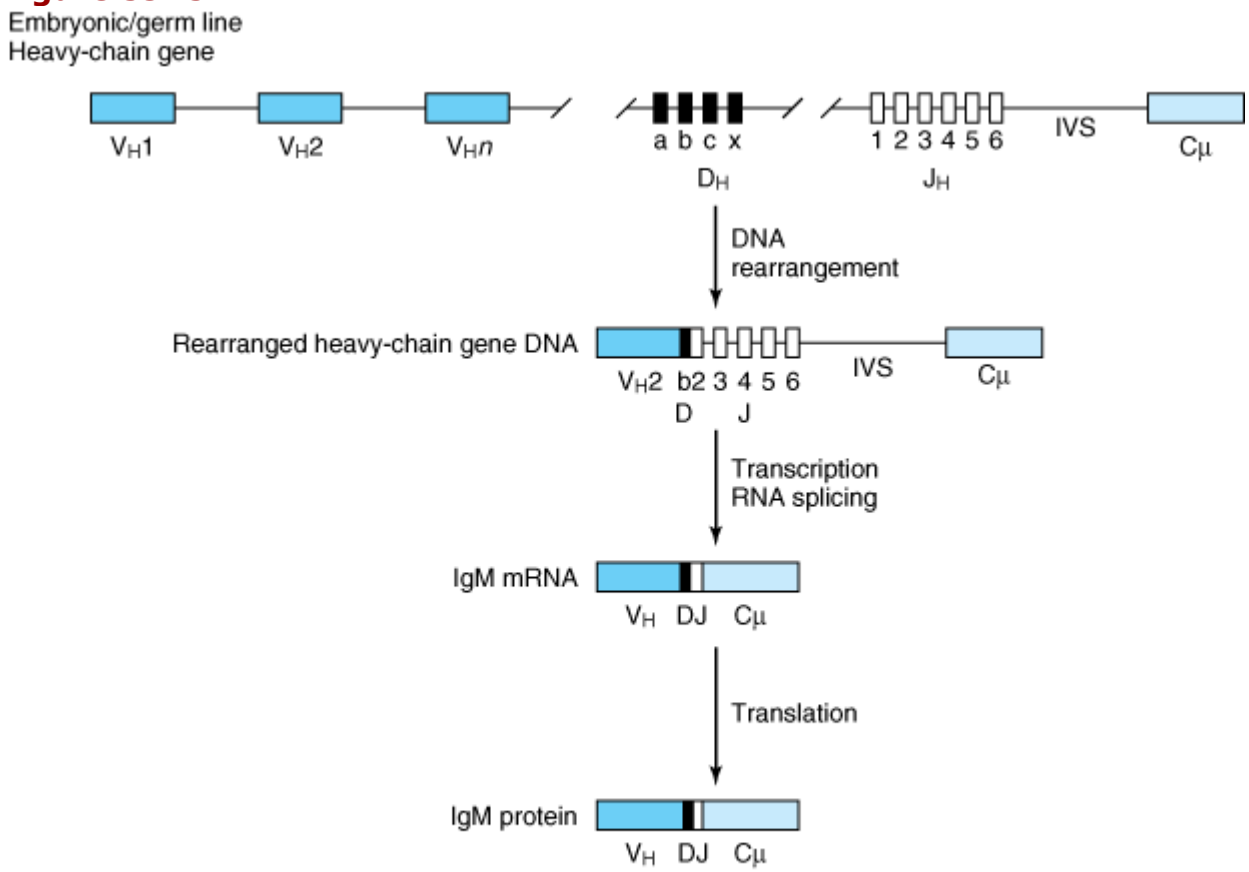
IMMUNOGLOBULIN GENES

To produce the very large number of different immunoglobulin molecules (10^6–10^9) without requiring excessive numbers of genes, special genetic mechanisms, e.g.,

DNA rearrangement and **RNA splicing,** are used. The DNA rearrangements are performed by **recombinases.** Two important genes that encode recombinases are RAG-1 and RAG-2 (recombination-activating genes). Mutations in these genes arrest the development of lymphocytes and result in severe combined immunodeficiency (see Severe Combined Immunodeficiency Disease).

Each of the four immunoglobulin chains consists of two distinct regions: a variable (V) and a constant (C) region. For each type of immunoglobulin chain, i.e., kappa light chain (κL), lambda light chain (λL), and the five heavy chains (γH, αH, μH, εH, and δH), there is a separate pool of gene segments located on different chromosomes.[7] Each pool contains a set of different V gene segments widely separated from the D (diversity, seen only in H chains), J (joining), and C gene segments (Figure 59–5). In the synthesis of an H chain, for example, a particular V region is translocated to lie close to a D segment, several J segments, and a C region. These genes are transcribed into mRNA, and all but one of the J segments are removed by splicing the RNA. During B-cell differentiation the first translocation brings a V_H gene near a $C\mu$ gene, leading to the formation of IgM as the first antibody produced in a primary response. Note that the J (joining) gene does *not* encode the J chain found in IgM and IgA. Note also that the DNA of the unused V, H, and J genes is discarded so a particular B cell is committed to making antibody with only one specificity.

Figure 59–5.



Source: Levinson W: *Review of Medical Microbiology and Immunology*, 10th Edition: http://www.accessmedicine.com

Gene rearrangement to produce a $^{\mu}$H chain. The antigen-binding site is formed by randomly choosing one of the V_H genes, one of the D_H genes, and one of the J_H genes. After transcription and RNA splicing, the mRNA is translated to produce an IgM heavy chain. V, variable regions; D, diversity segments; J, joining segments; C, constant region; IVS, intervening sequence. (Modified and reproduced, with permission, from Stites DP, Terr A, Parslow T [editors]: *Basic & Clinical Immunology,* 8th ed. Originally published by Appleton & Lange. Copyright © 1994 by The McGraw-Hill Companies, Inc.)

The V region of each L chain is encoded by two gene segments (V + J). The V region of each H chain is encoded by three gene segments (V + D + J). These various segments are united into one functional V gene by DNA rearrangement. Each of these assembled V genes is then transcribed with the appropriate C genes and spliced to produce an mRNA that codes for the complete peptide chain. L and H chains are synthesized separately on polysomes and then assembled in the cytoplasm by means of disulfide bonds to form H2L2 units. Finally, an oligosaccharide is added to the constant region of the heavy chain and the immunoglobulin molecule is released from the cell.

The gene organization mechanism outlined above permits the assembly of a very large number of different molecules. Antibody **diversity** depends on (1) multiple gene segments, (2) their rearrangement into different sequences, (3) the combining of different L and H chains in the assembly of immunoglobulin molecules, and (4) mutations. A fifth mechanism called junctional diversity applies primarily to the antibody heavy chain. Junctional diversity occurs by the addition of new nucleotides at the splice junctions between the V-D and D-J gene segments.

The diversity of the T-cell antigen receptor is also dependent on the joining of V, D, and J gene segments and the combining of different alpha and beta polypeptide chains. However, unlike antibodies, mutations do *not* play a significant role in the diversity of the T-cell receptor.

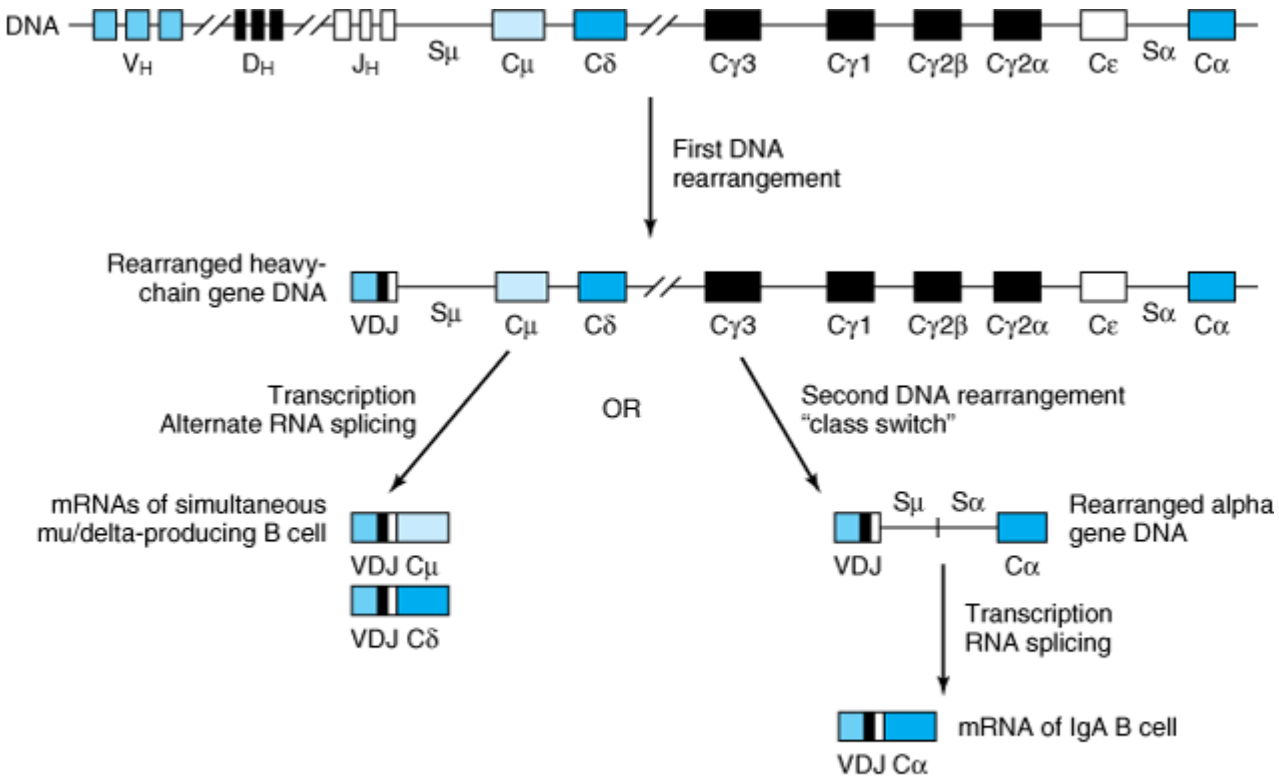
Several lymphoid cancers manifest chromosomal translocations involving the VDJ region and a cellular oncogene. For example, in Burkitt's lymphoma, the *c-myc* oncogene on chromosome 8 is translocated to a position adjacent to the VDJ region of a heavy-chain gene. The active promoter of the heavy-chain gene increases transcription of the *c-myc* oncogene, which predisposes to malignancy.

[7]The genes for κL, λL, and the five heavy chains are on chromosomes 2, 22, and 14, respectively.

IMMUNOGLOBULIN CLASS SWITCHING (ISOTYPE SWITCHING)

Initially, all B cells carry IgM specific for an antigen and produce IgM antibody in response to exposure to that antigen. Later, gene rearrangement permits the elaboration of antibodies of the same antigenic specificity but of different immunoglobulin classes (Figure 59–6). Note that the antigenic specificity **remains the same** for the lifetime of the B cell and plasma cell because the specificity is determined by the variable region genes (V, D, and J genes on the heavy chain and V and J genes on the light chain) no matter which heavy-chain constant region is being utilized.

Figure 59–6.



Source: Levinson W: *Review of Medical Microbiology and Immunology*, 10th Edition: http://www.accessmedicine.com

Gene rearrangement to produce different immunoglobulin classes. IgM is formed first because the $^{\mu}$constant region is closest to the VDJ DNA. Later the $^{\mu}$constant region can be switched with a γ, ε, or α constant region to form the heavy chain of IgG, IgE, or IgA, respectively. Note that the antigenic specificity of the B cell remains the same because the VDJ DNA remains the same. V, variable regions; D, diversity segments; J, joining segments; C, constant regions; S, switch sites. (Modified and reproduced, with permission, from Stites DP, Terr A, Parslow T [editors]: *Basic & Clinical Immunology,* 8th ed. Originally published by Appleton & Lange. Copyright © 1994 by The McGraw-Hill Companies, Inc.)

In **class switching,** the same assembled V_H gene can sequentially associate with different C_H genes so that the immunoglobulins produced later (IgG, IgA, or IgE) are specific for the same antigen as the original IgM but have different biologic characteristics. This is illustrated in the "class switch" section of Figure 59–6. A different molecular mechanism is involved in the switching from IgM to IgD. In this case, a single mRNA consisting of VDJ C^{μ}Cδ is initially transcribed and is then spliced into separate VDJ C^{μ} and VDJ Cδ mRNAs. Mature B cells can, in this manner, express both IgM and IgD (see Figure 59–6, alternative RNA splicing). Note that once a B cell has "class" switched past a certain H-chain gene, it can no longer make that class of

H chain because the intervening DNA is excised and discarded. Class switching occurs only with heavy chains; light chains do not undergo class switching. "Switch recombinase" is the enzyme that catalyzes the rearrangement of the VDJ genes during class switching.

The control of class switching is dependent on at least two factors. One is the concentration of various interleukins. For example, IL-4 enhances the production of IgE, whereas IL-5 increases IgA (see Table 58–7). The other is the interaction of the CD40 protein on the B cell with CD40 ligand protein on the helper T cell. In hyper-IgM syndrome, the failure to interact properly results in an inability of the B cell to switch to the production of IgG, IgA, or IgE. Therefore, only IgM is made (see Chapter 68).

ALLELIC EXCLUSION

A single B cell expresses only one L-chain gene (either κ or λ) and one H-chain gene. In theory, a B cell could express two sets of immunoglobulin genes, a maternal set and a paternal set. But this is *not* what happens. Only one set of genes is expressed, either maternal or paternal, and the other set is silent; i.e., it is excluded. This is called **allelic exclusion.** Each individual contains a mixture of B cells, some expressing the paternal genes and others the maternal ones. The mechanism of this exclusion is unknown.

CATALYTIC ANTIBODY

Antibody can act as an enzyme to catalyze the synthesis of ozone (O_3) that has microbicidal activity. Antibody can take the singlet oxygen produced by neutrophils and react it with water to produce hydrogen peroxide and O_3. The O_3 generated can kill *Escherichia coli.* The catalytic function of antibodies is independent of their antigen specificity and of the requirement to bind to any antigen. The importance of these observations to our host defenses remains to be determined.

Chapter 60. Humoral Immunity

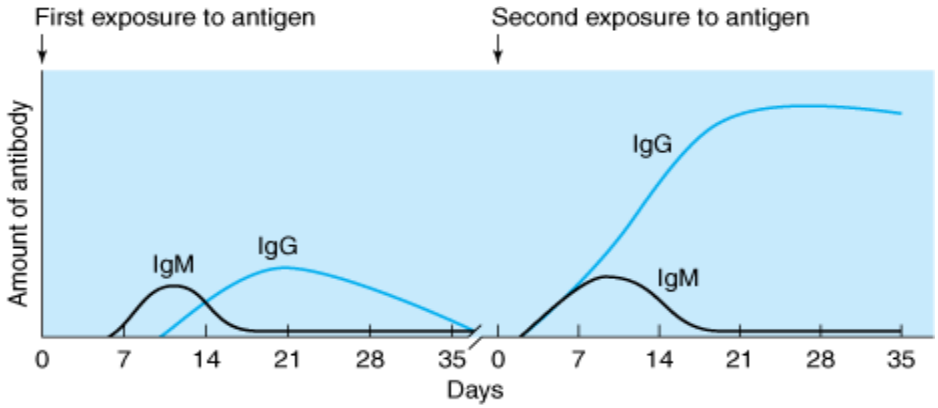
HUMORAL IMMUNITY: INTRODUCTION

Humoral (antibody-mediated) immunity is directed primarily against (1) exotoxin-mediated diseases such as tetanus and diphtheria, (2) infections in which virulence is related to polysaccharide capsules (e.g., pneumococci, meningococci, *Haemophilus influenzae*), and (3) certain viral infections. In this chapter the kinetics of antibody synthesis, i.e., the primary and secondary responses, are described. The functions of the various immunoglobulins are summarized in this chapter and described in detail in Chapter 59.

THE PRIMARY RESPONSE

When an antigen is first encountered, antibodies are detectable in the serum after a **longer lag period** than occurs in the secondary response. The lag period is typically **7–10 days** but can be longer depending on the nature and dose of the antigen and the route of administration (e.g., parenteral or oral). A small clone of B cells and plasma cells specific for the antigen is formed. The serum antibody concentration continues to rise for several weeks, then declines and may drop to very low levels (Figure 60–1). The **first** antibodies to appear are **IgM,** followed by IgG or IgA. IgM levels decline earlier than IgG levels.

Figure 60–1.



Source: Levinson W: *Review of Medical Microbiology and Immunology*, 10th Edition: http://www.accessmedicine.com

Antibody synthesis in the primary and secondary responses. In the primary response, IgM is the first type of antibody to appear. In the secondary response, IgG appears earlier and shows a more rapid rise and a higher final concentration than in the primary response. If at the time of the second exposure to the antigen (Ag1), a second, non-cross-reacting antigen (Ag2) was injected, a primary response to Ag2 would occur while a secondary response to Ag1 was occurring.

THE SECONDARY RESPONSE

When there is a second encounter with the same antigen or a closely related (or cross-reacting) one, months or years after the primary response, there is a **rapid** antibody response (the lag period is typically only **3–5 days**) to **higher** levels than the primary response. This is attributed to the persistence of antigen-specific "memory cells" after the first contact. These memory cells proliferate to form a large clone of specific B cells and plasma cells, which mediate the secondary antibody response.

During the secondary response, the amount of IgM produced is similar to that after the first contact with antigen. However, a much **larger** amount of **IgG** antibody is produced and the levels tend to persist much **longer** than in the primary response.

With each succeeding exposure to the antigen, the antibodies tend to bind antigen more firmly. Antibody binding improves because mutations occur in the DNA that encodes the antigen-binding site, a process called **somatic hypermutation.** Some mutations result in the insertion of different amino acids in the hypervariable region that result in a better fit and cause the antigen to be bound more strongly. The subset of plasma cells with these improved hypervariable regions are more strongly (and more frequently) selected by antigen and therefore constitute an increasingly larger part of the population of antibody-producing cells. This process is called **affinity maturation.** One important effect of booster doses of vaccines is to improve antibody binding by enhancing the affinity maturation process.

Affinity maturation occurs in the germinal centers of the follicles in the spleen and lymph nodes. Follicle dendritic cells capture antigen–antibody complexes on their surface via Fc receptors. The complexes interact with an activated B cell bearing the immunoglobulin that best fits the antigen, and it is that B cell that is stimulated to form a clone of many B cells capable of synthesizing the improved antibody.

RESPONSE TO MULTIPLE ANTIGENS ADMINISTERED SIMULTANEOUSLY

When two or more antigens are administered at the same time, the host reacts by producing antibodies to all of them. Competition of antigens for antibody-producing mechanisms occurs experimentally but appears to be of little significance in medicine. Combined immunization is widely used, e.g., the diphtheria, tetanus, and pertussis (DTP) vaccine or the measles, mumps, rubella (MMR) vaccine.

FUNCTION OF ANTIBODIES

The primary function of antibodies is to protect against infectious agents or their products (see Table 59–2). Antibodies provide protection because they can (1) **neutralize** toxins and viruses and (2) **opsonize** microorganisms. Opsonization is

the process by which antibodies make microorganisms more easily ingested by phagocytic cells. This occurs by either of two reactions: (1) The Fc portion of IgG interacts with its receptors on the phagocyte surface to facilitate ingestion or (2) IgG or IgM activates complement to yield C3b, which interacts with its receptors on the surface of the phagocyte.

Antibodies can be induced **actively** in the host or acquired **passively** and are thus immediately available for defense. In medicine, passive immunity is used in the neutralization of the toxins of diphtheria, tetanus, and botulism by antitoxins and in the inhibition of such viruses as rabies and hepatitis A and B viruses early in the incubation period.

ANTIBODIES IN THE FETUS

IgM is the antibody made in greatest amounts by the fetus. Small amounts of fetal IgG and IgA are made also. Note, however, that the fetus has more total IgG than IgM because maternal IgG passes the placenta in large amounts.

TESTS FOR EVALUATION OF HUMORAL IMMUNITY

Evaluation of humoral immunity consists primarily of measuring the amount of each of the three important immunoglobulins, i.e., IgG, IgM, and IgA, in the patient's serum. This is usually done by radial immunodiffusion. Immunoelectrophoresis can also provide valuable information. These techniques are described in Chapter 64.

Chapter 61. Cell-Mediated Immunity

CELL-MEDIATED IMMUNITY: INTRODUCTION

Although humoral (antibody-mediated immunity) is an important host defense against many bacterial and viral diseases, in many other bacterial infections (especially intracellular infections such as tuberculosis) and viral infections, it is primarily the cell-mediated arm that imparts resistance and aids in recovery. Furthermore, cell-mediated immunity is important in defense against fungi, parasites, and tumors and in the rejection of organ transplants. The strongest evidence for the importance of cell-mediated immunity comes from clinical situations in which its suppression (by immunosuppressive drugs or disease, e.g., AIDS) results in overwhelming infections or tumors.

The constituents of the cell-mediated immune system include several cell types: (1) **macrophages,** which present the antigen to T cells; (2) **helper T cells,** which participate in antigen recognition and in regulation (helper and suppressor) functions (see Chapter 58); (3) **natural killer (NK) cells,** which can inactivate pathogens; and (4) **cytotoxic T cells,** which can kill virus-infected cells with or without antibody. Macrophages and helper T cells produce cytokines that activate helper and cytotoxic T cells, leading to the killing of the pathogen or tumor cell.

Infection with some viruses, namely, measles virus and cytomegalovirus, can suppress cell-mediated immunity against other microorganisms. In particular, measles virus infection in people infected with *Mycobacterium tuberculosis* can result in a loss of PPD skin test reactivity, reactivation of dormant organisms, and clinical disease. A proposed explanation for these findings is that when measles virus binds to its receptor on the surface of human macrophages, the production of IL-12 by the macrophages, which is necessary for cell-mediated immunity to occur, is suppressed.

The terms primary and secondary response are associated primarily with antibody formation as described in Chapter 60, but the timing of the T-cell response also follows the same pattern. After the initial exposure to the antigen, the specific T cell proliferates to form a small clone of cells; i.e., a primary response occurs. Then, on subsequent exposure to the antigen, the small clone expands and many more specific T cells are formed. These cells constitute the secondary response.

Although the interactions between various cells and various cytokines are complex, the result is relatively simple: In the person with competent cellular immunity, opportunistic pathogens rarely or never cause disease, and the spread of other agents—for example, certain viruses (e.g., herpesviruses) or tumors (e.g., Kaposi's sarcoma)—is limited. The assessment of the competence of cell-mediated immunity is therefore important.

TESTS FOR EVALUATION OF CELL-MEDIATED IMMUNITY

Evaluation of the immunocompetence of persons depends either on the demonstration of delayed-type hypersensitivity to commonly present antigens (equating the ability to respond with the competence of cell-mediated immunity) or on laboratory assessments of T cells.

In Vivo Tests for Lymphoid Cell Competence (Skin Tests)

SKIN TESTS FOR THE PRESENCE OF DELAYED-TYPE HYPERSENSITIVITY

Most normal persons respond with delayed-type reactions to skin test antigens of *Candida,* streptokinase-streptodornase, or mumps virus because of past exposure to these antigens. Absence of reactions to several of these skin tests suggests impairment of cell-mediated immunity.

SKIN TESTS FOR THE ABILITY TO DEVELOP DELAYED-TYPE HYPERSENSITIVITY

Most normal persons readily develop reactivity to simple chemicals (e.g., dinitrochlorobenzene [DNCB]) applied to their skin in lipid solvents. When the same chemical is applied to the same area 7–14 days later, they respond with a delayed-type skin reaction. Immunocompromised persons with incompetent cell-mediated immunity fail to develop such delayed-type hypersensitivity.

