Chapter e106: Function and Evaluation of the Immune System

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KEY CONCEPTS

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1. Cells of the immune system are derived from the pluripotent stem cell. Hematopoiesis is closely regulated to maintain adequate numbers of different cell types. The development of these different cells or cell lineages depends on cell-to-cell interactions and hematopoietic growth factors.

2. Upon activation, dendritic cells (DCs) express higher concentrations of major histocompatibility complex class II molecules, B7-1, B7-2, CD40, ICAM-1, and LFA-3 molecules than other antigen-presenting cells (APCs). They also produce more IL-12. These differences may explain why DCs are the most efficient APC.

3. A T-lymphocyte expresses hundreds of T-cell receptors (TCRs). All the TCRs expressed on the surface of an individual T-lymphocyte have the same antigen specificity.

4. An immature B-lymphocyte expresses thousands of membrane-bound surface immunoglobulin (sIg) as IgM (monomeric) or IgD, all with the same specificity (ie, antigen-binding site). Upon antigen stimulation and T-cell help, the immature B-lymphocyte matures (proliferates, class-switches and becomes a plasma cell) to secrete different isotypes (eg, IgM [pentamer], IgA, immunoglobulin G [IgG], and IgE) with the same specificity as the original membrane-bound sIg.

5. Serum protein electrophoresis determines the total concentration of all circulating proteins, including the immunoglobulins (ie, IgG, IgA, IgM, IgD, and IgE). The concentration of the individual isotypes can be determined with isotype-specific quantification methods. Most clinical laboratories measure only IgG, IgM, and IgA because they are the most prevalent isotypes in the bloodstream. In patients with allergic disorders, measurement of IgE is rarely useful.

6. An understanding of the mechanism of action of immunomodulators allows a clinician to anticipate potential adverse drug reactions. The benefit of manipulating immune responses must be balanced with the potential consequences and long-term sequela (eg, tumor growth, infections, autoimmune reactions) of such manipulation.

BEYOND THE BOOK

BEYOND THE BOOK

Watch the following video to learn more about the different ways clinicians use the immune system to treat cancer. IMMUNOTHERAPY: The Path to a Cancer Cure (For Clinicians), https://www.youtube.com/watch?v=UbFjiWOBErA. As you read the chapter, note which strategies for modulating the immune response for therapeutic purposes were discussed in the video.

INTRODUCTION

The immune system is a complex network of barriers, organs, cellular elements, and molecules that interact to defend the body against invading pathogens. The *immune system* is actually composed of two distinct systems of immunity: innate immunity and adaptive immunity. Innate immunity includes a series of nonspecific barriers (physical and chemical), along with cellular and molecular elements strategically deployed and positioned to prevent or quickly neutralize infection. Adaptive immunity works in concert with the innate immune system. In contrast to innate immunity, adaptive immunity constantly evolves and adapts to invading pathogens. The hallmarks of the adaptive immune response are *diversity*, *memory*, *mobility*, *self-versus-nonself discrimination*, *redundancy*, *replication*, and *specificity*.[1] *Diversity* is the capability of the immune system to respond to many different pathogens or strains of pathogens. Immunological *memory* ensures a quicker and more vigorous response to a subsequent encounter with the same pathogen. If an individual has encountered something before, the odds are good that they will encounter it again, so the individual will make more of these cells and have them ready. *The mobility* of components of the immune system enables local reactions to provide systemic protection. *Discrimination of self-versus-nonself* helps prevent the immune system from responding to our own healthy tissues, and thus results in self-tolerance. *Redundancy* is the ability of the immune system to produce components with similar biological effects from multiple cell lines, such as inflammatory cytokines. *Replication* of the cellular components of the immune system amplifies the immune response. *Specificity* is the ability of the immune system to distinguish between dissimilar antigens.