In Vitro Tests for Lymphoid Cell Competence

LYMPHOCYTE BLAST TRANSFORMATION

When sensitized T lymphocytes are exposed to the specific antigen, they transform into large blast cells with greatly increased DNA synthesis, as measured by incorporation of tritiated thymidine. This *specific* effect involves relatively few cells. A larger number of T cells undergo *nonspecific* blast transformation when exposed to certain mitogens. The mitogens phytohemagglutinin and concanavalin A are plant extracts that stimulate T cells specifically. (Bacterial endotoxin, a lipopolysaccharide, stimulates B cells specifically.)

MACROPHAGE MIGRATION INHIBITORY FACTOR

Macrophage migration inhibitory factor is elaborated by cultured T cells when exposed to the antigen to which they are sensitized. Its effect can be measured by observing the reduced migration of macrophages in the presence of the factor compared with the level in controls.

ENUMERATION OF T CELLS, B CELLS, AND SUBPOPULATIONS

The number of each type of cell can be counted by use of a machine called a fluorescence-activated cell sorter (FACS) (see Chapter 64). In this approach, cells are labeled with monoclonal antibody tagged with a fluorescent dye, such as fluorescein or rhodamine. Single cells are passed through a laser light beam, and the number of cells that fluoresce is registered.

B cells (and plasma cells) making different classes of antibodies can be detected by using monoclonal antibodies against the various heavy chains. The total number of B cells can be counted by using fluorescein-labeled antibody against all immunoglobulin classes. Specific monoclonal antibodies directed against T-cell markers permit the enumeration of T-cells, CD4 helper cells, CD8 suppressor cells, and others. The normal ratio of CD4 to CD8 cells is 1.5 or greater, whereas in some immunodeficiencies (e.g., AIDS) it is less than 1.

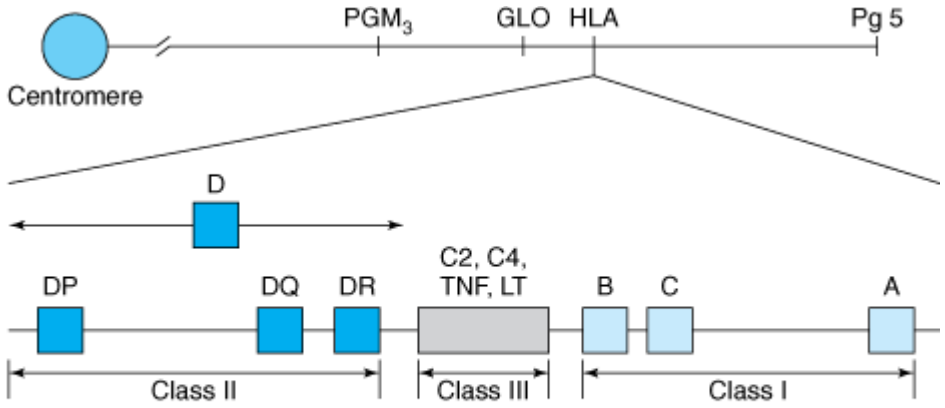
ROLE OF ADJUVANTS & LIPIDS IN ESTABLISHING CELL-MEDIATED REACTIVITY

Weak antigens or simple chemicals tend not to elicit cell-mediated hypersensitivity when administered alone, but they do so when given as a mixture with an adjuvant. The role of the **adjuvant** is to enhance the uptake of the antigen by antigen-presenting cells, e.g., macrophages, to stimulate the expression of costimulators, such as B7, and to enhance the production of cytokines, such as IL-12, that promotes the development of Th-1 cells. A common experimental adjuvant is a mixture of mineral oil, lanolin, and killed mycobacteria (Freund's adjuvant), which stimulates the formation of local granulomas. It is prohibited for human use.

MAJOR HISTOCOMPATIBILITY COMPLEX & TRANSPLANTATION: INTRODUCTION

The success of tissue and organ transplants depends on the donor's and recipient's **human leukocyte antigens** (HLA) encoded by the HLA genes. These proteins are alloantigens; i.e., they differ among members of the same species. If the HLA proteins on the donor's cells differ from those on the recipient's cells, an immune response occurs in the recipient. The genes for the HLA proteins are clustered in the major histocompatibility complex (MHC), located on the short arm of chromosome 6. Three of these genes (HLA-A, HLA-B, and HLA-C) code for the class I MHC proteins. Several HLA-D loci determine the class II MHC proteins, i.e., DP, DQ, and DR (Figure 62–1). The features of class I and class II MHC proteins are compared in Table 62–1.

Figure 62–1.



Source: Levinson W: *Review of Medical Microbiology and Immunology*, 10th Edition: http://www.accessmedicine.com

The human leukocyte antigen (HLA)-gene complex. A, B, and C are class I loci. DP, DQ, and DR are class II loci. C2 and C4 are complement loci. TNF is tumor necrosis factor; LT is lymphotoxin. PGM_3, GLO, and Pg 5 are adjacent, unrelated genes. (Reproduced, with permission, from Stites DP, Terr A, Parslow T [editors]: *Basic & Clinical Immunology,* 9th ed. Originally published by Appleton & Lange. Copyright © 1997 by The McGraw-Hill Companies, Inc.)

Table 62–1. Comparison of Class I and Class II MHC Proteins.

Feature	Class I MHC Proteins	Class II MHC Proteins
Present antigen to CD4-positive cells	No	Yes
Present antigen to CD8-positive cells	Yes	No
Found on surface of all nucleated cells	Yes	No
Found on surface of "professional" antigen-presenting cells, such as dendritic cells, macrophages, and B cells	Yes[1]	Yes
Encoded by genes in the HLA locus	Yes	Yes
Expression of genes is codominant	Yes	Yes
Multiple alleles at each gene locus	Yes	Yes
Composed of two peptides encoded in the HLA locus	No	Yes
Composed of one peptide encoded in the HLA locus and a β_2-microglobulin	Yes	No

[1]Note that class I MHC proteins are found on the surface of all nucleated cells, including those that have class II MHC proteins on their surface. Mature red blood cells are non-nucleated; therefore, they do synthesize class I MHC proteins.

Each person has two **haplotypes,** i.e., two sets of these genes, one on the paternal and the other on the maternal chromosome 6. These genes are very diverse (**polymorphic**) (i.e., there are many alleles of the class I and class II genes). For example, there are at least 47 HLA-A genes, 88 HLA-B genes, 29 HLA-C genes, and more than 300 HLA-D genes, but any individual inherits only a single allele at each locus from each parent and thus can make no more than 2 class I and II proteins at each gene locus. Expression of these genes is **codominant;** i.e., the proteins encoded by *both* the paternal and maternal genes are produced. Each person can make as many as 12 different HLA proteins: 3 at class I loci and 3 at class II loci, from both chromosomes. A person can make less than 12 different HLA proteins if the person is homozygous at any of the 6 loci, i.e., if both parents have the same HLA allele.

In addition to the major antigens encoded by the HLA genes, there are an unknown number of **minor** antigens encoded by genes at sites other than the HLA locus.

These minor antigens can induce a weak immune response that can result in slow rejection of a graft. The cumulative effect of several minor antigens can lead to a more rapid rejection response. These minor antigens are various normal body proteins that have one or more amino acid differences from one person to another; i.e., they are "allelic variants." Because these proteins have an amino acid difference, they are immunogenic when introduced as part of the donor graft tissue. There are no laboratory tests for minor antigens.

Between the class I and class II gene loci is a third locus (Figure 62–1), sometimes called class III. This locus contains several immunologically important genes, encoding two cytokines (tumor necrosis factor and lymphotoxin) and two complement components (C2 and C4), but it does not have any genes that encode histocompatibility antigens.

MHC PROTEINS

Class I MHC Proteins

These are glycoproteins found on the **surface of virtually all nucleated cells.** There are approximately 20 different proteins encoded by the allelic genes at the A locus, 40 at the B locus, and 8 at the C locus. The complete class I protein is composed of a 45,000-molecular-weight heavy chain noncovalently bound to a β_2-microglobulin. The heavy chain is highly polymorphic and is similar to an immunoglobulin molecule; it has hypervariable regions in its N-terminal region. The **polymorphism** of these molecules is important in the **recognition of self** and **nonself.** Stated another way, if these molecules were more similar, our ability to accept foreign grafts would be correspondingly improved. The heavy chain also has a constant region where the CD8 protein of the cytotoxic T cell binds.

Class II MHC Proteins

These are glycoproteins found on the surface of certain cells, including macrophages, B cells, dendritic cells of the spleen, and Langerhans' cells of the skin. They are highly polymorphic glycoproteins composed of two polypeptides (MW 33,000 and 28,000) that are noncovalently bound. Like class I proteins, they have hypervariable regions that provide much of the polymorphism. Unlike class I proteins, which have only one chain encoded by the MHC locus (β_2-microglobulin is encoded on chromosome 15), both chains of the class II proteins are encoded by the MHC locus. The two peptides also have a constant region where the CD4 proteins of the helper T cells bind.

BIOLOGIC IMPORTANCE OF MHC

The ability of T cells to recognize antigen is dependent on association of the antigen with either class I or class II proteins. For example, cytotoxic T cells respond to

antigen in association with class I MHC proteins. Thus, a cytotoxic T cell that kills a virus-infected cell will not kill a cell infected with the same virus if the cell does not also express the appropriate class I proteins. This finding was determined by mixing cytotoxic T cells bearing certain class I MHC proteins with virus-infected cells bearing different class I MHC proteins and observing that no killing of the virus-infected cells occurred. Helper T cells recognize class II proteins. Helper-cell activity depends in general on *both* the recognition of the antigen on antigen-presenting cells *and* the presence on these cells of "self" class II MHC proteins. This requirement to recognize antigen in association with a "self" MHC protein is called **MHC restriction.** Note that T cells recognize antigens only when the antigens are presented on the surface of cells (in association with either class I or II MHC proteins), whereas B cells do not have that requirement and can recognize soluble antigens in plasma with their surface monomer IgM acting as the antigen receptor.

MHC genes and proteins are also important in two other medical contexts. One is that many autoimmune diseases occur in people who carry certain MHC genes (see Chapter 66), and the other is that the success of organ transplants is, in large part, determined by the compatibility of the MHC genes of the donor and recipient (see below).

TRANSPLANTATION

An **autograft** (transfer of an individual's own tissue to another site in the body) is always permanently accepted, i.e., it always "takes." A **syngeneic graft**[1] is a transfer of tissue between genetically identical individuals, i.e., identical twins, and almost always "takes" permanently. A **xenograft,**[1] a transfer of tissue between different species, is always rejected by an immunocompetent recipient.

An **allograft**[1] is a graft between genetically different members of the same species, e.g., from one human to another. Allografts are usually rejected unless the recipient is given immunosuppressive drugs. The severity and rapidity of the rejection will vary depending on the degree of the differences between the donor and the recipient at the MHC loci.

Allograft Rejection

Unless immunosuppressive measures are taken, allografts are rejected by a process called the **allograft reaction.** In an acute allograft reaction, vascularization of the graft is normal initially, but in 11–14 days, marked reduction in circulation and mononuclear cell infiltration occurs, with eventual necrosis. This is called a **primary (first-set)** reaction. A **T-cell-mediated reaction is the main cause of rejection** of many types of grafts, e.g., skin, but antibodies contribute to the rejection of certain transplants, especially bone marrow. In experimental animals, rejection of

most types of grafts can be transferred by cells, not serum. Also, T-cell-deficient animals do not reject grafts but B-cell-deficient animals do.

If a second allograft from the same donor is applied to a sensitized recipient, it is rejected in 5–6 days. This **accelerated (second-set)** reaction is caused primarily by presensitized cytotoxic T cells.

The acceptance or rejection of a transplant is determined, in large part, by the class I and class II MHC proteins on the donor cells, with **class II** playing the **major** role. The proteins encoded by the DR locus are especially important. These alloantigens activate T cells, both helper and cytotoxic, which bear T-cell receptors specific for the alloantigens. The activated T cells proliferate and then react against the alloantigens on the donor cells. **CD8-positive cytotoxic T cells do most of the killing** of the allograft cells.

Foreign MHC proteins typically activate many more T cells (i.e., they elicit a much stronger reaction) than do foreign proteins that are not MHC proteins. The strength of the response to foreign MHC proteins can be explained by the observation that there are three processes by which the recipient's immune response is stimulated. These processes are (1) antigen-presenting cells (e.g., macrophages and dendritic cells) in the graft can present self (the donor's) proteins in association with their class I and class II MHC proteins and activate the recipient's immune response; (2) antigen-presenting cells in the graft can present the recipient's proteins and activate the recipient's immune response (because the recipient's proteins are recognized as foreign when presented by a foreign MHC protein); and (3) the donor's self proteins and class I and class II MHC proteins can be shed and subsequently processed by the recipient's antigen-presenting cells, which activates the recipient's immune response.

A graft that survives an acute allograft reaction can nevertheless become nonfunctional as a result of **chronic rejection.** This can occur months to years after engraftment. The main pathologic finding in grafts undergoing chronic rejection is atherosclerosis of the vascular endothelium. The immunologic cause of chronic rejection is unclear, but incompatibility of minor histocompatibility antigens and side effects of immunosuppressive drugs are likely to play a role.

In addition to acute and chronic rejection, a third type called **hyperacute rejection** can occur. Hyperacute rejection typically occurs within minutes of engraftment and is due to the reaction of **preformed** anti-ABO antibodies in the recipient with ABO antigens on the surface of the endothelium of the graft. Hyperacute rejection is often called the "white graft" reaction, because the graft turns white as a result of the loss of blood supply caused by spasm and occlusion of the vessels serving the graft. In

view of this severe rejection reaction, the ABO blood group of donors and recipients must be matched and a crossmatching test (see below) must be done.

HLA Typing in the Laboratory

Prior to transplantation surgery, laboratory tests, commonly called **HLA-typing** or **tissue-typing,** are performed to determine the closest MHC match between the donor and the recipient.

There are two methods commonly used in the laboratory to determine the haplotype (i.e., the class I and class II alleles on both chromosomes) of both the potential donors and the recipient. One method is **DNA sequencing** using polymerase chain reaction (PCR) amplification and specific probes to detect the different alleles. This method is highly specific and sensitive, and is the method of choice when available. The other method is **serologic assays,** in which cells from the donor and recipient are reacted with a battery of antibodies, each one of which is specific for a different class I and class II protein. Complement is then added, and any cell bearing an MHC protein homologous to the known antibody will lyse. This method is satisfactory in most instances but has failed to identify certain alleles that have been detected by DNA sequencing.

If sufficient data cannot be obtained by DNA sequencing or serologic assays, then additional information regarding the compatibility of the class II MHC proteins can be determined by using the **mixed lymphocyte culture (MLC)** technique. This test is also known as the **mixed lymphocyte reaction (MLR).** In this test, "stimulator" lymphocytes from a potential donor are first killed by irradiation and then mixed with live "responder" lymphocytes from the recipient; the mixture is incubated in cell culture to permit DNA synthesis, which is measured by incorporation of tritiated thymidine. The greater the amount of DNA synthesis in the responder cells, the more foreign are the class II MHC proteins of the donor cells. A large amount of DNA synthesis indicates an unsatisfactory "match"; i.e., donor and recipient class II (HLA-D) MHC proteins are *not* similar, and the graft is likely to be rejected. The best donor is, therefore, the person whose cells stimulated the incorporation of the **least** amount of tritiated thymidine in the recipient cells.

In addition to the tests used for matching, preformed cytotoxic antibodies in the recipient's serum reactive against the graft are detected by observing the lysis of donor lymphocytes by the recipient's serum plus complement. This is called **crossmatching** and is done to prevent hyperacute rejections from occurring. (The donor and recipient are also matched for the compatibility of their ABO blood groups.)

Among siblings in a single family, there is a 25% chance for both haplotypes to be shared, a 50% chance for one haplotype to be shared, and a 25% chance for no

haplotypes to be shared. For example, if the father is haplotype AB, the mother is CD, and the recipient child is AC, there is a 25% chance for a sibling to be AC, i.e., a two-haplotype match; a 50% chance for a sibling to be either BC or AD, i.e., a one-haplotype match; and a 25% chance for a sibling to be BD, i.e., a zero-haplotype match.

The Fetus Is an Allograft that Is Not Rejected

A fetus has MHC genes inherited from the father that are foreign to the mother, yet allograft rejection of the fetus does not occur. This is true despite many pregnancies from the same mother-father combination that produce offspring with the same MHC haplotypes. The reason that the mother fails to reject the fetus is unclear. The mother forms antibodies against the foreign paternal MHC proteins; therefore, the reason is not that the mother is not exposed to fetal antigens. One possible explanation is that the trophoblast layer of the placenta does not allow maternal T cells to enter the fetus.

Results of Organ Transplants

If the donor and recipient are well matched by mixed-lymphocyte culture and histocompatibility antigen-typing, the long-term survival of a transplanted organ or tissue is greatly enhanced. In 1986, the 5-year survival rate of two-haplotype-matched kidney transplants from related donors was near 95%, that of one-haplotype-matched kidney transplants was near 80%, and that of transplant of kidneys from cadaver donors was near 60%. The survival rate of the last category was higher if the graft recipient had had several previous blood transfusions. The reason for this is unknown (but may be associated with tolerance). The heart transplant survival rate for 5 years is near 50–60%; the liver transplant rate is lower. Corneas are easily grafted because they are avascular and the lymphatic supply of the eye prevents many antigens from triggering an immune response; consequently, the proportion of "takes" is very high. Because corneal transplants elicit a weak rejection response, immunosuppression is usually necessary for only a year. In contrast, most other transplants require lifelong immunosuppression, although the dose of immunosuppressive drugs typically decreases with time and in some recipients a state of tolerance ensues and the drugs can be stopped.

Graft-versus-Host Reaction

Well-matched transplants of bone marrow may establish themselves initially in 85% of recipients, but subsequently a **graft-versus-host (GVH)** reaction develops in about two-thirds of them.[2]

This reaction occurs because grafted immunocompetent T cells proliferate in the irradiated, immunocompromised host and reject cells with "foreign" proteins, resulting in severe organ dysfunction. The donor's cytotoxic T cells play a major role

in destroying the recipient's cells. Among the main symptoms are maculopapular rash, jaundice, hepatosplenomegaly, and diarrhea. Many GVH reactions end in overwhelming infections and death.

There are three requirements for a GVH reaction to occur: (1) the graft must contain immunocompetent T cells; (2) the host must be immunocompromised; and (3) the recipient must express antigens (e.g., MHC proteins) foreign to the donor; i.e., the donor T cells recognize the recipient cells as foreign. Note that even when donor and recipient have identical class I and class II MHC proteins, i.e., identical haplotypes, a GVH reaction can occur because it can be elicited by differences in minor antigens. The GVH reaction can be reduced by treating the donor tissue with antithymocyte globulin or monoclonal antibodies before grafting; this eliminates mature T cells from the graft. Cyclosporine (see below) is also used to reduce the GVH reaction.

[1]Previously used synonyms for these terms include isograft (syngeneic graft), heterograft (xenograft), and homograft (allograft).

[2]GVH reactions can also occur in immunodeficient patients given a blood transfusion, because there are immunocompetent T cells in the donor's blood that react against the recipient's cells.

EFFECT OF IMMUNOSUPPRESSION ON GRAFT REJECTION

To reduce the chance of rejection of transplanted tissue, immunosuppressive measures, e.g., cyclosporine, tacrolimus (FK506, Prograf), sirolimus (rapamycin, Rapamune), corticosteroids, azathioprine, monoclonal antibodies, and radiation, are used. Cyclosporine prevents the activation of T lymphocytes by inhibiting the synthesis of IL-2 and IL-2 receptor. It does so by inhibiting calcineurin, a protein (a serine phosphatase) involved in the activation of transcription of the genes for IL-2 and the IL-2 receptor. Cyclosporine is well-tolerated and is remarkably successful in preventing the rejection of transplants. Cyclosporine and tacrolimus have the same mode of action; tacrolimus is more immunosuppressive but causes more side effects. Rapamycin also inhibits signal transduction but at a site different from that of cyclosporine and tacrolimus.

Corticosteroids act primarily by inhibiting cytokine (e.g., IL-1 and tumor necrosis factor) production by macrophages and by lysing certain types of T cells. Corticosteroids inhibit cytokine production by blocking transcription factors, such as NFkB and AP-1, which prevents the mRNA for these cytokines from being synthesized. Azathioprine is an inhibitor of DNA synthesis and blocks the growth of T cells. Mycophenolate mofetil also inhibits DNA synthesis and has fewer side effects than azathioprine.

Monoclonal antibodies are used in immunosuppressive regimens, both to prevent rejection and to treat rejection episodes. Muromonab (OKT3) is a monoclonal antibody against CD3, and basiliximab and daclizumab are monoclonal antibodies against the IL-2 receptor. Table 62–2 describes these monoclonal antibodies as well as others in clinical use.

Table 62–2. Monoclonal Antibodies in Clinical Use.

Clinical Function	Name of the Monoclonal Antibody[1]	Target of Antibody	Specific Clinical Use
Transplant-related immunosuppression	1. Basiliximab, daclizumab	IL-2 receptor	Prevent or treat allograft rejection and graft-versus-host reaction
	2. Muromonab (OKT3)	CD3 on T cells	
Treatment of autoimmune disease	1. Infliximab	Tumor necrosis factor-α	Treat rheumatoid arthritis and Crohn's disease (regional ileitis)
	2. Adalimumab		
	3. Natalizumab	α-integrin	Treatment of multiple sclerosis and Crohn's disease
Prevention of infectious disease	Palivizumab	Fusion protein of respiratory syncytial virus	Prevent pneumonia in susceptible neonates
Treatment of cancer	1. Rituximab	CD20 protein on B cells	Treat non-Hodgkins lymphoma
	2. Trastuzumab	Epidermal growth factor receptor	Treat breast cancer

[1] Note that most of the names end in "mab," which is an abbreviation for monoclonal antibodies.

Unfortunately, immunosuppression greatly enhances the recipient's susceptibility to opportunistic infections and neoplasms. For example, some patients undergoing treatment for multiple sclerosis with the monoclonal antibody, natalizumab, developed progressive multifocal leukoencephalopathy (see Chapter 44 for a description of this viral disease). The incidence of cancer is increased as much as 100-fold in transplant recipients. Immunosuppressive drugs, e.g., cyclosporine, also reduce GVH reactions.

Note that although these drugs suppress the allograft reaction, tolerance to the graft tissue does not ensue. Therefore, most patients must take these drugs during their entire lives.

Chapter 63. Complement

COMPLEMENT: INTRODUCTION

The complement system consists of approximately 20 proteins that are present in normal human (and other animal) serum. The term "complement" refers to the ability of these proteins to complement, i.e., augment, the effects of other components of the immune system, e.g., antibody. Complement is an important component of our innate host defenses.

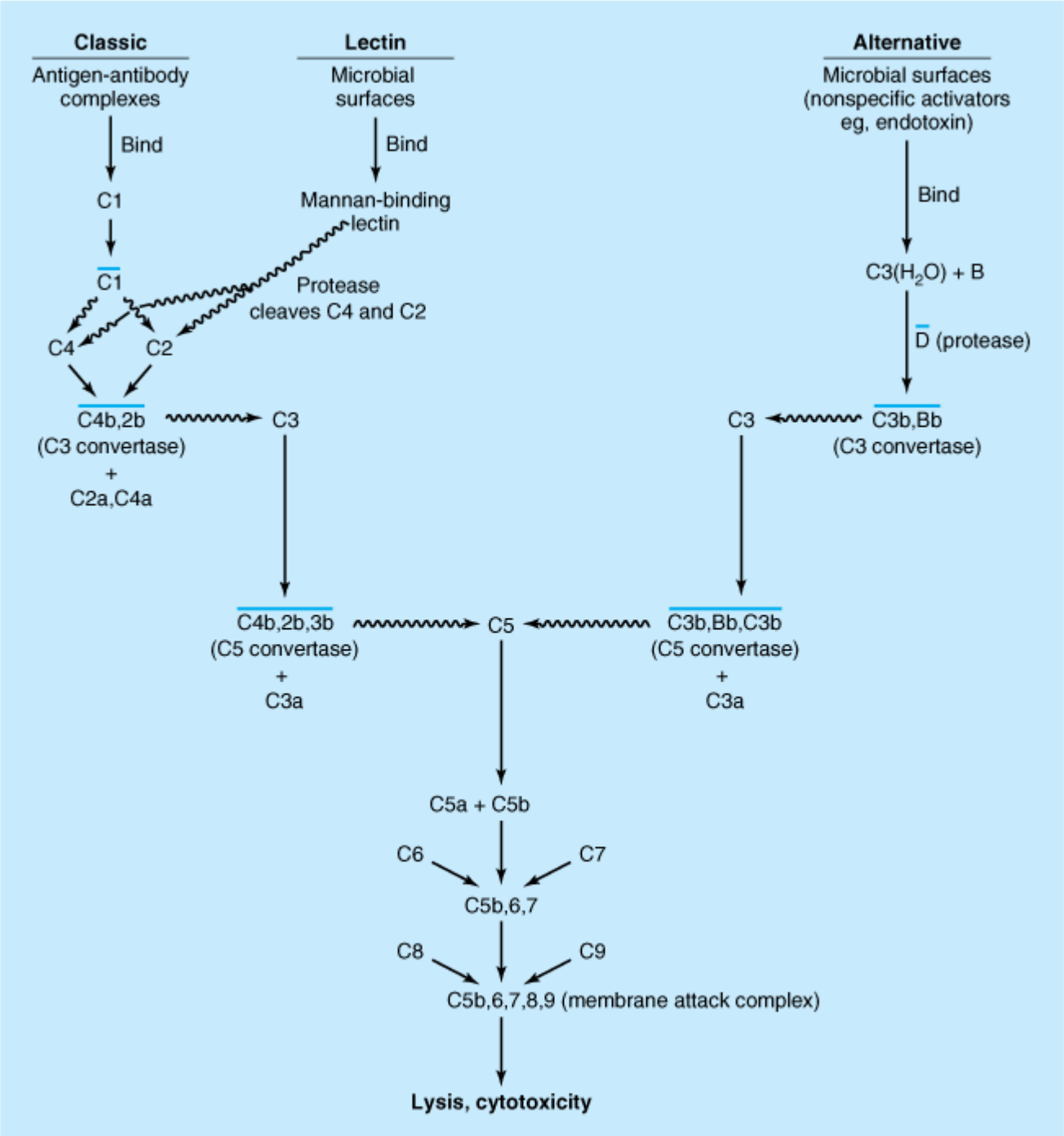
There are three main effects of complement: (1) **lysis** of cells such as bacteria, allografts, and tumor cells; (2) **generation of mediators** that participate in inflammation and attract neutrophils; and (3) **opsonization,** i.e., enhancement of phagocytosis. Complement proteins are synthesized mainly by the liver. Complement is heat-labile; i.e., it is inactivated by heating serum at 56°C for 30 minutes. Immunoglobulins are not inactivated at this temperature.

ACTIVATION OF COMPLEMENT

Several complement components are proenzymes, which must be cleaved to form active enzymes. Activation of the complement system can be initiated either by antigen–antibody complexes or by a variety of nonimmunologic molecules, e.g., endotoxin.

Sequential activation of complement components (Figure 63–1) occurs via one of three pathways: the classic pathway, the lectin pathway, and the alternative pathway (see below). Of these pathways, the **lectin and the alternative pathways are more important the first time** we are infected by a microorganism because the antibody required to trigger the classic pathway is not present. The lectin pathway and the alternative pathway are, therefore, participants in the innate arm of the immune system.

Figure 63–1.



Source: Levinson W: *Review of Medical Microbiology and Immunology*, 10th Edition: http://www.accessmedicine.com

The classic and alternative pathways of the complement system. ⇝ indicates that proteolytic cleavage of the molecule at the tip of the arrow has occurred; a line over a complex indicates that it is enzymatically active. Note that the nomenclature of the cleavage

products of C2 is undecided. Some call the large fragment C2a and others call it C2b. In this book, all small fragments are labeled "a," and all large fragments are labeled "b." Hence, the C3 convertase is depicted as C4b,2b. Note that proteases associated with the mannose-binding lectin cleave C4 as well as C2.

All three pathways lead to the production of **C3b, the central molecule** of the complement cascade. The presence of C3b on the surface of a microbe marks it as foreign and targets it for destruction. C3b has two important functions: (1) It combines with other complement components to generate C5 convertase, the enzyme that leads to the production of the membrane attack complex and (2) it opsonizes bacteria because phagocytes have receptors for C3b on their surface.

1. In the **classic** pathway, antigen–antibody complexes[1] activate C1[2] to form a protease, which cleaves C2 and C4 to form a C4b,2b complex. The latter is C3 convertase, which cleaves C3 molecules into two fragments, C3a and C3b. C3a, an **anaphylatoxin,** is discussed below. C3b forms a complex with C4b,2b, producing a new enzyme, C5 convertase (C4b,2b,3b), which cleaves C5 to form C5a and C5b. C5a is an anaphylatoxin and a chemotactic factor (see below). C5b binds to C6 and C7 to form a complex that interacts with C8 and C9 to produce the **membrane attack** complex (C5b,6,7,8,9), which causes cytolysis. Note that the "b" fragment continues in the main pathway, whereas the "a" fragment is split off and has other activities.
2. In the **lectin** pathway, mannan-binding lectin (MBL) (also known as mannose-binding protein) binds to the surface of microbes bearing mannan (a polymer of the sugar, mannose). This activates proteases associated with MBL that cleave C2 and C4 components of complement and activate the classic pathway. Note that this process bypasses the antibody-requiring step and so is protective early in infection before antibody is formed.
3. In the **alternative** pathway, many unrelated cell surface substances, e.g., bacterial lipopolysaccharides (endotoxin), fungal cell walls, and viral envelopes, can initiate the process by binding C3(H_2O) and factor B. This complex is cleaved by a protease, factor D, to produce C3b,Bb. This acts as a C3 convertase to generate more C3b.

[1]Only IgM and IgG fix complement. One molecule of IgM can activate complement; however, activation by IgG requires two cross-linked IgG molecules. C1 is bound to a site located in the Fc region of the heavy chain. Of the IgGs, only IgG1, IgG2, and IgG3 subclasses fix complement; IgG4 does not.

[2]C1 is composed of three proteins, C1q, C1r, and C1s. C1q is an aggregate of 18 polypeptides that binds to the Fc portion of IgG and IgM. It is multivalent and can cross-link several immunoglobulin molecules. C1s is a proenzyme that is cleaved to form an active protease. Calcium is required for the activation of C1.

REGULATION OF THE COMPLEMENT SYSTEM

The first regulatory step in the classic pathway is at the level of the antibody itself. The complement-binding site on the heavy chain of IgM and IgG is unavailable to the C1 component of complement if antigen is not bound to these antibodies. This means that complement is not activated by IgM and IgG despite being present in the blood at all times. However, when antigen binds to its specific antibody, a conformational shift occurs and the C1 component can bind and initiate the cascade.

Several serum proteins regulate the complement system at different stages.

1. C1 inhibitor is an important regulator of the classic pathway. It inactivates the protease activity of C1. Activation of the classic pathway proceeds past this point by generating sufficient C1 to overwhelm the inhibitor.
2. Regulation of the alternative pathway is mediated by the binding of factor H to C3b and cleavage of this complex by factor I, a protease. This reduces the amount of C5 convertase available. The alternative pathway can proceed past this regulatory point if sufficient C3b attaches to cell membranes. Attachment of C3b to cell membranes protects it from degradation by factors H and I. Another component that enhances activation of the alternative pathway is properdin, which protects C3b and stabilizes the C3 convertase.
3. Protection of human cells from lysis by the membrane attack complex of complement is mediated by **decay-accelerating factor** (DAF, CD55), a glycoprotein located on the surface of human cells. DAF acts by binding to C3b and C4b and limiting the formation of C3 convertase and C5 convertase. This prevents the formation of the membrane attack complex.

BIOLOGIC EFFECTS OF COMPLEMENT

Opsonization

Microbes, such as bacteria and viruses are phagocytized much better in the presence of C3b because there are C3b receptors on the surface of many phagocytes.

Chemotaxis

C5a and the C5, 6, 7 complex attract neutrophils. They migrate especially well toward C5a. C5a also enhances the adhesiveness of neutrophils to the endothelium.

Anaphylatoxin

C3a, C4a, and C5a cause degranulation of mast cells with release of mediators, e.g., histamine, leading to increased vascular permeability and smooth muscle contraction, especially contraction of the bronchioles leading to bronchospasm. Anaphylatoxins can also bind directly to smooth muscle cells of the bronchioles and cause bronchospasm. C5a is, by far, the most potent of these anaphylatoxins.

Anaphylaxis caused by these complement components is less common than anaphylaxis caused by type I (IgE-mediated) hypersensitivity (see Chapter 65).

Cytolysis

Insertion of the C5b,6,7,8,9 complex into the cell membrane leads to killing or lysis of many types of cells including erythrocytes, bacteria, and tumor cells. Cytolysis is not an enzymatic process; rather, it appears that insertion of the complex results in disruption of the membrane and the entry of water and electrolytes into the cell.

Enhancement of Antibody Production

The binding of C3b to its receptors on the surface of activated B cells greatly enhances antibody production compared with that by B cells that are activated by antigen alone. The clinical importance of this is that patients who are deficient in C3b produce significantly less antibody than do those with normal amounts of C3b. The low concentration of both antibody and C3b significantly impairs host defenses, resulting in multiple, severe pyogenic infections.

CLINICAL ASPECTS OF COMPLEMENT

1. Inherited (or acquired) deficiency of some complement components, especially C5–C8, greatly enhances susceptibility to ***Neisseria*bacteremia** and other infections. A deficiency of MBL also predisposes to severe ***Neisseria*** infections. A deficiency of C3 leads to severe, recurrent pyogenic sinus and respiratory tract infections.

2. Inherited deficiency of C1 esterase inhibitor results in **angioedema.** When the amount of inhibitor is reduced, an overproduction of esterase occurs. This leads to an increase in anaphylatoxins, which cause capillary permeability and edema.

3. Acquired deficiency of decay-accelerating factor on the surface of cells results in an increase in complement-mediated hemolysis. Clinically, this appears as the disorder paroxysmal nocturnal hemoglobinuria (see Chapter 68).

4. In transfusion mismatches, e.g., when type A blood is given by mistake to a person who has type B blood, antibody to the A antigen in the recipient binds to A antigen on the donor red cells, complement is activated, and large amounts of anaphylatoxins and membrane attack complexes are generated. The anaphylatoxins cause shock, and the membrane attack complexes cause red cell hemolysis.

5. Immune complexes bind complement, and thus complement levels are low in immune complex diseases, e.g., acute glomerulonephritis and systemic lupus erythematosus. Binding (activating) complement attracts polymorphonuclear leukocytes, which release enzymes that damage tissue.

6. Patients with severe liver disease, e.g., alcoholic cirrhosis or chronic hepatitis B, who have lost significant liver function and therefore cannot synthesize sufficient complement proteins, are predisposed to infections caused by pyogenic bacteria.

ANTIGEN–ANTIBODY REACTIONS IN THE LABORATORY: INTRODUCTION

Reactions of antigens and antibodies are highly specific. An antigen will react only with antibodies elicited by itself or by a closely related antigen. Because of the great specificity, reactions between antigens and antibodies are suitable for identifying one by using the other. This is the basis of serologic reactions. However, cross-reactions between related antigens can occur, and these can limit the usefulness of the test. The results of many immunologic tests are expressed as a **titer,** which is defined as the highest dilution of the specimen, e.g., serum, that gives a positive reaction in the test. Note that a patient's serum with an antibody titer of, for example, 1/64 contains **more** antibodies, i.e., is a higher titer, than a serum with a titer of, for example, 1/4.

Table 64–1 describes the medical importance of serologic (antibody-based) tests. Their major uses are in the diagnosis of infectious diseases, in the diagnosis of autoimmune diseases, and in the typing of blood and tissues prior to transplantation.

Table 64–1. Major Uses of Serologic (Antibody-Based) Tests.

I. Diagnosis of infectious diseases When the organism cannot be cultured, e.g., syphilis and hepatitis A, B, and C. When the organism is too dangerous to culture, e.g., rickettsial diseases. When culture techniques are not readily available, e.g., HIV, EBV. When the organism takes too long to grow, e.g., *Mycoplasma.*
One problem with this approach is that it takes time for antibodies to form, e.g., 7–10 days in the primary response. For this reason, acute and convalescent serum samples are taken and a 4-fold or greater rise in antibody titer is required to make a diagnosis. By this time the patient has often recovered and the diagnosis becomes a retrospective one. If a test is available that can detect IgM antibody in the patient's serum, it can be used to make a diagnosis of current infection. In certain infectious diseases, an arbitrary IgG antibody titer of sufficient magnitude is used to make a diagnosis.
II. Diagnosis of autoimmune diseases Antibodies against various normal body components are used, e.g., antibody against DNA in systemic lupus erythematosus, antibody against

human IgG (rheumatoid factor) in rheumatoid arthritis.
III. Determination of blood type and HLA type Known antibodies are used to determine ABO and Rh blood types. Known antibodies are used to determine class I and class II HLA proteins prior to transplantation, although DNA sequencing is also being used.

Microorganisms and other cells possess a variety of antigens and thus induce antisera containing many different antibodies; i.e., the antisera are polyclonal. Monoclonal antibodies excel in the identification of antigens because cross-reacting antibodies are absent; i.e., monoclonal antibodies are highly specific.

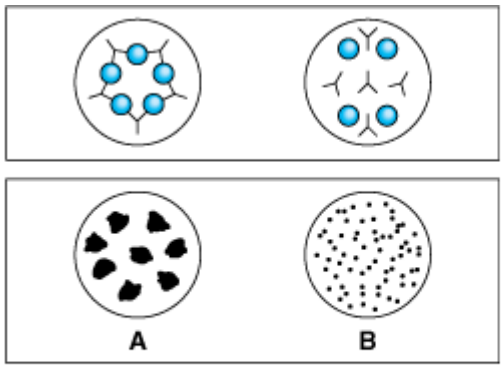
TYPES OF DIAGNOSTIC TESTS

Many types of diagnostic tests are performed in the immunology laboratory. Most of these tests can be designed to determine the presence of either antigen or antibody. To do this, one of the components, either antigen or antibody, is known and the other is unknown. For example, with a known antigen such as influenza virus, a test can determine whether antibody to the virus is present in the patient's serum. Alternatively, with a known antibody, such as antibody to herpes simplex virus, a test can determine whether viral antigens are present in cells taken from the patient's lesions.

Agglutination

In this test, the antigen is **particulate** (e.g., bacteria and red blood cells)[1] or is an inert particle (latex beads) coated with an antigen. Antibody, because it is divalent or multivalent, cross-links the antigenically multivalent particles and forms a latticework, and clumping (agglutination) can be seen. This reaction can be done in a small cup or tube or with a drop on a slide. One very commonly used agglutination test is the test that determines a person's ABO blood group (Figure 64–1; see the section on blood groups at the end of this chapter).

Figure 64–1.



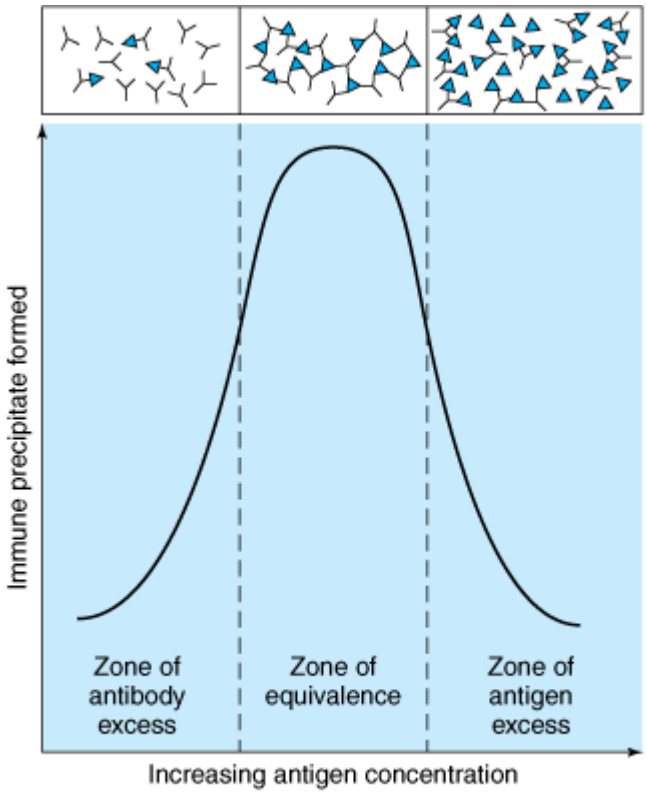
Source: Levinson W: *Review of Medical Microbiology and Immunology*, 10th Edition: http://www.accessmedicine.com

Agglutination test to determine ABO blood type. On the slide at the bottom of the figure, a drop of the patient's blood was mixed with antiserum against either type A (**left**) or type B (**right**) blood cells. Agglutination (clumping) has occurred in the drop on the left containing the type A antiserum but not in the drop containing the type B antiserum, indicating that the patient is type A, i.e., has A antigen on the red cells. The slide at the top shows that the red cells (circles) are cross-linked by the antibodies (**Y**-shapes) in the drop on the left but not in the drop on the right. If agglutination had occurred in the right side as well, it would indicate that the patient was producing B antigen as well as A and was type AB.

Precipitation (Precipitin)

In this test, the antigen is **in solution.** The antibody cross-links antigen molecules in variable proportions, and aggregates (precipitates) form. In the **zone of equivalence,** optimal proportions of antigen and antibody combine; the maximal amount of precipitates forms, and the supernatant contains neither an excess of antibody nor an excess of antigen (Figure 64–2). In the **zone of antibody excess,** there is too much antibody for efficient lattice formation, and precipitation is less than maximal.[2] In the **zone of antigen excess,** all antibody has combined, but precipitation is reduced because many antigen–antibody complexes are too small to precipitate; i.e., they are "soluble."

Figure 64–2.



Source: Levinson W: *Review of Medical Microbiology and Immunology*, 10th Edition: http://www.accessmedicine.com

Precipitin curve. In the presence of a constant amount of antibody, the amount of immune precipitate formed is plotted as a function of increasing amounts of antigen. In the top part of the figure, the binding of antigen (▲) and antibody (**Y**) in the three zones is depicted. In the zones of antibody excess and antigen excess, a lattice is not formed and precipitation does not occur, whereas in the equivalence zone, a lattice forms and precipitation is maximal. (Modified and reproduced, with permission, from Stites D, Terr A, Parslow T [editors]: *Basic & Clinical Immunology,* 9th ed. Originally published by Appleton & Lange. Copyright © 1997 by The McGraw-Hill Companies.)

Precipitin tests can be done in solution or in semisolid medium (agar).

PRECIPITATION IN SOLUTION

This reaction can be made quantitative; i.e., antigen or antibody can be measured in terms of micrograms of nitrogen present. It is used primarily in research.

PRECIPITATION IN AGAR

This is done as either single or double diffusion. It can also be done in the presence of an electric field.

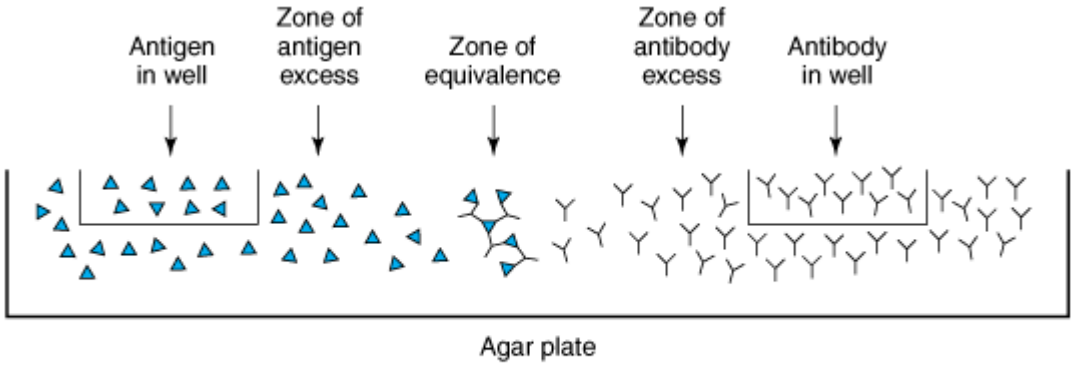
Single Diffusion

In single diffusion, antibody is incorporated into agar and antigen is measured into a well. As the antigen diffuses with time, precipitation rings form depending on the antigen concentration. The greater the amount of antigen in the well, the farther the ring will be from the well. By calibrating the method, such **radial immunodiffusion** is used to measure IgG, IgM, complement components, and other substances in serum. (IgE cannot be measured because its concentration is too low.)

Double Diffusion

In double diffusion, antigen and antibody are placed in different wells in agar and allowed to diffuse and form concentration gradients. Where optimal proportions (see zone of equivalence, above) occur, lines of precipitate form (Figure 64–3). This method (Ouchterlony) indicates whether antigens are identical, related but not identical, or not related (Figure 64–4).

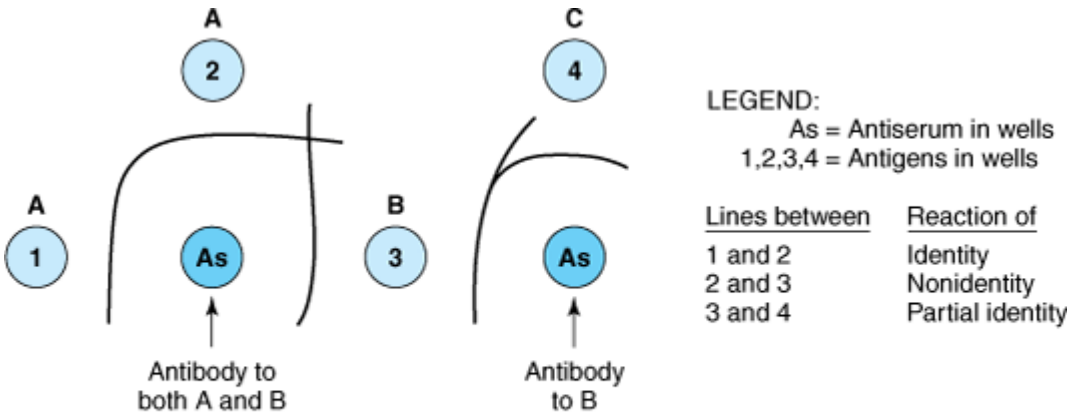
Figure 64–3.



Source: Levinson W: *Review of Medical Microbiology and Immunology*, 10th Edition: http://www.accessmedicine.com

Double diffusion in agar. Antigen is placed in the well on the left, and antibody is placed in the well on the right. The antigen and antibody diffuse through the agar and form a precipitate in the zone of equivalence. Close to the antigen-containing well is the zone of antigen excess, and close to the antibody-containing well is the zone of antibody excess. No precipitate forms in the zones of antigen and antibody excess.

Figure 64–4.