MAJOR TISSUES AND ORGANS OF THE IMMUNE SYSTEM

While numerous cells of the immune system have the ability to migrate to most body tissues, some tissues and organs serve as key members of the immune system. These include primary and secondary lymphoid tissues and organs. *Primary lymphoid tissues and organs*, the bone marrow and thymus, provide an environment for the development and maturation of select cells of the immune system. It is here that these select cells of the immune system mature and become tolerant of self and competent to respond to foreign antigens. Importantly, no immune response occurs in these sites. *Secondary lymphoid organs* provide an environment where various cells of the immune system interact with and respond to antigens.[2]

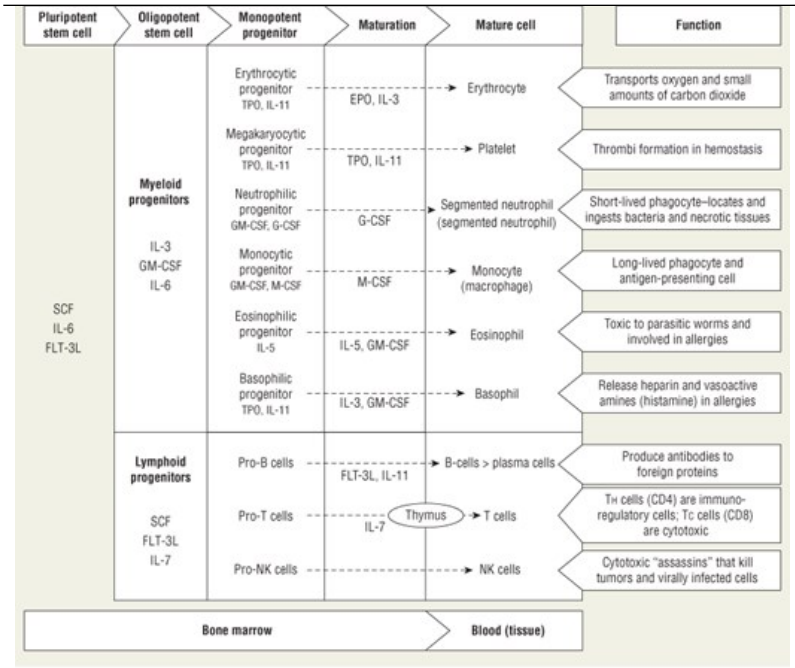
Primary Lymphoid Tissues

Bone Marrow

1 The bone marrow is the predominant primary lymphoid tissue of the body because it is the source of all cellular elements of the blood (erythrocytes, leukocytes, and thrombocytes [ie, platelets]). The few exceptions to this rule are mostly confined to fetal development when some blood cells are transiently produced in the yolk sac, liver, spleen, thymus, and lymph nodes.[3] Regardless of where they are formed, all blood cells arise from common self-renewing pluripotent stem cells via the process of *hematopoiesis* (Fig. e106-1). During hematopoiesis, pluripotent stem cells differentiate along particular myeloid and lymphoid lineages to produce the leukocytes of the immune system, erythrocytes, and thrombocytes.[4,5] Hematopoiesis is controlled by soluble mediators called hematopoietic growth factors (ie, cytokines) or colony-stimulating factors (CSFs) that are multifunctional and stimulate growth, survival, proliferation, differentiation, maturation, and functional activation.[6] The destiny of the leukocytes (if they survive the maturation process) is to become mature cells of the immune system directly from the bone marrow (all leukocytes except T-lymphocytes) or to migrate out of the bone marrow to continue their maturation elsewhere (T-lymphocytes in the thymus). Selected hematopoietic growth factors are identified in Fig. e106-1, and a more comprehensive list is given in Table e106-1. Four human hematopoietic cytokines have one or more recombinant products that are FDA-approved for clinical use: erythropoietin (EPO); granulocyte colony-stimulating factor (G-CSF); granulocyte-macrophage colony-stimulating factor (GM-CSF); and interleukin 11 (IL-11).[7] In addition, two small molecule thrombopoietin receptor agonists, eltrombopag and romiplostim, are FDA-approved for the treatment of various autoimmune-mediated platelet disorders.[8,9]

FIGURE e106-1

Basic model of hematopoiesis, outlining the various pathways blood cells taken from their origin as bone marrow stem cells through stages in which they are progressively selected to become monopotent mature cells with specific functions. Selected hematopoietic growth factors include EPO, erythropoietin; FLT-3L, fms-like tyrosine kinase ligand; GM-CSF, granulocyte-macrophage colony-stimulating factor; G-CSF, granulocyte colony-stimulating factor; IL, interleukin; M-CSF, macrophage colony-stimulating factor; NK, natural killer; SCF, stem cell factor; TPO, thrombopoietin.