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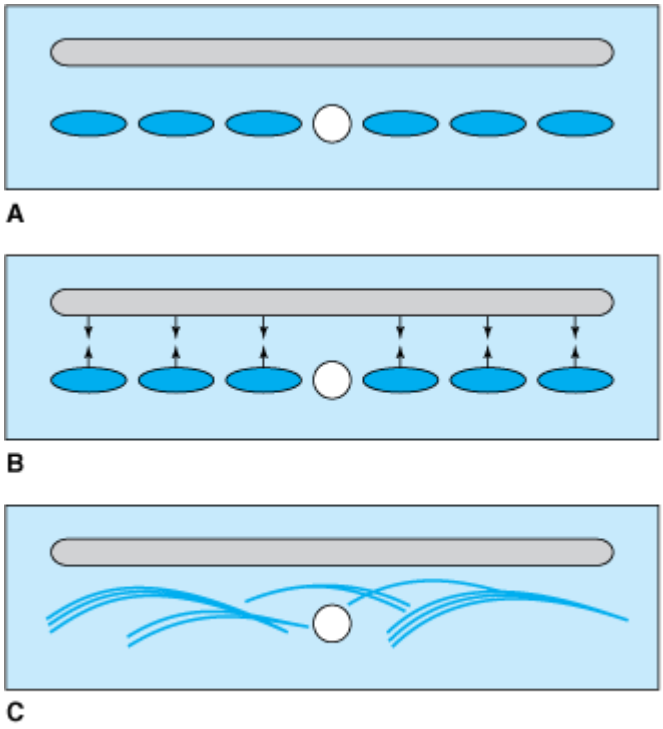
Double-diffusion (Ouchterlony) precipitin reactions. In these Ouchterlony reactions, wells are cut into an agar plate and various antigens and antisera are placed in the wells. The antigens and antibodies diffuse toward each other within the agar, and a line of precipitate forms in the zone of equivalence. Close to the antigen-containing well, a zone of antigen excess exists and no precipitate forms; close to the antibody-containing well, a zone of antibody excess exists and no precipitate forms. A and B are unrelated antigens; i.e., they have no epitopes in common. B and C are related antigens; i.e., they have some epitopes in common but some that are different. For example, chicken lysozyme (well B) and duck lysozyme (well C) share some epitopes because they are both lysozymes but have unique epitopes as well because they are from different species. The line of identity between B and C is caused by the reaction of the anti-B antibody with the shared epitopes on antigens B and C. The spur pointing toward well 4 is caused by the reaction of some of the anti-B antibody with the unique epitopes on antigen B in well 3. These lines of partial identity occur because antibody to B (chicken lysozyme) is polyclonal and has some immunoglobulins that react with the epitopes common to chicken and duck lysozyme and other immunoglobulins that react only with the epitopes unique to chicken lysozyme. (Modified and reproduced, with permission, from Brooks GF et al: *Medical Microbiology,* 19th ed. Originally published by Appleton & Lange. Copyright © 1991 by The McGraw-Hill Companies, Inc.)

PRECIPITATION IN AGAR WITH AN ELECTRIC FIELD

Immunoelectrophoresis

A serum sample is placed in a well in agar on a glass slide (Figure 64–5). A current is passed through the agar, and the proteins move in the electric field according to their charge and size. Then a trough is cut into the agar and filled with antibody. As the antigen and antibody diffuse toward each other, they form a series of arcs of precipitate. This permits the serum proteins to be characterized in terms of their presence, absence, or unusual pattern (e.g., human myeloma protein).

Figure 64–5.



Immunoelectrophoresis. **A:** Human serum placed in the central well is electrophoresed, and the proteins migrate to different regions (blue ellipses). Antiserum to human serum is then placed in the elongated trough (gray areas). **B:** Human serum proteins and antibodies diffuse into agar. **C:** Precipitate arcs (blue lines) form in the agar. (Modified and reproduced, with permission, from Stites D, Terr A, Parslow T [editors]: *Basic & Clinical Immunology*, 9th ed. Originally published by Appleton & Lange. Copyright © 1997 by The McGraw-Hill Companies, Inc.)

Counter-Immunoelectrophoresis

This method relies on movement of antigen toward the cathode and of antibody toward the anode during the passage of electric current through agar. The meeting of the antigen and antibody is greatly accelerated by this method and is made visible in 30–60 minutes. This has been applied to the detection of bacterial and fungal polysaccharide antigens in cerebrospinal fluid.

Radioimmunoassay (RIA)

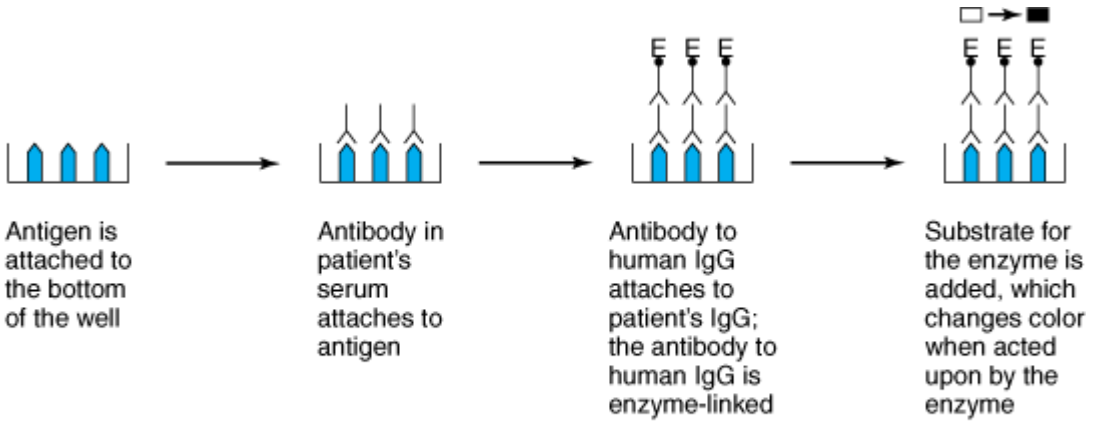
This method is used for the quantitation of antigens or haptens that can be radioactively labeled. It is based on the competition for specific antibody between the labeled (known) and the unlabeled (unknown) concentration of material. The

complexes that form between the antigen and antibody can then be separated and the amount of radioactivity measured. The more unlabeled antigen is present, the less radioactivity there is in the complex. The concentration of the unknown (unlabeled) antigen or hapten is determined by comparison with the effect of standards. RIA is a highly sensitive method and is commonly used to assay hormones or drugs in serum. The radioallergosorbent test (RAST) is a specialized RIA that is used to measure the amount of serum IgE antibody that reacts with a known allergen (antigen).

Enzyme-Linked Immunosorbent Assay (ELISA)

This method can be used for the quantitation of either antigens or antibodies in patient specimens. It is based on covalently linking an enzyme to a known antigen or antibody, reacting the enzyme-linked material with the patient's specimen, and then assaying for enzyme activity by adding the substrate of the enzyme. The method is nearly as sensitive as RIA yet requires no special equipment or radioactive labels (Figure 64–6).

Figure 64–6.



Source: Levinson W: *Review of Medical Microbiology and Immunology*, 10th Edition: http://www.accessmedicine.com

Enzyme-linked immunosorbent assay (ELISA).

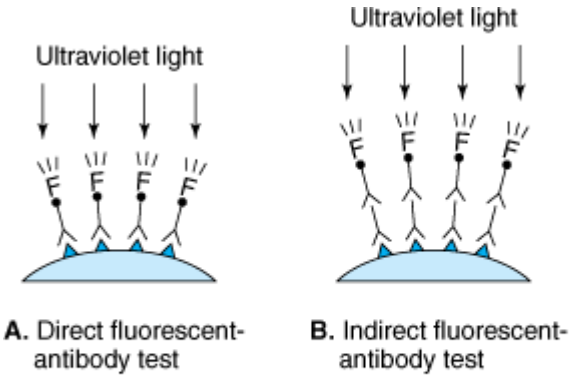
For measurement of antibody, known antigens are fixed to a surface (e.g., the bottom of small wells on a plastic plate), incubated with dilutions of the patient's serum, washed, and then reincubated with antibody to human IgG labeled with an enzyme, e.g., horseradish peroxidase. Enzyme activity is measured by adding the substrate for the enzyme and estimating the color reaction in a spectrophotometer. The amount of antibody bound is proportional to the enzyme activity. The titer of

antibody in the patient's serum is the highest dilution of serum that gives a positive color reaction.

Immunofluorescence (Fluorescent Antibody)

Fluorescent dyes, e.g., fluorescein and rhodamine, can be covalently attached to antibody molecules and made visible by UV light in the fluorescence microscope. Such "labeled" antibody can be used to identify antigens, e.g., on the surface of bacteria (such as streptococci and treponemes), in cells in histologic section, or in other specimens (Figure 64–7). The immunofluorescence reaction is **direct** when known labeled antibody interacts directly with unknown antigen and **indirect** when a two-stage process is used. For example, known antigen is attached to a slide, the patient's serum (unlabeled) is added, and the preparation is washed; if the patient's serum contains antibody against the antigen, it will remain fixed to it on the slide and can be detected on addition of a fluorescent dye–labeled antibody to human IgG and examination by UV microscopy. The indirect test is often more sensitive than direct immunofluorescence, because more labeled antibody adheres per antigenic site. Furthermore, the labeled antiglobulin becomes a "universal reagent"; i.e., it is independent of the nature of the antigen used because the antibody to IgG is reactive with all human IgG.

Figure 64–7.



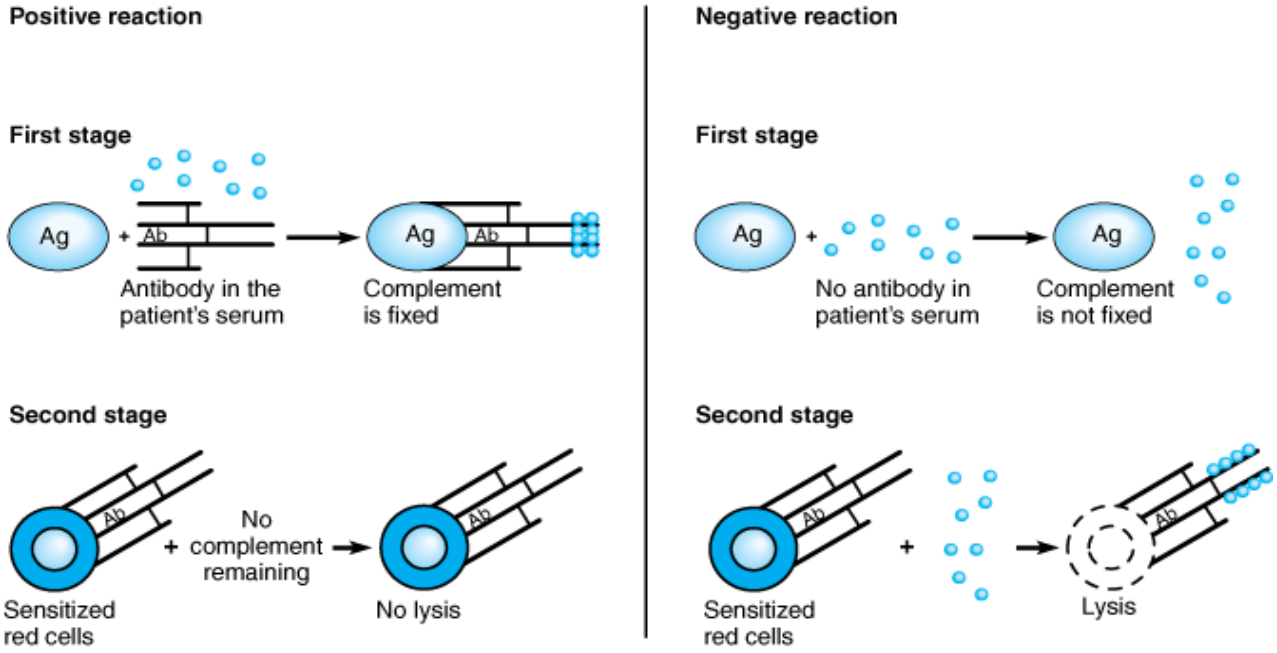
Fluorescent-antibody test. **A:** In the direct fluorescent-antibody test, the fluorescent dye is attached directly to the antibody that is interacting with the antigen (dark triangles) on the surface of the cell. **B:** In the indirect fluorescent-antibody test, the fluorescent dye is attached to antibody made against human IgG.

Complement Fixation

The complement system consists of 20 or more plasma proteins that interact with one another and with cell membranes. Each protein component must be activated sequentially under appropriate conditions for the reaction to progress. Antigen–antibody complexes are among the activators, and the complement fixation test can be used to identify one of them if the other is known.

The reaction consists of the following two steps (Figure 64–8): (1) Antigen and antibody (one known and the other unknown; e.g., use a known antigen and determine whether a patient's serum contains antibodies to that antigen) are mixed, and a measured amount of complement (usually from guinea pig) is added. If the antigen and antibody match, they will combine and use up ("fix") the complement. (2) An indicator system, consisting of "sensitized" red blood cells (i.e., red blood cells plus anti–red blood cell antibody), is added. If the antibody matched the antigen in the first step, complement was fixed and less (or none) is available to attach to the sensitized red blood cells. The red blood cells remain **unhemolyzed;** i.e., the test is **positive,** because the patient's serum had antibodies to that antigen. If the antibody did *not* match the antigen in the first step, complement is free to attach to the sensitized red blood cells and they are **lysed;** i.e., the test is **negative.**

Figure 64–8.



Source: Levinson W: *Review of Medical Microbiology and Immunology*, 10th Edition: http://www.accessmedicine.com

Complement fixation. **Left:** Positive reaction; i.e., the patient's serum contains antibody. If a known antigen is mixed with the patient's serum containing antibody against that antigen,

then complement (solid circles) will be fixed. Because no complement is left over, the sensitized red cells are *not* lysed. **Right:** Negative reaction. If a known antigen is mixed with the patient's serum that does *not* contain antibody against that antigen, complement (solid circles) is *not* fixed. Complement is left over and the sensitized red cells are lysed.

Complement must be carefully standardized, and the patient's serum must be heated to 56°C for 30 minutes to inactivate any human complement activity. The antigen must be quantitated. The result is expressed as the highest dilution of serum that gives positive results. Controls to determine whether antigen or antibody alone fixes complement are needed to make the test results valid. If antigen or antibody alone fixes complement, it is said to be anticomplementary.

Neutralization Tests

These use the ability of antibodies to block the effect of toxins or the infectivity of viruses. They can be used in cell culture (e.g., inhibition of cytopathic effect and plaque-reduction assays) or in host animals (e.g., mouse protection tests).

Immune Complexes

Immune complexes in tissue can be stained with fluorescent complement. Immune complexes in serum can be detected by binding to C1q or by attachment to certain (e.g., Raji lymphoblastoid) cells in culture.

Hemagglutination Tests

Many viruses clump red blood cells from one species or another (active hemagglutination). This can be inhibited by antibody specifically directed against the virus (hemagglutination inhibition) and can be used to measure the titer of such antibody. Red blood cells also can absorb many antigens and, when mixed with matching antibodies, will clump (this is known as passive hemagglutination, because the red cells are passive carriers of the antigen).

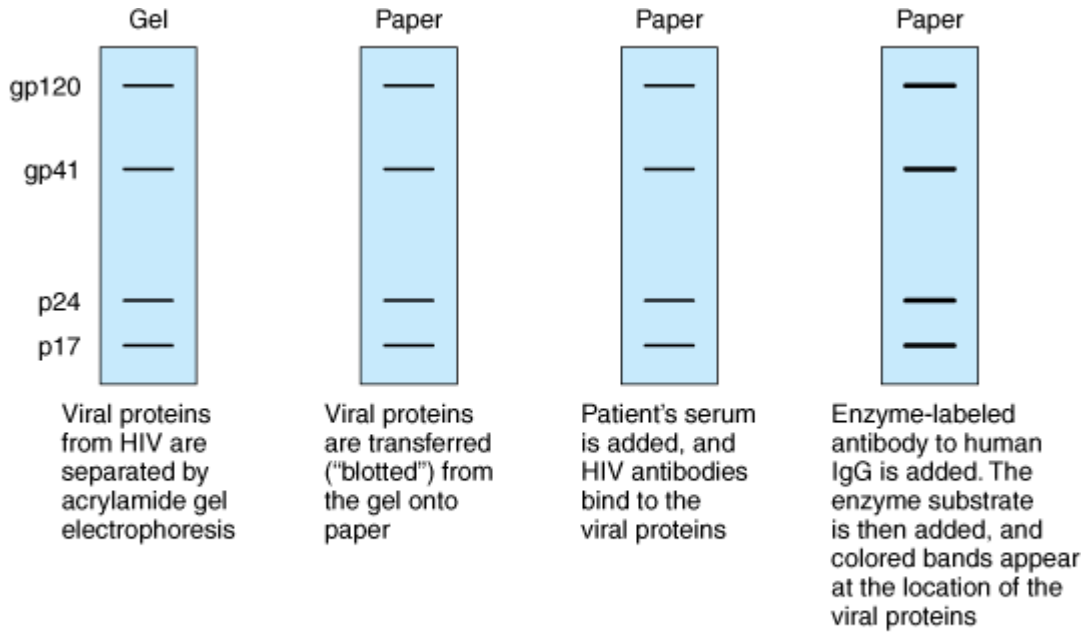
Antiglobulin (Coombs) Test

Some patients with certain diseases, e.g., hemolytic disease of the newborn (Rh incompatibility) and drug-related hemolytic anemias, become sensitized but do not exhibit symptoms of disease. In these patients, antibodies against the red cells are formed and bind to the red cell surface but do not cause hemolysis. These cell-bound antibodies can be detected by the direct antiglobulin (Coombs) test, in which antiserum against human immunoglobulin is used to agglutinate the patient's red cells. In some cases, antibody against the red cells is not bound to the red cells but is in the serum and the indirect antiglobulin test for antibodies in the patient's serum should be performed. In the indirect Coombs test, the patient's serum is mixed with normal red cells and antiserum to human immunoglobulins is added. If antibodies are present in the patient's serum, agglutination occurs.

Western Blot

This test is typically used to determine whether a positive result in a screening immunologic test is a true-positive or a false-positive result. For example, patients who are positive in the screening ELISA for HIV infection or for Lyme disease should have a Western blot test performed. Figure 64–9 illustrates a Western blot test for the presence of HIV antibodies in the patient's serum. In this test, HIV proteins are separated electrophoretically in a gel, resulting in discrete bands of viral protein. These proteins are then transferred from the gel, i.e., blotted, onto filter paper, and the person's serum is added. If antibodies are present, they bind to the viral proteins (primarily gp41 and p24) and can be detected by adding antibody to human IgG labeled with either radioactivity or an enzyme such as horseradish peroxidase, which produces a visible color change when the enzyme substrate is added.

Figure 64–9.



Source: Levinson W: *Review of Medical Microbiology and Immunology*, 10th Edition: http://www.accessmedicine.com

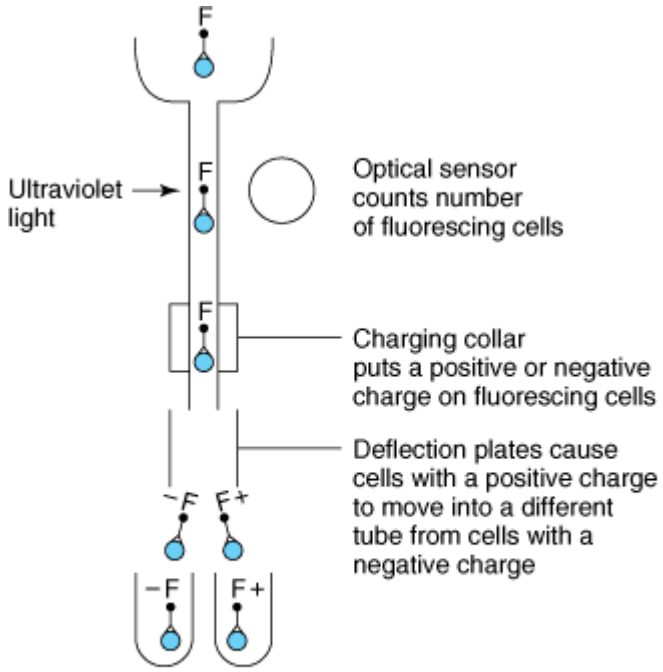
Western blot.

Fluorescence-Activated Cell Sorting (Flow Cytometry)

This test is commonly used to measure the number of the various types of immunologically active blood cells (Figure 64–10). For example, it is used in HIV-infected patients to determine the number of CD4-positive T cells. In this test, the patient's cells are labeled with monoclonal antibody to the protein specific to the cell

of interest, e.g., CD4 protein if the number of helper T cells is to be determined. The monoclonal antibody is tagged with a fluorescent dye, such as fluorescein or rhodamine. Single cells are passed through a laser light beam, and the number of cells that fluoresce is counted by use of a machine called a fluorescence-activated cell sorter (FACS).

Figure 64–10.



Source: Levinson W: *Review of Medical Microbiology and Immunology*, 10th Edition: http://www.accessmedicine.com

Flow cytometry. At the top of the figure, a cell has interacted with monoclonal antibody labeled with a fluorescent dye. As the cell passes down the tube, ultraviolet light causes the dye to fluoresce and a sensor counts the cell. Farther down the tube, an electrical charge can be put on the cell, which allows it to be deflected into a test tube and subjected to additional analysis.

[1]When red cells are used, the reaction is called hemagglutination.

[2]The term "prozone" refers to the failure of a precipitate or flocculate to form because too much antibody is present. For example, a false-negative serologic test for syphilis (VDRL) is occasionally reported because the antibody titer is too high. Dilution of the serum yields a positive result.

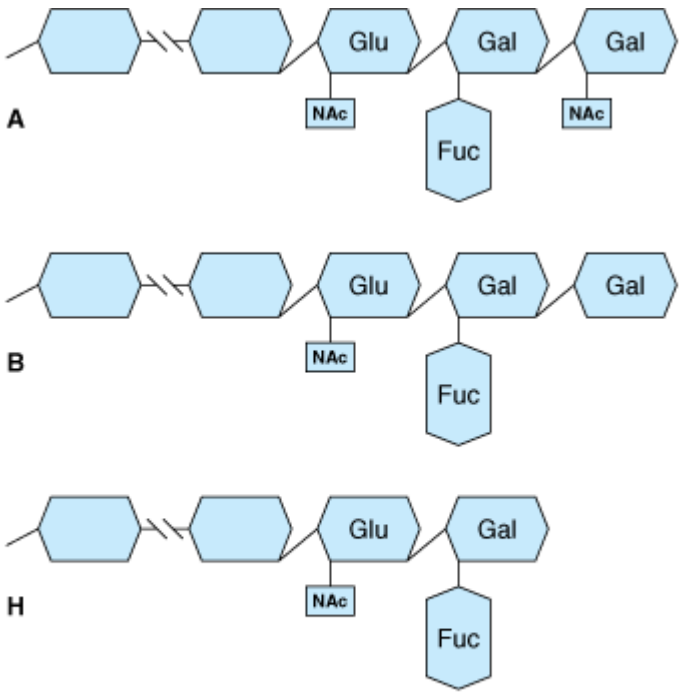
ANTIGEN–ANTIBODY REACTIONS INVOLVING RED BLOOD CELL ANTIGENS

Many different blood group systems exist in humans. Each system consists of a gene locus specifying antigens on the erythrocyte surface. The two most important blood groupings, ABO and Rh, are described below.

The ABO Blood Groups & Transfusion Reactions

All human erythrocytes contain alloantigens (i.e., antigens that vary among individual members of a species) of the ABO group. This is an important system, which is the basis for blood-typing and transfusions. The A and B genes encode enzymes that add specific sugars to the end of a polysaccharide chain on the surface of many cells, including red cells (Figure 64–11). People who inherit neither gene are type O. The genes are codominant, so people who inherit both genes are type AB. People who are either homozygous AA or heterozygous AO are type A, and, similarly, people who are either homozygous BB or heterozygous BO are type B.

Figure 64–11.



Source: Levinson W: *Review of Medical Microbiology and Immunology*, 10th Edition: http://www.accessmedicine.com

ABO blood groups. Structures of the terminal sugars that determine ABO blood groups are shown. Blood group O cells have H antigen on their surface; blood group A cells have *N*-acetylgalactosamine added to the end of the H antigen; and blood group B cells have galactosamine added to the end of the H antigen. (Reproduced, with permission, from Stites

The A and B antigens are carbohydrates that differ by a single sugar. Despite this small difference, A and B antigens do not cross-react. Erythrocytes have three terminal sugars in common on their surface: *N*-acetylglucosamine, galactose, and fucose. These three sugars form the H antigen (Figure 64–11). People who are blood group O have only the H antigen on the surface of their red cells. People who are blood group A have *N*-acetylgalactosamine added to the galactose of the H antigen, whereas people who are blood group B have galactose added to the galactose of the H antigen.

There are four combinations of the A and B antigens called A, B, AB, and O (Table 64–2). A person's blood group is determined by mixing the person's blood with antiserum against A antigen on one area on a slide and with antiserum against B antigen on another area (Figure 64–1). If agglutination occurs only with A antiserum, the blood group is A; if it occurs only with B antiserum, the blood group is B; if it occurs with both A and B antisera, the blood group is AB; and if it occurs with neither A nor B antisera, the blood group is O.

Table 64–2. ABO Blood Groups.

Group	Antigen on Red Cell	Antibody in Plasma
A	A	Anti-B
B	B	Anti-A
AB	A and B	No anti-A or anti-B
O	No A or B	Anti-A and anti-B

The plasma contains antibody against the absent antigens; i.e., people with blood group A have antibodies to B in their plasma. These antibodies are formed against cross-reacting bacterial or food antigens, are first detectable at 3–6 months of age, and are of the IgM class. Individuals are tolerant to their own blood group antigens and therefore a person with blood group A does not form antibodies to A antigen. The end result is that antigen and corresponding antibody do *not* coexist in the same person's blood. Transfusion reactions occur when incompatible donor red blood cells are transfused, e.g., if group A blood were transfused into a group B person (because anti-A antibody is present). The red cell–antibody complex activates complement, and a reaction consisting of shock caused by large amounts of C3a and

C5a (anaphylatoxins) and hemolysis caused by C5, C6, C7, C8, and C9 (membrane attack complex) occurs.

To avoid antigen–antibody reactions that would result in transfusion reactions, all blood for transfusions must be carefully **matched;** i.e., erythrocytes are typed for their surface antigens by specific sera. As shown in Table 64–2, persons with group O blood have no A or B antigens on their red cells and so are **universal donors;** i.e., they can give blood to people in all four groups (Table 64–3). Note that type O blood has A and B antibodies. Therefore when type O blood is given to a person with type A, B, or AB blood, you might expect a reaction to occur. A clinically detectable reaction does not occur because the donor antibody is rapidly diluted below a significant level. Persons with group AB blood have neither A nor B antibody and thus are **universal recipients.**

Table 64–3. Compatibility of Blood Transfusions between ABO Blood Groups.[1]

	Recipient			
Donor	**O**	**A**	**B**	**AB**
O	Yes	Yes	Yes	Yes
A (AA or AO)	No	Yes	No	Yes
B (BB or BO)	No	No	Yes	Yes
AB	No	No	No	Yes

[1]Yes indicates that a blood transfusion from a donor with that blood group to a recipient with that blood group is compatible, i.e., that no hemolysis will occur. No indicates that the transfusion is incompatible and that hemolysis of the donor's cells will occur.

In addition to red blood cells, the A and B antigens appear on the cells of many tissues. Furthermore, these antigens can be secreted in saliva and other body fluids. Secretion is controlled by a secretor gene. Approximately 85% of people carry the dominant form of the gene, which allows secretion to occur.

ABO blood group differences can lead to neonatal jaundice and anemia, but the effects on the fetus are usually less severe than those seen in Rh incompatibility (see below). For example, mothers with blood group O have antibodies against both A and B antigens. These IgG antibodies can pass the placenta and, if the fetus is blood group A or B, cause lysis of fetal red cells. Mothers with either blood group A or B

have a lower risk of having a neonate with jaundice because these mothers produce antibodies to either B or A antigens, respectively, that are primarily IgM, and IgM does not pass the placenta.

Rh Blood Type & Hemolytic Disease of the Newborn

About 85% of humans have erythrocytes that express the Rh(D) antigen, i.e., are $Rh(D)^+$. When an $Rh(D)^-$ person is transfused with $Rh(D)^+$ blood or when an $Rh(D)^-$ woman has an $Rh(D)^+$ fetus (the D gene being inherited from the father), the Rh(D) antigen will stimulate the development of antibodies (Table 64–4). This occurs most often when the $Rh(D)^+$ erythrocytes of the fetus leak into the maternal circulation during delivery of the first $Rh(D)^+$ child. Subsequent $Rh(D)^+$ pregnancies are likely to be affected by the mother's anti-D antibody, and hemolytic disease of the newborn (**erythroblastosis fetalis**) often results. This disease results from the passage of maternal IgG anti-Rh(D) antibodies through the placenta to the fetus, with subsequent lysis of the fetal erythrocytes. The direct antiglobulin (Coombs) test is typically positive (see above for a description of the Coombs test). The problem can be prevented by administration of **high-titer Rh(D) immune globulins (Rho-Gam)** to an $Rh(D)^-$ mother at 28 weeks of gestation and immediately upon the delivery of an $Rh(D)^+$ child. These antibodies promptly attach to $Rh(D)^+$ erythrocytes and prevent their acting as sensitizing antigen. This prophylaxis is widely practiced and effective.

Table 64–4. Rh Status and Hemolytic Disease of the Newborn.

Rh Status			
Father	**Mother**	**Child**	**Hemolysis[1]**
+	+	+ or –	No
+	–	+	No (1st child)
			Yes (2nd child and subsequent children)
+	–	–	No
–	+	+ or –	No
–	–	–	No

[1]No indicates that hemolysis of the newborn's red cells will not occur and that hemolytic disease will therefore not occur. Yes indicates that hemolysis of the newborn's red cells is likely to occur and that symptoms of hemolytic disease will therefore probably occur.

Chapter 65. Hypersensitivity (Allergy)

HYPERSENSITIVITY (ALLERGY): INTRODUCTION

Hypersensitivity is the term used when an immune response results in exaggerated or inappropriate reactions harmful to the host. The term "allergy" is often equated with hypersensitivity but more accurately should be limited to the IgE-mediated reactions discussed below in the section Type I: Immediate (Anaphylactic) Hypersensitivity.

The clinical manifestations of these reactions are typical in a given individual and occur on contact with the specific antigen to which the individual is hypersensitive. The first contact of the individual with the antigen sensitizes, i.e., induces the antibody, and the subsequent contacts elicit the allergic response.

Hypersensitivity reactions can be subdivided into four main types. Types I, II, and III are **antibody-mediated,** whereas type IV is **cell-mediated** (Table 65–1). Type I reactions are mediated by IgE, whereas types II and III are mediated by IgG.

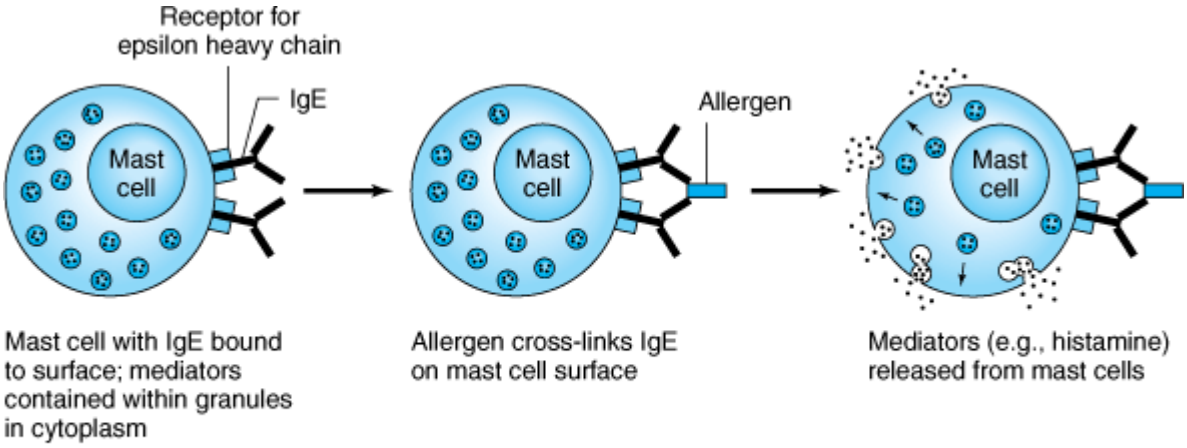
Table 65–1. Hypersensitivity Reactions.

Mediator	Type	Reaction
Antibody (IgE)	I (immediate, anaphylactic)	IgE antibody is induced by allergen and binds to mast cells and basophils. When exposed to the allergen again, the allergen cross-links the bound IgE, which induces degranulation and release of mediators, e.g., histamine.
Antibody (IgG)	II (cytotoxic)	Antigens on a cell surface combine with antibody; this leads to complement-mediated lysis, e.g., tranfusion or Rh reactions, or autoimmune hemolytic anemia.
Antibody (IgG)	III (immune complex)	Antigen–antibody immune complexes are deposited in tissues, complement is activated, and polymorphonuclear cells are attracted to the site. They release lysosomal enzymes, causing tissue damage.
Cell	IV (delayed)	Helper T lymphocytes sensitized by an antigen release lymphokines upon second contact with the same antigen. The lymphokines induce inflammation and activate macrophages, which, in turn, release various mediators.

TYPE I: IMMEDIATE (ANAPHYLACTIC) HYPERSENSITIVITY

An immediate hypersensitivity reaction occurs when an antigen (allergen) binds to IgE on the surface of mast cells with the consequent release of several mediators (see list of mediators below) (Figure 65–1). The process begins when an antigen induces the formation of **IgE antibody,** which binds firmly by its Fc portion to receptors on the surface of basophils and mast cells. Reexposure to the same antigen results in cross-linking of the cell-bound IgE, degranulation, and release of pharmacologically active mediators within minutes (**immediate phase**). Cyclic nucleotides and calcium play essential roles in release of the mediators.[1] Symptoms such as edema and erythema ("wheal and flare") and itching appear rapidly because these mediators, e.g., histamine, are preformed.

Figure 65–1.



Immediate (anaphylactic) hypersensitivity.

The **late phase** of IgE-mediated inflammation occurs approximately 6 hours after exposure to the antigen and is due to mediators, e.g., leukotrienes (SRS-A), that are synthesized after the cell degranulates. These mediators cause an influx of inflammatory cells, such as neutrophils and eosinophils, and symptoms such as erythema and induration occur. Complement is not involved with either the immediate or late reactions because IgE does not activate complement.

Note that the allergens involved in hypersensitivity reactions are substances, such as pollens, animal danders, foods (nuts, shellfish), and various drugs, to which most people do *not* exhibit clinical symptoms. However, some individuals respond to those substances by producing large amounts of IgE and, as a result, manifest various allergic symptoms. The increased IgE is the result of increased class switching to IgE in B cells caused by large amounts of IL-4 produced by Th-2 cells. Nonallergic individuals respond to the same antigen by producing IgG, which does not cause the release of mediators from mast cells and basophils. (There are no receptors for IgG on those cells.) There is a genetic predisposition to immediate hypersensitivity reactions, which is discussed in the Atopy section below.

The clinical manifestations of type I hypersensitivity can appear in various forms, e.g., urticaria (also known as hives), eczema, rhinitis and conjunctivitis (also known as hay fever), and asthma. Which clinical manifestation occurs depends in large part on the route of entry of the allergen and on the location of the mast cells bearing the IgE specific for the allergen. For example, some individuals exposed to pollens in the air get hay fever, whereas others who ingest allergens in food get diarrhea. Furthermore, people who respond to an allergen with urticaria have the allergen-specific IgE on mast cells in the skin, whereas those who respond with rhinitis have the allergen-specific mast cells in the nose.

The most severe form of type I hypersensitivity is **systemic anaphylaxis,** in which severe bronchoconstriction and hypotension (shock) can be life-threatening. The most common causes of anaphylaxis are foods, such as peanuts and shellfish, bee venom, and drugs. Of particular interest to medical personnel are type I hypersensitivity reactions to the wearing of latex rubber gloves, which include urticaria, asthma, and even systemic anaphylaxis. Table 65–2 summarizes some of the important clinical aspects of immediate hypersensitivities.

Table 65–2. Important Clinical Aspects of Immediate Hypersensitivities.

Main Organ Affected	Disease	Main Symptoms	Typical Allergens	Route of Acquisition
Lung	Asthma	Wheezing, dyspnea, tachypnea	Pollens, house dust (feces of dust mite), animal danders, many occupational airborne allergens	Inhalation

Nose and eyes	Rhinitis, conjunctivitis, "hay fever"	Runny nose, redness and itching of eyes	Pollens	Contact with mucous membranes
Skin	1. Eczema (atopic dermatitis)	Pruritic, vesicular lesions	Uncertain	Uncertain
	2. Urticaria (hives)	Pruritic, bullous lesions	1. Various foods	Ingestion
			2. Drugs	Various
Intestinal tract	Allergic gastroenteropathy	Vomiting, diarrhea	Various foods	Ingestion
Systemic	Anaphylaxis	Shock, hypotension, wheezing	1. Insect venom, e.g., bee venom	Sting
			2. Drugs, e.g., penicillin	Various
			3. Foods, e.g., peanuts	Ingestion

No single mediator accounts for all the manifestations of type I hypersensitivity reactions. Some important mediators and their effects are as follows:

1. **Histamine** occurs in granules of tissue mast cells and basophils in a preformed state. Its release causes vasodilation, increased capillary permeability, and smooth-muscle contraction. Clinically, disorders such as allergic rhinitis (hay fever), urticaria, and angioedema can occur. The bronchospasm so prominent in acute anaphylaxis results, in part, from histamine release. Antihistamine drugs block histamine receptor sites and can be relatively effective in allergic rhinitis but not in asthma (see below).
2. **Slow-reacting substance of anaphylaxis (SRS-A)** consists of several **leukotrienes,** which do not exist in a preformed state but are produced during anaphylactic reactions. This accounts for the slow onset of the effect of SRS-A. Leukotrienes are formed from arachidonic acid by the lipoxygenase pathway and cause increased vascular permeability and smooth-muscle contraction. They are the principal mediators in the bronchoconstriction of asthma and are not influenced by antihistamines.

3. **Eosinophil chemotactic factor of anaphylaxis (ECF-A)** is a tetrapeptide that exists preformed in mast cell granules. When released during anaphylaxis, it attracts eosinophils that are prominent in immediate allergic reactions. The role of eosinophils in type I hypersensitivity reactions is uncertain, but they do release histaminase and arylsulfatase, which degrade two important mediators, histamine and SRS-A, respectively. Eosinophils may therefore reduce the severity of the type I response.
4. **Serotonin** (hydroxytryptamine) is preformed in mast cells and blood platelets. When released during anaphylaxis, it causes capillary dilation, increased vascular permeability, and smooth-muscle contraction but is of minor importance in human anaphylaxis.
5. **Prostaglandins and thromboxanes** are related to leukotrienes. They are derived from arachidonic acid via the cyclooxygenase pathway. Prostaglandins cause dilation and increased permeability of capillaries and bronchoconstriction. Thromboxanes aggregate platelets.

The above-mentioned mediators are active only for a few minutes after release; they are enzymatically inactivated and resynthesized slowly. Manifestations of anaphylaxis vary among species, because mediators are released at different rates in different amounts and tissues vary in their sensitivity to them. For example, the respiratory tract (bronchospasm, laryngeal edema) is a principal shock organ in humans, but the liver (hepatic veins) plays that role in dogs.

In allergic airway disease (asthma), the airway hyperactivity appears to be caused by IL-13. IL-13 is made by Th-2 cells and binds to a receptor that shares a chain with the IL-4 receptor. IL-13 does not increase the amount of IgE.

In contrast to anaphylactic reactions, which are IgE-mediated, **anaphylactoid** reactions, which appear clinically similar to anaphylactic ones, are not IgE-mediated. In anaphylactoid reactions, the inciting agents, usually drugs or iodinated contrast media, directly induce the mast cells and basophils to release their mediators without the involvement of IgE.

Atopy

Atopic disorders, such as hay fever, asthma, eczema, and urticaria, are immediate-hypersensitivity reactions that exhibit a strong **familial predisposition** and are associated with **elevated IgE levels.** Several processes seem likely to play a role in atopy, for example, failure of regulation at the T-cell level (e.g., increased production of interleukin-4 leads to increased IgE synthesis), enhanced uptake and presentation of environmental antigens, and hyperreactivity of target tissues. Target tissues often contain large numbers of **Th-2 cells,** and these are thought to play a major role in the pathogenesis of atopic reactions.

It is estimated that up to 40% of people in the United States have experienced an atopic disorder at some time in their lives. The incidence of allergic diseases, such as asthma, is increasing markedly in the developed countries of North America and Europe. One hypothesis that might explain this increase is that the parasite burden is low in those countries. IgE evolved as a host defense against parasites. In regions where the parasite burden is high, IgE is used for host defense against those organisms. But in developed regions where the parasite burden is low, IgE is available to cause allergic diseases. This is called the "hygiene" hypothesis, which states that people who live in countries with a high parasite burden have fewer allergic diseases, whereas those who live in countries with a low parasite burden have more allergic diseases.

The symptoms of these atopic disorders are induced by exposure to the specific allergens. These antigens are typically found in the environment (e.g., pollens released by plants and dust mite feces often found in bedding and carpet) or in foods (e.g., shellfish and nuts). Exposure of nonatopic individuals to these substances does not elicit an allergic reaction. Many sufferers give immediate-type reactions to skin tests (injection, patch, or scratch) containing the offending antigen.

Atopic hypersensitivity is transferable by serum (i.e., it is antibody-mediated), not by lymphoid cells. In the past, this observation was used for diagnosis in the passive cutaneous anaphylaxis (Prausnitz-Küstner) reaction, which consists of taking serum from the patient and injecting it into the skin of a normal person. Some hours later the test antigen, injected into the "sensitized" site, will yield an immediate wheal-and-flare reaction. This test is now impractical because of the danger of transmitting certain viral infections. Radioallergosorbent tests (RAST) permit the identification of specific IgE against potentially offending allergens if suitable specific antigens for in vitro tests are available.

Several genes associated with atopy have been identified. Mutations in the gene encoding the alpha chain of the IL-4 receptor strongly predispose to atopy. These mutations enhance the effectiveness of IL-4, resulting in an increased amount of IgE synthesis by B cells. Other genes identified include the gene for IL-4 itself, the gene for the receptor for the epsilon heavy chain, and several class II MHC genes.

Drug Hypersensitivity

Drugs, particularly antimicrobial agents such as penicillin, are now among the most common causes of hypersensitivity reactions. Usually it is not the intact drug that induces antibody formation. Rather, a metabolic product of the drug, which acts as a hapten and binds to a body protein, does so. The resulting antibody can react with the hapten or the intact drug to give rise to type I hypersensitivity.[2]

When reexposed to the drug, the person may exhibit rashes, fevers, or local or systemic anaphylaxis of variable severity. Reactions to very small amounts of the drug can occur, e.g., in a skin test with the hapten. A clinically useful example is the skin test using penicilloyl-polylysine to reveal an allergy to penicillin.

Desensitization

Major manifestations of anaphylaxis occur when large amounts of mediators are suddenly released as a result of a massive dose of antigen abruptly combining with IgE on many mast cells. This is systemic anaphylaxis, which is potentially fatal. Desensitization can prevent systemic anaphylaxis.

Acute desensitization involves the administration of very small amounts of antigen at 15-minute intervals. Antigen-IgE complexes form on a small scale, and not enough mediator is released to produce a major reaction. This permits the administration of a drug or foreign protein to a hypersensitive person, but the hypersensitive state returns because IgE continues to be made.

Chronic desensitization involves the long-term weekly administration of the antigen to which the person is hypersensitive. This stimulates the production of IgA- and IgG-blocking antibodies, which can prevent subsequent antigen from reaching IgE on mast cells, thus preventing a reaction.

Treatment & Prevention

Treatment of anaphylactic reactions includes drugs to counteract the action of mediators, maintenance of an airway, and support of respiratory and cardiac function. Epinephrine, antihistamines, corticosteroids, or cromolyn sodium, either singly or in combination, should be given. Cromolyn sodium prevents release of mediators, e.g., histamine, from mast cell granules. Prevention relies on identification of the allergen by a skin test and avoidance of that allergen.

There are several approaches to the treatment of asthma. Inhaled β-adrenergic bronchodilators, such as albuterol, are commonly used. Corticosteroids, such as prednisone, are also effective. Aminophylline, a bronchodilator, is effective but not commonly used. A monoclonal anti-IgE antibody (omalizumab, Xolair) is indicated for patients with severe asthma whose symptoms are not controlled by corticosteroids. For the prevention of asthma, leukotriene receptor inhibitors, such as montelukast (Singulair), and cromolyn sodium are effective.

The treatment of allergic rhinitis typically involves antihistamines along with nasal decongestants. For allergic conjunctivitis, eye drops containing antihistamines or vasoconstrictors are effective. Avoidance of the inciting allergens, such as pollens, is helpful in prophylaxis. Desensitization can also be helpful.

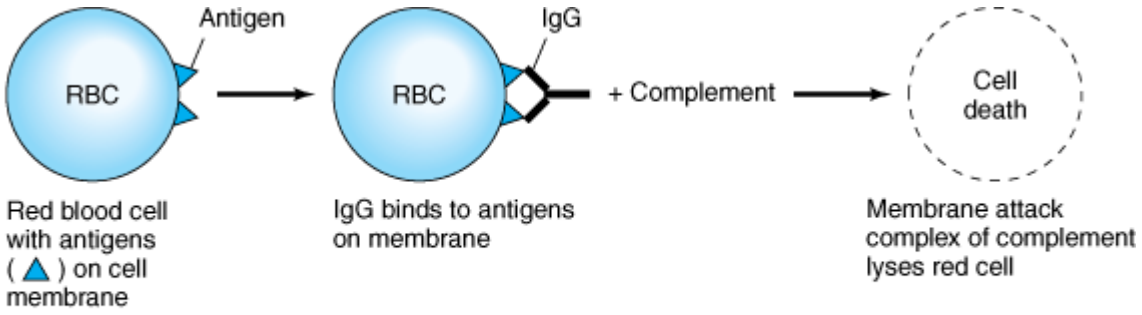
[1]An increase in cyclic GMP within these cells increases mediator release, whereas an increase in cyclic AMP decreases the release. Therefore, drugs that increase intracellular cyclic AMP, such as epinephrine, are used to treat type I reactions. Epinephrine also has sympathomimetic activity, which is useful in treating type I reactions.

[2]Some drugs are involved in cytotoxic hypersensitivity reactions (type II) and in serum sickness (type III).

TYPE II: CYTOTOXIC HYPERSENSITIVITY

Cytotoxic hypersensitivity occurs when antibody directed at antigens of the **cell membrane** activates complement (Figure 65–2). This generates a membrane attack complex (see Chapter 63), which damages the cell membrane. The antibody (IgG or IgM) attaches to the antigen via its Fab region and acts as a bridge to complement via its Fc region. As a result, there is complement-mediated lysis as in hemolytic anemias, ABO transfusion reactions, or Rh hemolytic disease. In addition to causing lysis, complement activation attracts phagocytes to the site, with consequent release of enzymes that damage cell membranes.

Figure 65–2.



Cytotoxic hypersensitivity.