Source: Joseph T. DiPiro, Gary C. Yee, Stuart T. Haines, Thomas D. Nolin, Vicki L. Ellingrod, L. Michael Posey: *DiPiro's Pharmacotherapy: A Pathophysiologic Approach, 12e*

TABLE e106-1

Hematopoietic Growth Factors or Colony-Stimulating Factors

Cytokine	Sources	Principal Effects
EPO	Kidney, liver	Erythrocyte production and maturation
GM-CSF	T-lymphocytes, macrophages, bone marrow stromal cells	Maturation and activation of granulocytes, monocytes/macrophages, and eosinophils
G-CSF	Macrophages, bone marrow stromal cells	Maturation and activation of neutrophils
M-CSF	Macrophages, bone marrow stromal cells	Maturation and activation of monocytes/macrophages
TPO	Liver, kidney	Platelet production
SCF	Bone marrow stromal cells, constitutively	Stem cell and progenitor cells activation
FLT-3L	Bone marrow stromal cells	Early acting growth factor
IL-3	T-lymphocytes, macrophages	Maturation and differentiation of hematopoietic and mast cells
IL-5	Activated T-lymphocytes	Eosinophil production
IL-1	Activated T-lymphocytes, bone marrow stromal cells	Progenitor cell stimulation
IL-7	Bone marrow stromal cells	T-cell maturation/survival
IL-11	Bone marrow stromal cells	Growth factor for B-lymphocytes and megakaryocytes

EPO, erythropoietin; FLT-3L, fms-like tyrosine kinase ligand; GM-CSF, granulocyte-macrophage colony-stimulating factor; G-CSF, granulocyte colony-stimulating factor; IL, interleukin; M-CSF, macrophage colony-stimulating factor; SCF, stem cell factor; TPO, thrombopoietin.

Thymus

The thymus is a bilobed primary lymphoid organ located in the superior mediastinum between the aorta and the sternum. Its primary function is to produce mature T-cells (thymus-dependent lymphocytes), the leukocytes responsible for cell-mediated immunity, including cytotoxic actions and immunoregulation. Through an intricate multistep process called thymic education, T-cells that do not react to self and have the appropriate receptors are considered beneficial to the immune system and leave the thymus. T-cells that fail the thymic education test (estimated at >99% of all T-cells) are eliminated via apoptosis.[1]

Secondary Lymphoid Tissues

Spleen

The spleen is a slender elongated secondary lymphoid organ located in the upper left quadrant of the abdomen that receives blood from the splenic artery. Although it is not a vital organ, it functions as an immunological filter of the blood and destroys defective and old erythrocytes. It contains compartments designated as red pulp and white pulp. The white pulp provides an environment for the interaction of B-cells and T-cells where debris in the blood can interact with antigen-presenting cells (APCs), T-cells, and B-cells to initiate cell-mediated immune responses (T-cells) and antibody

production (B-cells). The red pulp serves as a site of red blood cell degradation. The spleen sequesters many cellular elements (leukocytes, erythrocytes, and platelets) and can become dangerously congested during a strong inflammatory response and result in *splenomegaly*.

Lymph Nodes

Lymph nodes are normally small BB-sized lymphoid organs widely distributed between the groin and the neck. While the spleen filters blood, lymph nodes act as immunological filters for interstitial lymphatic fluid from the body's tissues. Lymph nodes provide an environment for the interaction of debris with APCs and other immune cells (T-cells and B-cells).[2] Lymph nodes may sequester activated immune cells (or tumor cells) and become inflamed and engorged (ie, lymphadenopathy).

Mucosa-Associated Lymphoid Tissue

Mucosa-associated lymphoid tissue (MALT) is the most extensive component of human lymphoid tissue and is distributed along mucosal linings of the body.[10] MALT may consist of well-defined networks of primary lymphoid follicles and other associated immunocompetent cells (adenoids, appendix, intestinal Peyer's patches, and tonsils), small solitary lymph nodes, or loosely organized clusters of lymphoid cells that are found in intestinal villi. The primary function of these tissues is to filter, trap, and remove pathogens that breach mucosal surfaces. In addition to neutralizing pathogens, MALT generates plasma cells (activated B-cells) that secrete antibodies, some of which are of the secretory IgA class of immunoglobulins with unique components to enable increased longevity in mucosal sites.