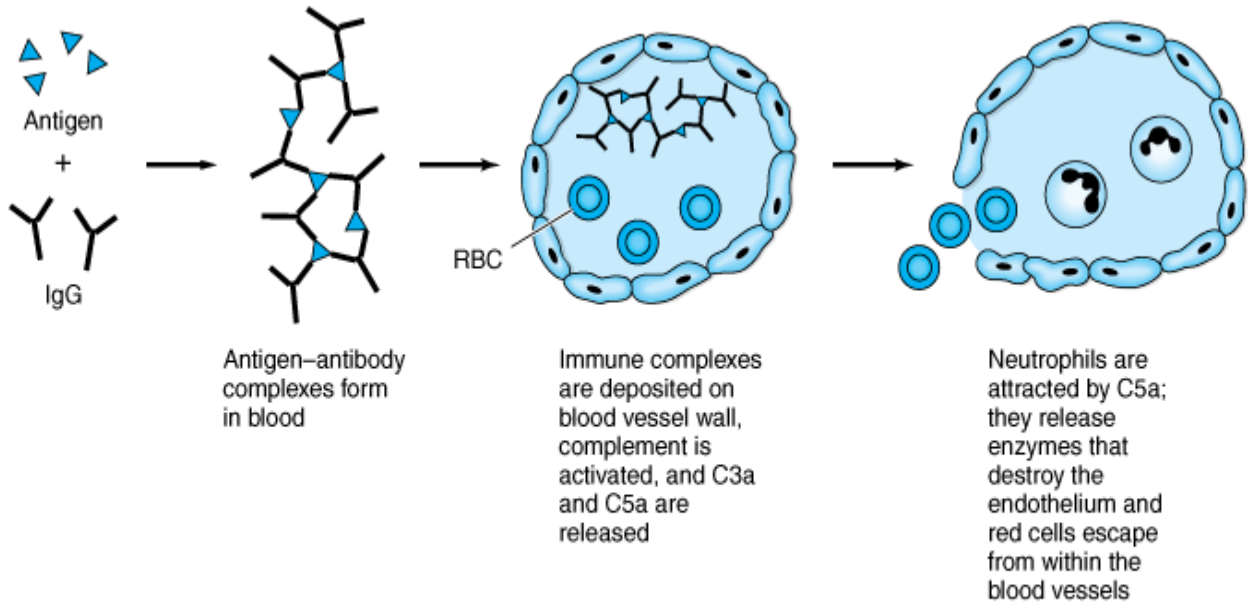
Drugs (e.g., penicillins, phenacetin, quinidine) can attach to surface proteins on red blood cells and initiate antibody formation. Such autoimmune antibodies (IgG) then interact with the red blood cell surface and result in hemolysis. The direct antiglobulin (Coombs) test is typically positive (see Chapter 64). Other drugs (e.g., quinine) can attach to platelets and induce autoantibodies that lyse the platelets, producing thrombocytopenia and, as a consequence, a bleeding tendency. Others (e.g., hydralazine) may modify host tissue and induce the production of autoantibodies directed at cell DNA. As a result, disease manifestations resembling

those of systemic lupus erythematosus occur. Certain infections, e.g., *Mycoplasma pneumoniae* infection, can induce antibodies that cross-react with red cell antigens, resulting in hemolytic anemia. In rheumatic fever, antibodies against the group A streptococci cross-react with cardiac tissue. In Goodpasture's syndrome, antibody to basement membranes of the kidneys and lungs bind to those membranes and activate complement. Severe damage to the membranes is caused by proteases released from leukocytes attracted to the site by complement component C5a (see Clinical Aspects of Complement).

TYPE III: IMMUNE-COMPLEX HYPERSENSITIVITY

Immune-complex hypersensitivity occurs when antigen–antibody complexes induce an inflammatory response in tissues (Figure 65–3). Normally, immune complexes are promptly removed by the reticuloendothelial system, but occasionally they persist and are **deposited in tissues,** resulting in several disorders. In persistent microbial or viral infections, immune complexes may be deposited in organs, e.g., the kidneys, resulting in damage. In autoimmune disorders, "self" antigens may elicit antibodies that bind to organ antigens or deposit in organs as complexes, especially in joints (arthritis), kidneys (nephritis), or blood vessels (vasculitis).

Figure 65–3.



Source: Levinson W: *Review of Medical Microbiology and Immunology*, 10th Edition: http://www.accessmedicine.com

Immune-complex hypersensitivity.

Wherever immune complexes are deposited, they activate the complement system. Polymorphonuclear cells are attracted to the site, and inflammation and tissue injury occur. Two typical type III hypersensitivity reactions are the Arthus reaction and serum sickness.

Arthus Reaction

Arthus reaction is the name given to the inflammation caused by the deposition of immune complexes at a localized site. It is named for Dr. Arthus, who first described the inflammatory response that occurs under the following conditions. If animals are given an antigen repeatedly until they have high levels of IgG antibody[3] and that antigen is then injected subcutaneously or intradermally, intense edema and hemorrhage develop, reaching a peak in 3–6 hours.

Antigen, antibody, and complement are deposited in vessel walls; polymorphonuclear cell infiltration and intravascular clumping of platelets then occur. These reactions can lead to vascular occlusion and necrosis.

A clinical manifestation of the Arthus reaction is hypersensitivity pneumonitis (allergic alveolitis) associated with the inhalation of thermophilic actinomycetes ("farmer's lung") growing in plant material such as hay. There are many other occupation-related examples of hypersensitivity pneumonitis, such as "cheese-workers lung," "woodworker's lung," and "wheat-millers lung." Most of these are caused by the inhalation of some microorganism, either bacterium or fungus, growing on the starting material. An Arthus reaction can also occur at the site of tetanus immunizations if they are given at the same site with too short an interval between immunizations. (The minimum interval is usually 5 years.)

[3]Much more antibody is typically needed to elicit an Arthus reaction than an anaphylactic reaction.

Serum Sickness

In contrast to the Arthus reaction, which is localized inflammation, serum sickness is a systemic inflammatory response to the presence of immune complexes deposited in many areas of the body. After the injection of foreign serum (or, more commonly these days, certain drugs), the antigen is excreted slowly. During this time, antibody production starts. The simultaneous presence of antigen and antibody leads to the formation of immune complexes, which may circulate or be deposited at various sites. Typical serum sickness results in fever, urticaria, arthralgia, lymphadenopathy, splenomegaly, and eosinophilia a few days to 2 weeks after injection of the foreign serum or drug. Although it takes several days for symptoms to appear, serum sickness is classified as an immediate reaction because symptoms occur promptly after immune complexes form. Symptoms improve as the immune system removes the antigen and subside when the antigen is eliminated. Nowadays, serum sickness

is caused more commonly by drugs, e.g., penicillin, than by foreign serum because foreign serum is used so infrequently.

Immune-Complex Diseases

Many clinical disorders associated with immune complexes have been described, although the antigen that initiates the disease is often in doubt. Several representative examples are described below.

GLOMERULONEPHRITIS

Acute poststreptococcal glomerulonephritis is a well-accepted immune-complex disease. Its onset follows several weeks after a group A beta-hemolytic streptococcal infection, particularly of the skin, and often with nephritogenic serotypes of *Streptococcus pyogenes.* Typically, the complement level is low, suggesting an antigen–antibody reaction. Lumpy deposits of immunoglobulin and C3 are seen along glomerular basement membranes by immunofluorescence, suggesting the presence of antigen–antibody complexes. It is assumed that streptococcal antigen–antibody complexes, after being deposited on glomeruli, fix complement and attract neutrophils, which start the inflammatory process.

Similar lesions with "lumpy" deposits containing immunoglobulin and C3 occur in infective endocarditis, serum sickness, and certain viral infections, e.g., hepatitis B and dengue hemorrhagic fever. Lesions containing immune complexes also occur in autoimmune diseases, e.g., the nephritis of systemic lupus erythematosus, in which the "lumpy" deposits contain DNA as the antigen (see below and Systemic Lupus Erythematosus).

IgA nephropathy is one of the most common forms of immune-complex glomerulonephritis worldwide. This disease is characterized by deposits of IgA on the glomeruli. The cause is unknown; no infectious agent has been associated with this disease. The course of the disease varies widely. Some patients are asymptomatic, some have mild symptoms, and others progress rapidly to kidney failure. Diagnosis is made by doing renal biopsy and demonstrating IgA deposits by immunohistologic testing.

RHEUMATOID ARTHRITIS

Rheumatoid arthritis is a chronic inflammatory autoimmune disease of the joints seen commonly in young women. It is a systemic disease involving not only the joints but other organs as well, most often the lung and pericardium. Serum and synovial fluid of patients contain "rheumatoid factor," i.e., IgM and IgG antibodies that bind to the Fc fragment of normal human IgG. Deposits of immune complexes (containing the normal IgG and rheumatoid factor) on synovial membranes and in blood vessels activate complement and attract polymorphonuclear cells, causing inflammation. Patients have high titers of rheumatoid factor and low titers of

complement in serum especially during periods when their disease is most active (see Systemic Lupus Erythematosus).

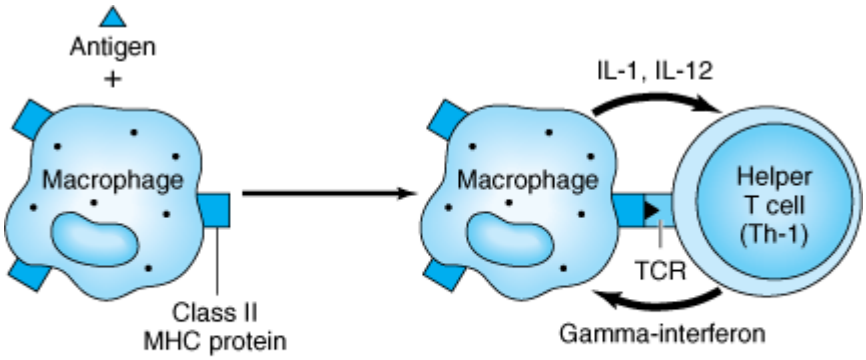
SYSTEMIC LUPUS ERYTHEMATOSUS

Systemic lupus erythematosus is a chronic inflammatory autoimmune disease that affects several organs, especially the skin of the face, the joints, and the kidneys. Antibodies are formed against DNA and other components of the nucleus of cells. These antibodies form immune complexes that activate complement. Complement activation produces C5a, which attracts neutrophils that release enzymes, thereby damaging tissue (see Systemic Lupus Erythematosus).

TYPE IV: DELAYED (CELL-MEDIATED) HYPERSENSITIVITY

Delayed hypersensitivity is a function of **T lymphocytes, not antibody** (Figure 65–4). It can be transferred by immunologically committed (sensitized) T cells, not by serum. The response is "delayed"; i.e., it starts hours (or days) after contact with the antigen and often lasts for days.

Figure 65–4.



Source: Levinson W: *Review of Medical Microbiology and Immunology*, 10th Edition: http://www.accessmedicine.com

Delayed (cell-mediated) hypersensitivity. The macrophage ingests the antigen, processes it, and presents an epitope on its surface in association with class II MHC protein. The helper T (Th-1) cell is activated and produces gamma interferon, which activates macrophages. These two types of cells mediate delayed hypersensitivity.

In certain contact hypersensitivities, such as poison oak, the pruritic, vesicular skin rash is caused by CD8-positive cytotoxic T cells that attack skin cells that display the plant oil as a foreign antigen. In the tuberculin skin test, the indurated skin rash is caused by CD4-positive helper T cells and macrophages that are attracted to the

injection site. Table 65–3 describes some of the important clinical aspects of delayed hypersensitivities.

Table 65–3. Important Clinical Aspects of Delayed Hypersensitivities.

Main Immune Cells Involved	Important Disease or Skin Test	Pathologic or Clinical Feature	Common Inducing Agents
CD4 (helper) T cells and macrophages	1. Tuberculosis, coccidioidomycosis	Granuloma	Constituents of bacterium or fungus
	2. Tuberculin or coccidioidin (or spherulin) skin tests	Induration	PPD (purified protein derivative) or coccidioidin (or spherulin)
CD8 (cytotoxic) T cells	Contact dermatitis	Pruritic, vesicular rash	Oil of poison oak or poison ivy, topical drugs, soaps, heavy metals (in jewelry)

Clinically Important Delayed Hypersensitivity Reactions

CONTACT HYPERSENSITIVITY

This manifestation of cell-mediated hypersensitivity occurs after sensitization with simple chemicals (e.g., nickel, formaldehyde), plant materials (e.g., poison ivy, poison oak), topically applied drugs (e.g., sulfonamides, neomycin), some cosmetics, soaps, and other substances. In all cases, the small molecules acting as haptens enter the skin, attach to body proteins, and become complete antigens. It is thought that these normal skin proteins to which the immune system is tolerant now can act as a carrier protein, because the hapten alters the protein enough that the immune system recognizes it as foreign. Cell-mediated hypersensitivity is induced, particularly in the skin. Upon a later skin contact with the offending agent, the sensitized person develops erythema, itching, vesicles, eczema, or necrosis of skin within 12–48 hours caused by the attack of cytotoxic T cells. Patch testing on a small area of skin can sometimes identify the offending antigen. Subsequent avoidance of the material will prevent recurrences.

TUBERCULIN-TYPE HYPERSENSITIVITY

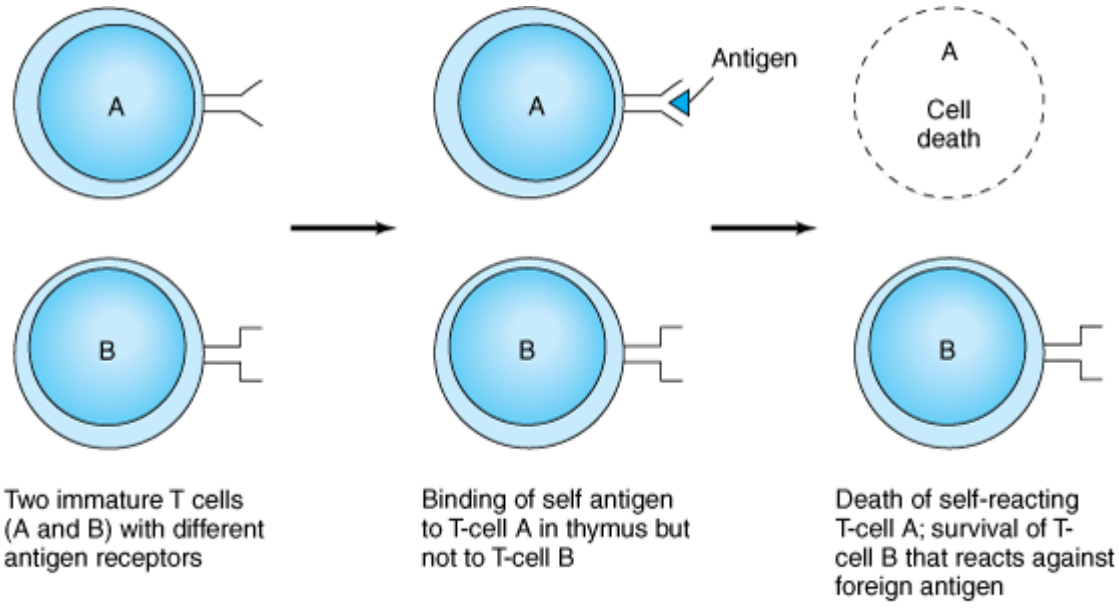
Delayed hypersensitivity to antigens of microorganisms occurs in many infectious diseases and has been used as an aid in diagnosis. It is typified by the tuberculin reaction. When a patient previously exposed to *Mycobacterium tuberculosis* is injected with a small amount of tuberculin (PPD) intradermally, there is little reaction in the first few hours. Gradually, however, induration and redness develop and reach a peak in 48–72 hours. A positive skin test indicates that the person **has been infected** with the agent, but it does *not* confirm the presence of current disease. However, if the skin test converts from negative to positive, it suggests that the patient has been recently infected. Infected persons do not always have a positive skin test, because overwhelming infection, disorders that suppress cell-mediated immunity (e.g., uremia, measles, sarcoidosis, lymphoma, and AIDS), or the administration of immunosuppressive drugs (e.g., corticosteroids, antineoplastics) may cause anergy.

A positive skin test response assists in diagnosis and provides support for chemoprophylaxis or chemotherapy. In leprosy, a positive lepromin test indicates the presence of tuberculoid leprosy with competent cell-mediated immunity, whereas a negative lepromin test suggests the presence of lepromatous leprosy with impaired cell-mediated immunity. In systemic mycotic infections (e.g., coccidioidomycosis and histoplasmosis), a positive skin test with the specific antigen indicates exposure to the organism. Cell-mediated hypersensitivity develops in many viral infections; however, serologic tests are more specific than skin tests both for diagnosis and for assessment of immunity. In protozoan and helminthic infections, skin tests may be positive, but they are generally not as useful as specific serologic tests.

TOLERANCE

Tolerance is **specific immunologic unresponsiveness;** i.e., an immune response to a certain antigen (or epitope) does not occur, although the immune system is otherwise functioning normally. In general, antigens that are present during embryonic life are considered "self" and **do not stimulate** an immunologic response, i.e., we are tolerant to those antigens. The lack of an immune response in the fetus is caused by the **deletion of self-reactive T-cell precursors** in the thymus (Figure 66–1). On the other hand, antigens that are not present during the process of maturation, i.e., that are encountered first when the body is immunologically mature, are considered "nonself" and usually elicit an immunologic response. Although both B cells and T cells participate in tolerance, it is **T-cell tolerance** that plays the primary role.

Figure 66–1.



Source: Levinson W: *Review of Medical Microbiology and Immunology*, 10th Edition: http://www.accessmedicine.com

Production of T-cell tolerance in the thymus.

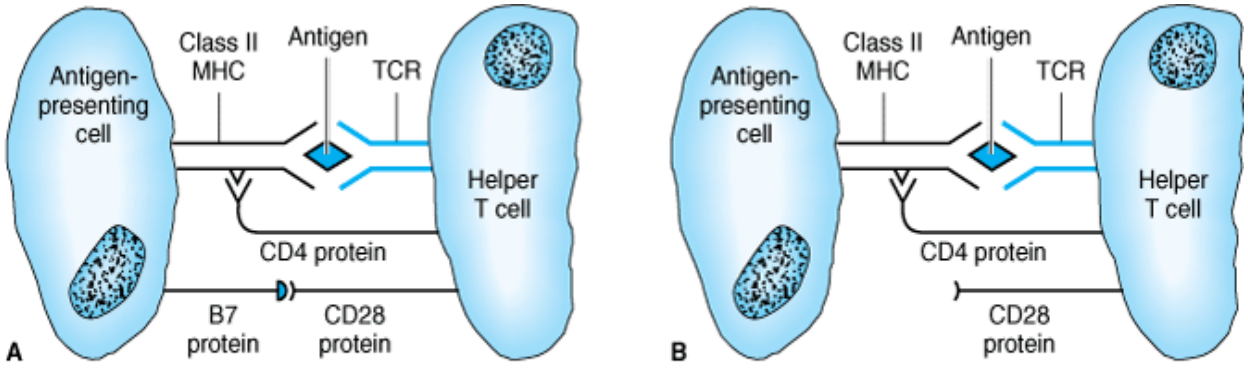
T-Cell Tolerance

The main process by which T lymphocytes acquire the ability to distinguish self from nonself occurs in the fetal thymus (see Chapter 58). This process, called **clonal deletion,** involves the killing of T cells ("negative selection") that react against

antigens (primarily self MHC proteins) present in the fetus at that time. (Note that exogenous substances injected into the fetus early in development are treated as self.) The self-reactive cells die by a process of programmed cell death called **apoptosis.** Tolerance to self acquired within the thymus is called **central tolerance,** whereas tolerance acquired outside the thymus is called **peripheral tolerance.**

Peripheral tolerance is necessary because some antigens do not reach the thymus and therefore some self-reactive T cells are not killed in the thymus. There are several mechanisms involved in peripheral tolerance: some self-reactive T cells are killed, some are not activated, and others are suppressed by regulatory T cells producing inhibitory cytokines. **Clonal anergy** is the term used to describe self-reactive T cells that are not activated because proper costimulation does not occur (Figure 66–2). **Clonal ignorance** refers to self-reactive T cells that ignore self antigens. These self-reactive T cells are either kept ignorant by physical separation from the target antigens, e.g., the blood-brain barrier, or ignore self antigens because the antigens are present in such small amounts.

Figure 66–2.



Source: Levinson W: *Review of Medical Microbiology and Immunology*, 10th Edition: http://www.accessmedicine.com

Clonal anergy outside the thymus. **A:** B7 protein on the antigen-presenting cell interacts with CD28 on the helper T cell, and full activation of the helper T cell occurs. **B:** B7 protein on the antigen-presenting cell is not produced; therefore, CD28 on the helper T cell does not give a costimulatory signal. Anergy of the helper T cell occurs despite interaction of the T-cell receptor (TCR) with the epitope.

Although T cells that are clonally anergic cells are nonfunctional, they can become functional and initiate an autoimmune disease if conditions change later in life. The mechanism of clonal anergy involves the inappropriate presentation of antigen, leading to a failure of interleukin-2 (IL-2) production. Inappropriate presentation is

due to a failure of "costimulatory signals"; e.g., sufficient amounts of IL-1 might not be made, or cell surface proteins, such as CD28 on the T cell and B7 on the B cell, might not interact properly, leading to a failure of signal transduction by *ras* proteins. For example, the inhibitory protein CTL-4 on the surface of the T cells may displace CD28 and interact with B7, resulting in a failure of T-cell activation. Furthermore, B7 is an inducible protein, and failure to induce it in sufficient amounts can lead to anergy. In addition, the costimulatory proteins, CD40 on the B cell and CD40L on the helper T cell, may fail to interact properly.

The failure of costimulatory signals most often occurs when there is an insufficient inflammatory response at the site of infection. The presence of microbes typically stimulates the production of proinflammatory cytokines such as TNF and IL-1. However, if the inflammatory response is insufficient, i.e., if the adjuvant effect of the cytokines is inadequate, the T cells will die instead of being activated.

B-Cell Tolerance

B cells also become tolerant to self by two mechanisms: (1) clonal deletion, probably while the B-cell precursors are in the bone marrow and (2) clonal anergy of B cells in the periphery. However, tolerance in B cells is less complete than in T cells, an observation supported by the finding that most autoimmune diseases are mediated by antibodies.

INDUCTION OF TOLERANCE

Whether an antigen will induce tolerance rather than an immunologic response is largely determined by the following:

1. The immunologic **maturity** of the host; e.g., neonatal animals are immunologically immature and do not respond well to foreign antigens (for instance, neonates will accept allografts that would be rejected by mature animals).
2. The **structure** and **dose** of the antigen; e.g., a very simple molecule induces tolerance more readily than a complex one, and very high or very low doses of antigen may result in tolerance instead of an immune response. Purified polysaccharides or amino acid copolymers injected in very large doses result in "immune paralysis"—a lack of response.

Other aspects of the induction or maintenance of tolerance are as follows:

1. T cells become tolerant more readily and remain tolerant longer than B cells.
2. Administration of a cross-reacting antigen tends to terminate tolerance.
3. Administration of immunosuppressive drugs enhances tolerance, e.g., in patients who have received organ transplants.

4. Tolerance is maintained best if the antigen to which the immune system is tolerant continues to be present.

AUTOIMMUNE DISEASES

The adult host usually exhibits tolerance to tissue antigens present during fetal life that are recognized as "self." However, in certain circumstances tolerance may be lost and immune reactions to host antigens may develop, resulting in autoimmune diseases. The most important step in the production of autoimmune disease is the **activation of self-reactive helper (CD4) T cells.** These self-reactive Th-1 or Th-2 cells can induce either cell-mediated or antibody-mediated autoimmune reactions, respectively. As described in Table 66–1, **most autoimmune diseases are antibody-mediated.**

Table 66–1. Important Autoimmune Diseases.

Type of Immune Response	Autoimmune Disease	Target of the Immune Response
Antibody to receptors	Myasthenia gravis	Acetylcholine receptor
	Graves' disease	TSH[1] receptor
	Insulin-resistant diabetes	Insulin receptor
	Lambert-Eaton myasthenia	Calcium channel receptor
Antibody to cell components other than receptors	Systemic lupus erythematosus	dsDNA, histones
	Rheumatoid arthritis[2]	IgG in joints
	Rheumatic fever	Heart and joint tissue
	Hemolytic anemia	RBC membrane
	Idiopathic thrombocytopenic purpura	Platelet membranes
	Goodpasture's syndrome	Basement membrane of kidney and lung
	Pernicious anemia	Intrinsic factor and parietal cells

	Hashimoto's thyroiditis[2]	Thyroglobulin
	Insulin-dependent diabetes mellitus[2]	Islet cells
	Addison's disease	Adrenal cortex
	Acute glomerulonephritis	Glomerular basement membrane
	Periarteritis nodosa	Small and medium-sized arteries
	Guillain-Barré syndrome	Myelin protein
	Wegener's granulomatosis	Cytoplasmic enzymes of neutrophils
	Pemphigus	Desmoglein in tight junctions of skin
	IgA nephropathy	Glomerulus
Cell-mediated	Allergic encephalomyelitis and multiple sclerosis	Reaction to myelin protein causes demyelination of brain neurons
	Celiac disease	Enterocytes

[1]TSH, thyroid-stimulating hormone.

[2]These diseases involve a significant cell-mediated as well as antibody-mediated response.

Genetic Factors

Many autoimmune diseases exhibit a marked familial incidence, which suggests a **genetic predisposition** to these disorders. There is a strong association of some diseases with certain human leukocyte antigen (HLA) specificities, especially the class II genes. For example, rheumatoid arthritis occurs predominantly in individuals carrying the HLA-DR4 gene. Ankylosing spondylitis is 100 times more likely to occur in people who carry HLA-B27, a class I gene, than in those who do not carry that gene.

There are two hypotheses offered to explain the relationship between certain HLA genes and autoimmune diseases. One is that those genes encode class I or class II

MHC proteins that present autoantigens with greater efficiency than do the MHC proteins that are not associated with autoimmune diseases. The other hypothesis is that autoreactive T cells escape negative selection in the thymus because they bind poorly to those class I or class II MHC proteins on the surface of the thymic epithelium.

It should be noted, however, that whether a person develops an autoimmune disease or not is clearly multifactorial, because people with HLA genes known to predispose to certain autoimmune diseases nevertheless do not develop the disease; e.g., many people carrying the HLA-DR4 gene do not develop rheumatoid arthritis. That is to say, HLA genes appear to be necessary but not sufficient to cause autoimmune diseases. In general, class II MHC-related diseases, e.g., rheumatoid arthritis, Graves' disease (hyperthyroidism), and systemic lupus erythematosus, occur more commonly in women, whereas class I MHC-related diseases, e.g., ankylosing spondylitis and Reiter's syndrome, occur more commonly in men.

Hormonal Factors

Approximately **90% of all autoimmune diseases occur in women.** Although the explanation for this markedly unequal gender ratio is unclear, there is some evidence from animal models that estrogen can alter the B-cell repertoire and enhance the formation of antibody to DNA. Clinically, the observation that systemic lupus erythematosus either appears or exacerbates during pregnancy (or immediately postpartum) supports the idea that hormones play an important role in predisposing women to autoimmune diseases.

Environmental Factors

There are several environmental agents that trigger autoimmune diseases, most of which are either bacteria or viruses. For example, pharyngitis caused by *Streptococcus pyogenes* predisposes to rheumatic fever. Other examples are described in Table 66–2. It is speculative at this time, but members of the normal flora of the bowel are thought to play a role in the genesis of inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis.

Table 66–2. Microbial Infections Associated with Autoimmune Diseases.

Microbe	Autoimmune Disease
1. Bacteria	
Streptococcus pyogenes	Rheumatic fever

Campylobacter jejuni	Guillain-Barré syndrome
Escherichia coli	Primary biliary cirrhosis
Chlamydia trachomatis	Reiter's syndrome
Shigella species	Reiter's syndrome
Yersinia enterocolitica	Graves' disease
Borrelia burgdorferi	Lyme arthritis
2. Viruses	
Hepatitis B virus[1]	Multiple sclerosis
Hepatitis C virus	Mixed cryoglobulinemia
Measles virus	Allergic encephalitis
Coxsackievirus B3[2]	Myocarditis
Coxsackievirus B4[3]	Type 1 diabetes mellitus
Cytomegalovirus	Scleroderma
Human T-cell leukemia virus	HTLV-associated myelopathy

[1]Other viruses, such as Epstein-Barr virus, human herpes virus-6, influenza A virus, and measles virus are also implicated as the possible cause of multiple sclerosis. No virus has definitely been shown to be the environmental trigger at this time.

[2]Coxsackievirus infects and kills cardiac myocytes causing the acute symptoms, but the late phase is caused by the attack of cytotoxic T cells on the myocytes.

[3]Causes diabetes mellitus in mice, but it is uncertain whether it is a cause in humans.

Certain infections cause autoimmune diseases in animals, e.g., coxsackievirus infection in mice causes type I diabetes, but have not been established as a cause in humans. Other environmental triggers include certain drugs such as procainamide, which causes systemic lupus erythematosus, and certain heavy metals such as gold and mercury, which cause autoimmune diseases in experimental animals.

There are two main mechanisms by which environmental factors could trigger autoimmune diseases. One is molecular mimicry, which proposes that infectious agents possess antigens that elicit an immune response that cross-reacts with

components of human cells. The other is that tissue injury releases intracellular (sequestered) antigens that elicit an immune response. These mechanisms are described in more detail in the next section.

In summary, the current model is that autoimmune diseases occur in people (1) with a genetic predisposition that is determined by their MHC genes and (2) who are exposed to an environmental agent that triggers a cross-reacting immune response against some component of normal tissue. Furthermore, because autoimmune diseases increase in number with advancing age, another possible factor is a decline in the number of regulatory T cells, which allows any surviving autoreactive T cells to proliferate and cause disease.

Mechanisms

The following main mechanisms for autoimmunity have been proposed.

MOLECULAR MIMICRY

Various bacteria and viruses are implicated as the source of cross-reacting antigens that trigger the activation of autoreactive T cells or B cells. For example, Reiter's syndrome occurs following infections with *Shigella* or *Chlamydia,* and Guillain-Barré syndrome occurs following infections with *Campylobacter.* The concept of **molecular mimicry** is used to explain these phenomena; i.e., the environmental trigger resembles (mimics) a component of the body sufficiently that an immune attack is directed against the cross-reacting body component. One of the best-characterized examples of molecular mimicry is the relationship between the M protein of *S. pyogenes* and the myosin of cardiac muscle. Antibodies against certain M proteins cross-react with cardiac myosin, leading to rheumatic fever.

Additional evidence supporting the molecular mimicry hypothesis includes the finding that there are identical amino acid sequences in certain viral proteins and certain human proteins. For example, there is an identical six–amino acid sequence in the hepatitis B viral polymerase and the human myelin basic protein.

ALTERATION OF NORMAL PROTEINS

Drugs can bind to normal proteins and make them immunogenic. Procainamide-induced systemic lupus erythematosus is an example of this mechanism.

RELEASE OF SEQUESTERED ANTIGENS

Certain tissues, e.g., sperm, central nervous system, and the lens and uveal tract of the eye, are sequestered so that their antigens are **not exposed** to the immune system. These are known as **immunologically privileged sites.** When such antigens enter the circulation accidentally, e.g., after damage, they elicit both humoral and cellular responses, producing aspermatogenesis, encephalitis, or endophthalmitis, respectively. Sperm, in particular, must be in a sequestered,

immunologically privileged site, because they develop after immunologic maturity has been reached and yet are normally not subject to immune attack.

Intracellular antigens, such as DNA, histones, and mitochondrial enzymes, are normally sequestered from the immune system. However, bacterial or viral infection may damage cells and cause the release of these sequestered antigens, which then elicit an immune response. Once autoantibodies are formed, subsequent release of sequestered antigens results in the formation of immune complexes and the symptoms of the autoimmune disease. In addition to infection, radiation and chemicals can also damage cells and release sequestered intracellular components. For example, sunlight is known to exacerbate the skin rash in patients with systemic lupus erythematosus. It is thought that UV radiation damages cells, which releases the normally sequestered DNA and histones that are the major antigens in this disease.

EPITOPE SPREADING

Epitope spreading is the term used to describe the new exposure of sequestered autoantigens as a result of damage to cells caused by viral infection. These newly exposed autoantigens stimulate autoreactive T cells, and autoimmune disease results. In an animal model, a multiple sclerosis–like disease was caused by infection with an encephalomyelitis virus. Note that the self-reactive T cells were directed against cellular antigens rather than the antigens of the virus.

Diseases

Table 66–1 describes several important autoimmune diseases according to the type of immune response causing the disease and the target affected by the autoimmune response. Some examples of autoimmune disease are described in more detail below.

DISEASES INVOLVING PRIMARILY ONE TYPE OF CELL OR ORGAN

Allergic Encephalitis

A clinically important example of allergic encephalitis occurs when people are injected with rabies vaccine made in rabbit brains. The immune response against the foreign myelin protein in the vaccine cross-reacts with human myelin, leading to inflammation of the brain. Although rare, this is a serious disease, and rabies vaccine made in rabbit brain is no longer used in the United States (see Chapter 39). Allergic encephalitis can also occur following certain viral infections, e.g., measles or influenza, or following immunizations against these infections. These reactions are rare, and the basis for the autoimmune reaction is uncertain. Allergic encephalitis can be reproduced in the laboratory by injecting myelin basic protein into a rodent's brain, which initiates a cell-mediated response leading to demyelination.

Multiple Sclerosis

In this disease, autoreactive T cells and activated macrophages cause demyelination of the white matter of the brain. The trigger that stimulates the autoreactive T cells is thought to be a viral infection. There is molecular evidence that the polymerase of Epstein-Barr virus may be the trigger. People with certain alleles in the HLA-DR region have an increased risk of contracting multiple sclerosis.

The clinical findings in multiple sclerosis typically wax and wane and affect both sensory and motor functions. MRI of the brain reveals plaques in the white matter. Oligoclonal bands of IgG are found in the spinal fluid of most patients. Immunosuppressive drugs, e.g., prednisone, methotrexate, or beta interferon, are effective in reducing the severity of some of the symptoms.

Chronic Thyroiditis

When animals are injected with thyroid gland material, they develop humoral and cell-mediated immunity against thyroid antigens and chronic thyroiditis. Humans with Hashimoto's chronic thyroiditis have antibodies to thyroglobulin, suggesting that these antibodies may provoke an inflammatory process that leads to fibrosis of the gland.

Hemolytic Anemias, Thrombocytopenias, and Granulocytopenias

Various forms of these disorders have been attributed to the attachment of autoantibodies to cell surfaces and subsequent cell destruction. Pernicious anemia is caused by antibodies to intrinsic factor, a protein secreted by parietal cells of the stomach that facilitates the absorption of vitamin B12. Idiopathic thrombocytopenic purpura is caused by antibodies directed against platelets. Platelets coated with antibody are either destroyed in the spleen or lysed by the membrane attack complex of complement.

Several drugs, acting as haptens, bind to the platelet membrane and form a "neoantigen" that induces the cytotoxic antibody that results in platelet destruction. Penicillins, cephalothin, tetracyclines, sulfonamides, isoniazid, and rifampin as well as drugs that are not antimicrobials can have this effect. Autoimmune hemolytic anemia caused by penicillins and cephalosporins is due to the same mechanism.

Insulin-Dependent Diabetes Mellitus (IDDM)

In this disease, autoreactive T cells destroy the islet cells of the pancreas. The main antigen against which the T-cell attack is directed is the islet cell enzyme, glutamic acid decarboxylase. Infection with coxsackievirus B4 has been shown to be a trigger of IDDM in mice, but it has yet to be established as a cause in human diabetes. There is a six–amino acid sequence in common between a coxsackievirus protein and glutamic acid decarboxylase. Antibodies against various antigens of the beta cells also are produced, but the major damage is T-cell mediated.

Insulin-Resistant Diabetes, Myasthenia Gravis, and Hyperthyroidism (Graves' Disease)

In these diseases, antibodies to receptors play a pathogenic role. In insulin-resistant diabetes, antibodies to insulin receptors have been demonstrated that interfere with insulin binding. In myasthenia gravis, which is characterized by severe muscular weakness, antibodies to acetylcholine receptors of neuromuscular junctions are found in the serum. Muscular weakness also occurs in Lambert-Eaton syndrome, in which antibodies form against the proteins in calcium channels. Some patients with Graves' disease have circulating antibodies to thyrotropin receptors, which, when they bind to the receptors, resemble thyrotropin in activity and stimulate the thyroid to produce more thyroxine.

Guillain-Barré Syndrome

This disease is the most common cause of acute paralysis in the United States. It follows a variety of infectious diseases such as viral illnesses (e.g., upper respiratory tract infections, HIV infection, and mononucleosis caused by Epstein-Barr virus and cytomegalovirus) and diarrhea caused by *Campylobacter jejuni.* Infection with *C. jejuni*, either symptomatic or asymptomatic, is considered to be the most common antecedent to Guillain-Barre syndrome. Antibodies against myelin protein are formed and result in a demyelinating polyneuropathy. The main symptoms are those of a rapidly progressing ascending paralysis. The treatment involves either intravenous immunoglobulins or plasmapheresis.

Pemphigus

Pemphigus is a skin disease characterized by bullae (blisters). It is caused by autoantibodies against desmoglein, a protein in the desmosomes that forms the tight junctions between epithelial cells in the skin. When the tight junctions are disrupted, fluid fills the spaces between cells and forms the bullae. One form of pemphigus, pemphigus foliaceus, is endemic in rural areas of South America, which lends support to the idea that infection with an endemic pathogen is the environmental trigger for this disease.

Reactive Arthritis

Reactive arthritis is an acute inflammation of the joints that follows infection with various bacteria, but the joints are sterile; i.e., the inflammation is a "reaction" to the presence of bacterial antigen elsewhere in the body. Reactive arthritis is associated with enteric infections caused by *Shigella, Campylobacter, Salmonella,* and *Yersinia* and with urethritis caused by *Chlamydia trachomatis.* The arthritis is usually oligoarticular and asymmetric. The bacterial infection precedes the arthritis by a few weeks. People who are HLA-B27-positive are at higher risk for reactive arthritis. Antibiotics directed against the organism have no effect. Anti-inflammatory

agents are typically used. (Reiter's syndrome includes a reactive arthritis, but the syndrome affects multiple organs and is described in the next section.)

Celiac Disease

Celiac disease (also known as celiac sprue and gluten enteropathy) is characterized by diarrhea, painful abdominal distention, fatty stools, and failure to thrive. Symptoms are induced by ingestion of gliadin, a protein found primarily in wheat, barley, and rye grains. Gliadin is the antigen that stimulates a cytotoxic T-cell attack on enterocytes, resulting in villous atrophy. A gluten-free diet typically results in marked improvement.

Inflammatory Bowel Disease (Crohn's Disease and Ulcerative Colitis)

These diseases are characterized by diarrhea, often bloody, and crampy lower abdominal pain. These symptoms arise from chronic inflammation, primarily in the ileum in Crohn's disease and in the colon in ulcerative colitis. It is thought that the chronic inflammation is caused by an abnormal immune response to the presence of normal flora of the bowel. There is evidence that a type of helper T cell called Th-17 and interleukin 23 are involved in the pathogenesis of these diseases.

IgA Nephropathy

This disease is one of the most common types of glomerulonephritis and is characterized primarily by hematuria, but proteinuria and progression to end-stage renal disease can occur. Immune complexes containing IgA are found lining the glomeruli. Symptoms are temporally related to viral infections, especially pharyngitis, but no specific virus has been identified. No treatment regimen is clearly effective. Fish oil has been tried, with variable results.

DISEASES INVOLVING MULTIPLE ORGANS (SYSTEMIC DISEASES)

Systemic Lupus Erythematosus

In this disease, autoantibodies are formed against DNA, histones, nucleolar proteins, and other components of the cell nucleus. Antibodies against double-stranded DNA are the hallmark of systemic lupus erythematosus. The disease affects primarily women between the ages of 20 and 60 years. Individuals with HLA-DR2 or -DR3 genes are predisposed to systemic lupus erythematosus. The agent that induces these autoantibodies in most patients is unknown. However, two drugs, procainamide and hydralazine, are known to cause systemic lupus erythematosus.

Most of the clinical findings are caused by immune complexes that activate complement and, as a consequence, damage tissue. For example, the characteristic rash on the cheeks is the result of a vasculitis caused by immune complex deposition. The arthritis and glomerulonephritis commonly seen in systemic lupus erythematosus are also caused by immune complexes. The immune complexes found on the glomerulus contain antibodies (IgG, IgM, or IgA) and the C3

component of complement but not fibrinogen. However, the anemia, leukopenia, and thrombocytopenia are caused by cytotoxic antibodies rather than immune complexes.

The diagnosis of systemic lupus erythematosus is supported by detecting antinuclear antibodies (ANA) with fluorescent antibody tests and anti-double-stranded DNA antibodies with ELISA. Antibodies to several other nuclear components are also detected, as is a reduced level of complement. Treatment of systemic lupus erythematosus varies depending upon the severity of the disease and the organs affected. Aspirin, nonsteroidal anti-inflammatory drugs, or corticosteroids are commonly used.

Rheumatoid Arthritis

In this disease, autoantibodies are formed against IgG. These autoantibodies are called rheumatoid factors and are of the IgM class. Rheumatoid arthritis affects primarily women between the ages of 30 and 50 years. People with HLA-DR4 genes are predisposed to rheumatoid arthritis. The agent that induces these autoantibodies is unknown. Within the inflamed joints, the synovial membrane is infiltrated with T cells, plasma cells, and macrophages, and the synovial fluid contains high levels of macrophage-produced inflammatory cytokines such as tumor necrosis factor (TNF), IL-1, and IL-8.

The main clinical finding is inflammation of the small joints of the hands and feet. Other organs, such as the pleura, pericardium, and skin, can also be involved. Most of the clinical findings are caused by immune complexes that activate complement and, as a consequence, damage tissue. The diagnosis of rheumatoid arthritis is supported by detecting rheumatoid factors in the serum. Detection of antibody to citrullinated peptide in the serum also supports the diagnosis.

Treatment of rheumatoid arthritis typically involves aspirin, nonsteroidal anti-inflammatory drugs, immunosuppressive drugs (especially methotrexate), or corticosteroids. Anticytokine therapy consisting of a fusion protein of TNF receptor and the Fc fragment of human IgG (etanercept, Enbrel) is also available. The soluble TNF receptor neutralizes TNF, which is an important inflammatory mediator in rheumatoid arthritis. Etanercept is particularly effective in combination with methotrexate in reducing the severity of joint inflammation in patients with persistently active rheumatoid arthritis. The monoclonal antibodies infliximab (Remicade) and adalimumab (Humira) are useful for the treatment of rheumatoid arthritis. These antibodies neutralize TNF, thereby decreasing the joint inflammation. Table 62–1 describes infliximab and other monoclonal antibodies that have different clinical uses.

Patients who have an inadequate response to these anti-TNF drugs have demonstrated significant improvement with abatacept (Orencia). Abatacept is CTLA-4-IG, a fusion protein composed of CTLA-4 and a fragment of the Fc domain of human IgG. CTLA-4 binds strongly to B7, which displaces CD-28 from its binding to B7. This results in a reduction of the helper T cell activity and the inflammatory response.

Rheumatic Fever

Group A streptococcal infections regularly precede the development of rheumatic fever. Antibodies against the M protein of group A streptococci that cross-react with myosin in cardiac muscle and proteins in joint and brain tissue are involved in the pathogenesis of rheumatic fever.

Reiter's Syndrome

This syndrome is characterized by the triad of arthritis, conjunctivitis, and urethritis. Cultures of the affected areas do not reveal a causative agent. Infection by one of the intestinal pathogens, e.g., *Shigella, Salmonella, Yersinia,* and *Campylobacter,* as well as other organisms such as *Chlamydia* predisposes to the disease. Most patients are men who are HLA-B27-positive. The pathogenesis of the disease is unclear, but immune complexes may play a role.

Goodpasture's Syndrome

In this syndrome, autoantibodies are formed against the collagen in basement membranes of the kidneys and lungs. Goodpasture's syndrome (GS) affects primarily young men, and those with HLA-DR2 genes are at risk for this disease. The agent that induces these autoantibodies is unknown, but GS often follows a viral infection.

The main clinical findings are hematuria, proteinuria, and pulmonary hemorrhage. The clinical findings are caused by cytotoxic antibodies that activate complement. As a consequence, C5a is produced, neutrophils are attracted to the site, and enzymes are released by the neutrophils that damage the kidney and lung tissue. The diagnosis of GS is supported by detecting antibody and complement bound to basement membranes in fluorescent-antibody test. Because this is a rapidly progressive, often fatal disease, treatment, including plasma exchange to remove the antibodies and the use of immunosuppressive drugs, must be instituted promptly.

Wegener's Granulomatosis

The main pathologic finding in this disease is a necrotizing granulomatous vasculitis that primarily affects the upper and lower respiratory tracts and the kidneys. Common clinical findings include sinusitis, otitis media, cough, sputum production, and arthritis. Glomerulonephritis is one of the main features of this disease. The diagnosis is supported by finding antineutrophil cytoplasmic antibodies (ANCA) in the

patient's serum. Immunosuppressive therapy with cyclophosphamide and prednisone is effective.

Other Collagen Vascular Diseases

Other diseases in this category include ankylosing spondylitis, which is very common in people carrying the HLA-B27 gene, polymyositis-dermatomyositis, scleroderma, periarteritis nodosa, and Sjögren's syndrome.

Treatment

The conceptual basis for the treatment of autoimmune diseases is to reduce the patient's immune response sufficiently to eliminate the symptoms. Corticosteroids, such as prednisone, are the mainstay of treatment, to which antimetabolites, such as azathioprine and methotrexate, can be added. The latter are nucleoside analogues that inhibit DNA synthesis in the immune cells. Immunosuppressive therapy must be given cautiously because of the risk of opportunistic infections.

Two approaches to therapy that do not involve systemic suppression of the immune system include antibody to TNF and soluble receptor for TNF that acts as a decoy. Both infliximab and adalimumab (antibody to TNF) as well as etanercept (TNF receptor) have been shown to ameliorate the joint inflammation of rheumatoid arthritis. However, these anti-TNF therapies increase the risk of tuberculosis and skin and soft tissue infections caused by pyogenic bacteria.

Certain antibody-mediated autoimmune diseases, such as Guillain-Barré syndrome and myasthenia gravis, can be treated either with plasmapheresis, which removes autoimmune antibodies, or with high doses of IgG pooled from healthy donors. One hypothesis regarding the mode of action of high-dose intravenous IgG is that it binds to Fc receptors on the surface of neutrophils and blocks the attachment of immune complexes that activate the neutrophils. Another hypothesis is that excess IgG saturates the FcRn receptor on the surface of vascular endothelial cells, which accelerates the catabolism of IgG, thereby reducing the level of autoimmune antibodies.

Chapter 67. Tumor Immunity

TUMOR-ASSOCIATED ANTIGENS

Animals carrying a chemically or virally induced malignant tumor can develop an immune response to that tumor and cause its **regression.** In the course of neoplastic transformation, **new antigens,** called **tumor-associated antigens (TAA),** develop at the cell surface and the host recognizes such cells as "nonself." An immune response then causes the tumor to regress.

In chemically induced tumors in experimental animals, TAAs are highly specific; i.e., cells of one tumor will have different TAAs from those on cells of another tumor even when they arise within the same animal. In contrast, virally induced tumors possess TAAs that cross-react with one another if induced by the same virus. TAAs on tumor cells induced by different viruses do not cross-react.