As mentioned earlier, the immune system includes two functional divisions: (a) The *innate* or nonspecific immune response, which encodes evolutionary genes aimed at providing rapid responses against nonmammalian targets; and (b) the *adaptive* or specific immune response, which uses cells that can rearrange their DNA to create specific structures on the T-cell receptor (on T-cells) and surface immunoglobulin (sIg) (on B-cells) which bind individual antigens or proteins (Table e106-2).[11] While we tend to classify these as two separate models for simplicity, these divisions extensively interact with the adaptive immune response driving the innate immune response.[12] An understanding of each component of the immune system and the consequences of disrupting homeostasis is essential to appropriately dose, administer, and monitor the effect of medications given to manipulate immune responses.

TABLE e106-2

Functional Divisions of the Immune System

	Innate	Adaptive
Exterior defenses	Skin, mucus, cilia, normal flora, saliva, low pH of the stomach, skin, genitourinary tract	None
Specificity	Limited and fixed	Extensive
Memory	None	Yes
Time to response	Hours	Days
Soluble factors	Lysozymes, complement, C-reactive protein, interferons, mannose-binding lectin, antimicrobial peptides[a]	Antibodies, cytokines
Cells	Neutrophils, monocytes, macrophages, natural killer cells, eosinophils	B-lymphocytes, T-lymphocytes

[a]Cathelicidins α-defensins, β-defensins.

METHODS TO DISTINGUISH SELF FROM NONSELF

The immune system is designed to attack and destroy a broad spectrum of foreign antigens/pathogens. However, the immune system must distinguish self from nonself, through a process now known as *self-tolerance*. If this did not occur, the immune system could direct an immune response against self-tissues.[13] The body employs many measures to avoid attacking itself, but when self-tolerance fails, the development of an autoimmune disease is often the result.

Innate Immune System

Physical Defense

Physical and chemical defenses are the most rudimentary form of innate immunity and the first line of defense against invading pathogens. The skin, the largest organ of the body, has the primary role of providing a physical defense. Alterations in the skin, such as burns or abrasions, allow a potential portal of entry for pathogens. The rapid turnover of intestinal cells also limits systemic infection as cells, including infected cells, are sloughed frequently. Drugs, such as cell-cycle, phase-specific antineoplastic agents, can disrupt the sloughing process and leave the patient at an increased risk for infections. Likewise, the respiratory tract has its forms of physical defense. The mucus coating the epithelial cells prevents microorganisms from adhering to cell surfaces, and the cilia lining the epithelium of the lungs repels inhaled organisms. The combination of cilia, mucus, and reactive coughing provides a natural barrier to invasion via the respiratory tract. The low pH of the stomach (pH 1-2) is inhospitable to most organisms and is a chemical defense resulting in the death of microorganisms. Other examples of mechanical or chemical defenses include normal urine flow through the urethra, lysozymes in tears and saliva, and the normal flora in the throat, the lower GI tract, and the genitourinary tract. Disruption of the normal physical and chemical defense systems through mechanical ventilation, for example, places the host at substantial risk for penetration by a pathogenic organism.[14]

Phagocytosis and Opsonization

If an infectious pathogen invades and infiltrates through a host's physical and chemical defense systems, the cells of the innate immune system are activated to halt the progression of the infection. These cells are present from birth and use a preexisting, but limited, repertoire of unique receptors to recognize and destroy pathogens. Innate immune cells include subgroups of leukocytes: monocytes/macrophages, neutrophils, basophils, mast cells, and eosinophils. When stimulated by a foreign pathogen, mast cells and basophils secrete inflammatory mediators. Monocytes/macrophages, neutrophils, mast cells, and eosinophils act as phagocytes. Phagocytes are cells that recognize, internalize, and degrade the invading pathogens. This process may occur in two ways: opsonin-dependent or opsonin-independent phagocytosis. For opsonin-dependent phagocytosis, opsonins like antibody (eg, IgG), complement (eg, C3b), or lectin (eg, C-reactive protein) coat the infectious pathogen by sticking to conserved structures on the infectious pathogens. Once the pathogen is opsonized, the opsonin (antibody, complement, or lectin) binds to the specific receptors on the phagocyte (Fig. e106-2) and activates the phagocytic process. For opsonin-independent phagocytosis, innate leukocytes use pattern recognition receptors (PRRs), which bind to highly conserved structures present on a large number of different microorganisms. PRRs on the phagocytes directly recognize the conserved ligands, also known as Pathogen Associated Molecular Patterns (PAMPs), on the surfaces of infectious pathogens (Table e106-3), leading to the immediate phagocytosis of the pathogen (see Fig. e106-2). PRRs include the macrophage mannose receptor, macrophage scavenger receptor, and members of the toll-like receptor family. Toll-like receptors are a family of PRRs on the cell- surface of innate leukocytes. To date, at least 10 toll-like receptors have been identified in humans. They recognize a broad spectrum of conserved structures ranging from lipopolysaccharide and flagellin on bacteria, to zymosan on yeast, to double-stranded RNA from RNA viruses (see Table e106-3). Binding of the PAMPs to the toll-like receptors (a PRR) allows the phagocyte to recognize and engulf the pathogen. Binding of toll-like receptors (PRRs) to their corresponding PAMPs also results in the secretion of chemokines, inflammatory cytokines, and antimicrobial peptides; increased expression of costimulatory proteins (eg, B7) and major histocompatibility complex (MHC) molecules by the phagocyte; and the recruitment and activation of antigen-specific lymphocytes.[12,15,16]