MECHANISM OF TUMOR IMMUNITY

Cell-mediated reactions attack these "nonself" tumor cells and limit their proliferation. Such immune responses probably act as a **surveillance** system to detect and eliminate newly arising clones of neoplastic cells. In general, the immune response against tumor cells is weak and can be overcome experimentally by a large dose of tumor cells. Some tumor cells can escape surveillance by "modulation," i.e., internalizing the surface antigen so that it no longer presents a target for immune attack.

The cell-mediated immune responses that affect tumor cells in vitro include natural killer (NK) cells, which act without antibody; killer (K) cells, which mediate antibody-dependent cytolysis (antibody-dependent cellular cytotoxicity); cytotoxic T cells; and activated macrophages. Whether these immune responses function to prevent or control tumors in vivo is unknown.

Tumor antigens can stimulate the development of specific antibodies as well. Some of these antibodies are cytotoxic, but others, called blocking antibodies, enhance tumor growth, perhaps by blocking recognition of tumor antigens by the host. Spontaneously arising human tumors may have new cell surface antigens against which the host develops both cytotoxic antibodies and cell-mediated immune responses. Enhancement of these responses can contain the growth of some tumors. For example, the administration of BCG vaccine (bacillus Calmette-Guérin, a bovine mycobacterium) into surface melanomas can lead to their partial regression. Immunomodulators, such as interleukins and interferons, are also being tested in such settings. One interleukin, tumor necrosis factor-α (cachectin), is experimentally effective against a variety of solid tumors (see Chapter 58). In addition, lymphocytes

activated by interleukin-2 (lymphokine-activated killer [LAK] cells) may be useful in cancer immunotherapy.

Another approach to cancer immunotherapy involves the use of tumor-infiltrating lymphocytes (TIL). The basis for this approach is the observation that some cancers are infiltrated by lymphocytes (NK cells and cytotoxic T cells) that seem likely to be trying to destroy the cancer cells. These lymphocytes are recovered from the surgically removed cancer, grown in cell culture until large numbers of cells are obtained, activated with interleukin-2, and returned to the patient in the expectation that the TIL will "home in" specifically on the cancer cells and kill them.

CARCINOEMBRYONIC ANTIGEN & ALPHA FETOPROTEIN

Some human tumors contain antigens that normally occur in fetal but not in adult human cells.

1. **Carcinoembryonic antigen** circulates at elevated levels in the serum of many patients with carcinoma of the colon, pancreas, breast, or liver. It is found in fetal gut, liver, and pancreas and in very small amounts in normal sera. Detection of this antigen (by radioimmunoassay) is not helpful in diagnosis but may be helpful in the management of such tumors. If the level declines after surgery, it suggests that the tumor is not spreading. Conversely, a rise in the level of carcinoembryonic antigen in patients with resected carcinoma of the colon suggests recurrence or spread of the tumor.
2. **Alpha fetoprotein** is present at elevated levels in the sera of hepatoma patients and is used as a marker for this disease. It is produced by fetal liver and is found in small amounts in some normal sera. It is, however, nonspecific; it occurs in several other malignant and nonmalignant diseases.

Monoclonal antibodies directed against new surface antigens on malignant cells (e.g., B-cell lymphomas) can be useful in diagnosis. Monoclonal antibodies coupled to toxins, such as diphtheria toxin or ricin, a product of the *Ricinus* plant, can kill tumor cells in vitro and someday may be useful for cancer therapy.

Chapter 68. Immunodeficiency

IMMUNODEFICIENCY: INTRODUCTION

Immunodeficiency can occur in any of the four major components of the immune system: (1) B cells (antibody), (2) T cells, (3) complement, and (4) phagocytes. The deficiencies can be either congenital or acquired (Table 68–1). Clinically, recurrent or opportunistic infections are commonly seen. **Recurrent infections with pyogenic bacteria, e.g., staphylococci, indicate a B-cell deficiency, whereas recurrent infections with certain fungi, viruses, or protozoa indicate a T-cell deficiency.**

Table 68–1. Important Congenital Immunodeficiencies.

Deficient Component and Name of Disease	Specific Deficiency	Molecular Defect	Clinical Features
B cell			
X-linked (Bruton's)	Absence of B cells; very low Ig levels	Mutant tyrosine kinase	Pyogenic infections
Selective IgA	Very low IgA levels	Failure of heavy-chain gene switching	Sinus and lung infections
T cell			
Thymic aplasia (DiGeorge's)	Absence of T cells	Defective development of pharyngeal pouches; not a genetic disease	Viral, fungal, and protozoal infections; tetany
Chronic mucocutaneous candidiasis	Deficient T-cell response to *Candida*	Unknown	Skin and mucous membrane infections with *Candida*
Combined B and T cell			
Severe combined immunodeficiency	Deficiency of both B-cell and	Either defective IL-2 receptor, defective recombinases, defective	Bacterial, viral, fungal, and protozoal

(SCID)	T-cell function	kinases, absence of class II MHC proteins, or ADA or PNP deficiency	infections
Complement			
Hereditary angioedema	Deficiency of C1 protease inhibitor	Too much C3a, C4a, and C5a generated	Edema, especially laryngeal edema
C3b	Insufficient C3	Unknown	Pyogenic infections, especially with *S. aureus*
C6,7,8	Insufficient C6,7,8	Unknown	*Neisseria* infections
Phagocytes			
Chronic granulomatous disease	Defective bactericidal activity because no oxidative burst	Deficient NADPH oxidase activity	Pyogenic infections, especially with *S. aureus*

CONGENITAL IMMUNODEFICIENCIES

B-Cell Deficiencies

X-LINKED HYPOGAMMAGLOBULINEMIA (BRUTON'S AGAMMAGLOBULINEMIA)

Very low levels of all immunoglobulins (IgG, IgA, IgM, IgD, and IgE) and a virtual **absence of B cells** are found in young **boys;** female carriers are immunologically normal. Pre-B cells are present, but they fail to differentiate into B cells. This failure is caused by a mutation in the gene encoding tyrosine kinase, an important signal transduction protein. Cell-mediated immunity is relatively normal. Clinically, recurrent pyogenic bacterial infections, e.g., otitis media, sinusitis, and pneumonia caused by *Streptococcus pneumoniae* and *Haemophilus influenzae,* occur in infants at about 6 months of age, when maternal antibody is no longer present in sufficient amount to be protective. Treatment with pooled gamma globulin reduces the number of infections.

SELECTIVE IMMUNOGLOBULIN DEFICIENCIES

IgA deficiency is the **most common** of these; IgG and IgM deficiencies are rarer. Patients with a deficiency of IgA typically have recurrent sinus and lung infections. (However, some individuals with IgA deficiency do not have frequent infections, possibly because their IgG and IgM levels confer protection.) The cause of IgA deficiency may be a failure of heavy-chain gene switching, because the amounts of IgG and IgM are normal. Patients with a deficiency of IgA should not be treated with gamma globulin preparations, because these patients may form antibodies against the foreign IgA and, by cross-reaction, deplete their already low level of IgA.

Patients with selective IgM deficiency or deficiency of one or more of the IgG subclasses also have recurrent sinopulmonary infections caused by pyogenic bacteria such as *S. pneumoniae, H. influenzae,* or *Staphylococcus aureus.*

T-Cell Deficiencies

THYMIC APLASIA (DIGEORGE'S SYNDROME)

Severe viral, fungal, or protozoal infections occur in affected infants early in life as a result of a profound **deficit of T cells.** Pneumonia caused by *Pneumocystis carinii* and thrush caused by *Candida albicans* are two common infections in these patients. Antibody production may be decreased or normal. If decreased, severe pyogenic bacterial infections can occur.

Both the **thymus and the parathyroids fail to develop properly** as a result of a defect in the third and fourth pharyngeal pouches. The most common presenting symptom is **tetany due to hypocalcemia** caused by hypoparathyroidism. Other congenital abnormalities are common. A transplant of fetal thymus may reconstitute T-cell immunity. A thymus from a child older than 14 weeks should not be used, because a graft-versus-host reaction may occur.

CHRONIC MUCOCUTANEOUS CANDIDIASIS

In this disease, the skin and mucous membranes of children are infected with *C. albicans,* which in immunocompetent individuals is a nonpathogenic member of the normal flora. These children have a T-cell deficiency **specifically** for this organism; other T-cell and B-cell functions are normal. Treatment consists primarily of antifungal drugs.

HYPER-IGM SYNDROME

In this syndrome, severe, recurrent pyogenic bacterial infections resembling those seen in X-linked hypogammaglobulinemia begin early in life. Patients have a high concentration of IgM but very little IgG, IgA, and IgE. They have normal numbers of T cells and B cells. Although the main manifestations of this syndrome are alterations in antibodies, the mutation is in the gene encoding the CD40 ligand in the CD4-positive helper T cells. As a result, the helper T cells have a defect in the surface

protein (CD40 ligand) that interacts with CD40 on the B-cell surface. The failure to properly interact with CD40 results in an inability of the B cell to switch from the production of IgM to the other classes of antibodies. Treatment with pooled gamma globulin results in fewer infections.

INTERLEUKIN-12 RECEPTOR DEFICIENCY

Patients with a deficiency of IL-12 receptor have disseminated mycobacterial infections. The absence of the receptor prevents IL-12 from initiating a Th-1 response, which is required to limit mycobacterial infections.

Combined B-Cell & T-Cell Deficiencies

SEVERE COMBINED IMMUNODEFICIENCY DISEASE (SCID)

Recurrent infections caused by bacteria, viruses, fungi, and protozoa occur in early infancy (3 months of age), because **both B cells and T cells** are defective. In some children, the B and T cells are absent; in others, the number of cells is normal but they do not function properly. Immunoglobulin levels are very low and tonsils and lymph nodes are absent. *Pneumocystis* pneumonia is the most common presenting infection in these infants. Infections caused by *C. albicans* and viruses such as varicella-zoster virus, cytomegalovirus, and respiratory syncytial virus are common and often fatal.

This is a group of inherited diseases, all of which are due to a defect in the differentiation of an early stem cell. There are two types: X-linked and autosomal; the X-linked form constitutes about 75% of cases. Some patients with X-linked SCID have a defect in the IL-2 receptor on T cells. They lack the γchain of the IL-2 receptor that is essential for the development of T cells. This is the most common form of SCID in the United States. Some patients with the autosomal form have a mutation in the gene encoding a tyrosine kinase called ZAP-70 that plays a role in signal transduction in T cells. Another autosomal form has mutations in the gene for a different kinase called Janus kinase 3. Other SCID patients with the autosomal form have a mutation in the RAG-1 or RAG-2 genes that encode the recombinase enzymes that catalyze the recombination of the DNA required to generate the T-cell antigen receptor and the IgM monomer on the B cell that acts as the antigen receptor.

Because immunity is so profoundly depressed, these children must be protected from exposure to microorganisms, usually by being enclosed in a plastic "bubble." Live, attenuated viral vaccines should *not* be given. Bone marrow transplantation may restore immunity. It is interesting that because infants with SCID do not reject allografts, bone marrow transplants do not require immunosuppressive drugs.

Patients with a hereditary absence of **adenosine deaminase (ADA) and purine nucleoside phosphorylase (PNP)** can have a severe deficiency of B cells and T

cells, causing SCID, although some have only mild dysfunction. The absence of these enzymes results in an accumulation of dATP, an inhibitor of ribonucleotide reductase, and a consequent decrease in the deoxynucleoside triphosphate precursors of DNA. This reduces the formation of B-cell and T-cell precursors in the bone marrow. Bone marrow transplantation can be helpful. Injections of ADA conjugated to polyethylene glycol reduce the number and severity of infections. Several patients with ADA deficiency have benefited from gene therapy. A retroviral vector carrying a normal copy of the ADA gene was allowed to infect the patient's bone marrow cells. The ADA gene functioned within some of these cells, and the patient's immune status improved.

Patients with **bare lymphocyte syndrome** exhibit the signs and symptoms of a severe combined immunodeficiency and are especially susceptible to viral infections. These patients have defective class I or class II MHC proteins or both. Mutations resulting in the inability to synthesize a transcription factor required for the synthesis of the mRNA for class II MHC proteins are an important cause of the failure to produce those proteins. Mutations in the gene encoding the TAP protein have been identified as one cause of the inability to display antigens on class I MHC proteins. (The TAP transporter protein is described in T Cells Recognize Only Peptides.)

WISKOTT-ALDRICH SYNDROME

Recurrent pyogenic infections, eczema, and bleeding caused by thrombocytopenia characterize this syndrome. These symptoms typically appear during the first year of life. It is an X-linked disease and thus occurs only in male infants. The most important defect is the inability to mount an IgM response to the capsular polysaccharides of bacteria, such as pneumococci. IgG levels and IgA levels are normal, but cell-mediated immunity is variable. The defect appears to be in the ability of T cells to provide help to B cells. The mutant gene encodes a protein involved in actin filament assembly. Bone marrow transplantation may be helpful.

ATAXIA-TELANGIECTASIA

In this disease, ataxia (staggering), telangiectasia (enlarged small blood vessels of the conjunctivas and skin), and recurrent infections appear by 2 years of age. It is an autosomal recessive disease caused by mutations in the genes that encode DNA repair enzymes. Lymphopenia and IgA deficiency commonly occur. Treatment designed to correct the immunodeficiency has not been successful.

Complement Deficiencies

HEREDITARY ANGIOEDEMA

This is an uncommon autosomal dominant disease caused by a deficiency of C1 inhibitor. In the absence of inhibitor, C1 continues to act on C4 to generate C4a and subsequently additional vasoactive components such as C3a and C5a. This leads to

capillary permeability and edema in several organs. Laryngeal edema can be fatal. Steroid drugs, such as oxymetholone and danazol, can be useful in increasing the concentration of C1 inhibitor.

RECURRENT INFECTIONS

Patients with deficiencies in C1, C3, or C5 or the later components C6, C7, or C8 have an increased susceptibility to bacterial infections. Patients with C3 deficiency are particularly susceptible to sepsis with pyogenic bacteria such as *S. aureus.* Those with reduced levels of C6, C7, or C8 are especially prone to bacteremia with *Neisseria meningitidis* or *Neisseria gonorrhoeae.*

AUTOIMMUNE DISEASES

Patients with C2 and C4 deficiencies have diseases resembling systemic lupus erythematosus or other autoimmune diseases. C2 deficiency is the most common complement defect and is frequently asymptomatic.

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

This rare disease is characterized by episodes of brownish urine (hemoglobinuria), particularly upon arising. The hemoglobinuria is due to complement-mediated hemolysis. This occurs especially at night because the lower oxygen concentration in the blood during sleep increases the susceptibility of the red cells to lyse. Hemolysis occurs because there is a deficiency of decay-accelerating factor (DAF) on the surface of blood cell precursors, leading to an increased activation of complement (see Chapter 63). These patients have a defect in the gene for the molecules that anchor DAF and other proteins to the cell membrane. There is no specific treatment. Iron can be given for the anemia, and prednisone can be helpful.

Phagocyte Deficiencies

CHRONIC GRANULOMATOUS DISEASE (CGD)

Patients with this disease are very susceptible to opportunistic infections with certain bacteria and fungi, e.g., *S. aureus,* enteric gram-negative rods, especially *Serratia* and *Burkholderia,* and *Aspergillus fumigatus.* Recurrent infections with catalase-positive bacteria, such as staphylococci, are common in these patients; whereas infections with catalase-negative bacteria, such as streptococci, are rare. Viral, mycobacterial, and protozoal infections are not a major concern. In 60–80% of cases, this is an X-linked disease that appears by the age of 2 years. (In the remaining patients, the disease is autosomal.)

CGD is due to a defect in the intracellular microbicidal activity of neutrophils as a result of a **lack of NADPH oxidase** activity (or similar enzymes). As a result, no hydrogen peroxide or superoxides are produced (i.e., no oxidative burst occurs), and the organisms, although ingested, are not killed. B-cell and T-cell functions are usually normal. In the laboratory, diagnosis can be confirmed by the **nitroblue**

tetrazolium dye reduction test or by the dichlorofluorescein (DCF) test. The DCF test is the more informative of the two because the analysis is done by flow cytometry, which provides information regarding the oxidative ability of single cells. For example, in the mothers of boys with CGD who are carriers, half of their neutrophils show normal oxidative activity because the X-chromosome carrying the mutant gene has been inactivated, whereas the other half show no oxidative activity because the X-chromosome carrying the normal gene has been inactivated.

Prompt, aggressive treatment of infection with the appropriate antibiotics is important. Chemoprophylaxis using trimethoprim-sulfamethoxazole can reduce the number of infections. Gamma interferon significantly reduces the frequency of recurrent infections, probably because it increases phagocytosis by macrophages.

The name chronic granulomatous disease arises from the widespread granulomas seen in these patients, even in the absence of clinically apparent infection. These granulomas can become large enough to cause obstruction of the stomach, esophagus, or bladder. The cause of these granulomas is unknown.

CHÉDIAK-HIGASHI SYNDROME

In this autosomal recessive disease, recurrent pyogenic infections, caused primarily by staphylococci and streptococci, occur. This is due to the failure of the **lysosomes** of neutrophils to fuse with phagosomes. The degradative enzymes in the lysosomes are, therefore, not available to kill the ingested organisms. Large granular inclusions composed of abnormal lysosomes are seen. In addition, the neutrophils do not function correctly during chemotaxis as a result of faulty microtubules. The mutant gene in this disease encodes a cytoplasmic protein involved in protein transport. Peroxide and superoxide formation is normal, as are B-cell and T-cell functions. Treatment involves antimicrobial drugs. There is no useful therapy for the phagocyte defect.

JOB'S SYNDROME (HYPER-IGE SYNDROME)

Patients with this syndrome have recurrent "cold"[1] staphylococcal abscesses, eczema, skeletal effects, and high levels of IgE.

The main immunologic defect is a failure to produce gamma interferon by helper T cells, which reduces the ability of macrophages to kill bacteria. This leads to an increase in Th-2 cells and, as a consequence, a high IgE level. The increased IgE causes histamine release, which blocks certain aspects of the inflammatory response, hence the "cold" abscesses. Histamine also inhibits neutrophil chemotaxis, another feature of this syndrome. Treatment consists of antimicrobial drugs.

LEUKOCYTE ADHESION DEFICIENCY SYNDROME

Patients with this syndrome have severe pyogenic infections early in life because they have defective adhesion (LFA-1) proteins on the surface of their phagocytes.

This is an autosomal recessive disease in which there is a mutation in the gene encoding the β chain of an integrin that mediates adhesion. As a result, neutrophils adhere poorly to endothelial cell surfaces and phagocytosis of the bacteria is inadequate.

CYCLIC NEUTROPENIA

In this autosomal dominant disease, patients have a very low neutrophil count (less than 200/μL) for 3 to 6 days of a 21-day cycle. During the neutropenic stage, patients are susceptible to life-threatening bacterial infections, but when neutrophil counts are normal, patients are not susceptible. Mutations in the gene encoding neutrophil elastase have been identified in these patients, but it is unclear how these contribute to the cyclic nature of the disease. It is hypothesized that irregular production of granulocyte colony-stimulating factor may play a role in the cyclic aspect of the disease.

MYELOPEROXIDASE DEFICIENCY

Deficiency of myeloperoxidase (either reduced amount or reduced function) is quite common but has little clinical importance. Surprisingly, most patients with this deficiency do not have a significant increase in infectious diseases. Myeloperoxidase catalyzes the production of hypochlorite, an important microbicidal agent, so an increase in infections would be expected. However, other intracellular killing mechanisms are intact and must be sufficient to kill the ingested microbes.

INTERFERON-GAMMA RECEPTOR DEFICIENCY

Patients with this deficiency have severe infections with atypical mycobacteria or with bacillus Calmette-Guérin (BCG), the attenuated mycobacterium in the BCG vaccine. They have a mutation in the gene encoding either the ligand-binding portion or the signal-transducing portion of the receptor for interferon-gamma. As a result, macrophages are not activated and severe mycobacterial infections occur. Defects in the production of interleukin-12 or in the receptor for interleukin-12 cause the same clinical picture.

[1]"Cold" refers to the absence of inflammation of the lesions; i.e., the lesions are not warm and red.

ACQUIRED IMMUNODEFICIENCIES

B-Cell Deficiencies

COMMON VARIABLE HYPOGAMMAGLOBULINEMIA

Patients present with recurrent infections caused by pyogenic bacteria, e.g., sinusitis and pneumonia caused by pyogenic bacteria such as *S. pneumoniae* and *H. influenzae.* The infections usually occur in persons between the ages of 15 and 35 years. The number of B cells is usually normal, but the ability to synthesize IgG (and other immunoglobulins) is greatly reduced. Cell-mediated immunity is usually

normal. The cause of the failure to produce IgG is unknown but appears to be due to defective T-cell signaling. Intravenous gamma globulin given monthly reduces the number of infections.

MALNUTRITION

Severe malnutrition can reduce the supply of amino acids and thereby reduce the synthesis of IgG. This predisposes to infection by pyogenic bacteria.

T-Cell Deficiencies

ACQUIRED IMMUNODEFICIENCY SYNDROME

Patients with acquired immunodeficiency syndrome (AIDS) present with opportunistic infections caused by certain bacteria, viruses, fungi, and protozoa (e.g., *Mycobacterium avium-intracellulare,* herpesviruses, *C. albicans,* and *P. carinii*). This is due to greatly reduced helper T-cell numbers caused by infection with the retrovirus human immunodeficiency virus (HIV; see Chapter 45). This virus specifically infects and kills cells bearing the CD4 protein as a surface receptor. The response to specific immunizations is poor; this is attributed to the loss of helper T-cell activity. AIDS patients also have a high incidence of tumors such as lymphomas, which may be the result of a failure of immune surveillance. See Chapter 45 for information on treatment and prevention.

MEASLES

Patients with measles have a transient suppression of delayed hypersensitivity as manifested by a loss of PPD skin test reactivity. Quiescent tuberculosis can become active. In these patients, T-cell function is altered but immunoglobulins are normal.

Complement Deficiencies

LIVER FAILURE

Liver failure caused by alcoholic cirrhosis or by chronic hepatitis B or hepatitis C can reduce the synthesis of complement proteins by the liver to a level that severe pyogenic infections can occur.

MALNUTRITION

Severe malnutrition can reduce the supply of amino acids and thereby reduce the synthesis of complement proteins by the liver. This predisposes to infection by pyogenic bacteria.

Phagocyte Deficiencies

NEUTROPENIA

Patients with neutropenia present with severe infections caused by pyogenic bacteria such as *S. aureus* and *S. pneumoniae* and enteric gram-negative rods. Neutrophil counts below 500/μL predispose to these infections. Common causes of neutropenia include cytotoxic drugs, such as those used in cancer chemotherapy; leukemia, in

which the bone marrow is "crowded out" by leukemic cells; and autoimmune destruction of the neutrophils. Ciprofloxacin is used to try to prevent infections in neutropenic patients.

Chronic Fatigue Syndrome (Chronic Fatigue Immune Dysfunction Syndrome)

The predominant finding in patients with chronic fatigue syndrome (CFS) is persistent, debilitating fatigue that has lasted for at least 6 months and is not relieved by bed rest. Because fatigue is a nonspecific symptom, all other causes of fatigue, including physical (e.g., cancer, autoimmune disease, and infection) and psychiatric (e.g., depression and neurosis), as well as prolonged use of drugs (e.g., tranquilizers), must be ruled out. The cause of CFS is unknown; attempts to isolate a causative organism from these patients have failed. A proposed relationship between CFS and chronic Epstein-Barr virus infection remains unsubstantiated.

There is a similarity between the symptoms of CFS and the symptoms that occur when alpha interferon or interleukin-2 is administered to patients. Abnormalities in various components of the immune system have been reported, e.g., loss of delayed hypersensitivity reactivity in skin tests and increased levels of cytotoxic T cells, but no definitive findings have emerged. There is no specific laboratory test for CFS. The approach to therapy involves treating the symptoms. Treatment with various antimicrobial drugs, such as acyclovir, ketoconazole, and gamma globulin, had no effect.