FIGURE e106-2

Phagocytosis of bacteria by macrophages, dendritic cells (DCs), and neutrophils. Macrophages, DCs, and neutrophils recognize bacteria opsonized (coated) with antibody or complement (C3b). On the surface of macrophages, DCs, and neutrophils reside receptors for antibody (Fc receptors) and complement (CR1, CR3, and CR4). In addition, these cells may recognize the bacteria by pattern recognition receptors on the surface of macrophages, DCs, and neutrophils. Pattern recognition receptors include toll-like receptors, scavenger receptors, and mannose receptors.

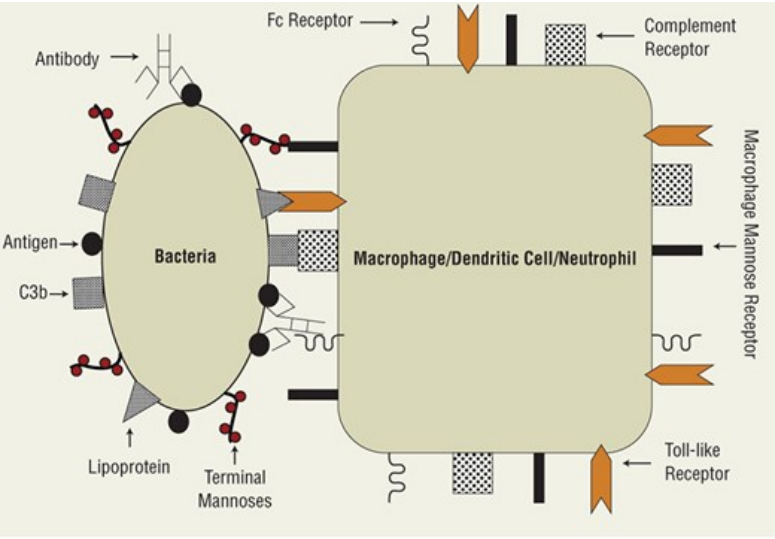


TABLE e106-3

Ligands for Pattern Recognition Receptors

Pathogen Ligand	Type of Organism
Lipoteichoic acid	Gram-positive organisms
Lipopolysaccharide	Gram-negative organisms
Mannose	Fungi, gram-positive, gram-negative
Double-stranded RNA	RNA viruses
Triacyl lipopeptides	Gram-positive, gram-negative
Peptidoglycans	Gram-positive
Bacterial flagella	Various

Cells of the Innate Immune System

Neutrophils, eosinophils, and basophils are considered granulocytes because they have numerous cytoplasmic granules that contain inflammatory mediators or digestive enzymes. Their names are derived from their staining characteristics. For example, neutrophils are named because they stain a neutral pink while basophils are named because they are easily stained by dyes with a high (basic) pH. *Neutrophils* comprise most of the total leukocytes in the bloodstream. They are polymorphonuclear cells, which serve as the primary human defense against invasive bacteria. Neutrophils migrate from the bloodstream into infected or inflamed tissue in response to chemotactic factors, such as IL-8 and breakdown products of complement (C3a and C5a). In this migration, a process termed *chemotaxis*, neutrophils reach the site of inflammation and then recognize (through the PRRs and PAMPs), adhere to, and phagocytose pathogens. In addition, complement and antibody can bind to specific epitopes on a pathogen (opsonize) and then bind to their corresponding receptors on neutrophils to phagocytize the pathogen. During phagocytosis, the engulfed pathogen is internalized within the phagocyte into a cytoplasmic lysosome. The neutrophil then releases its granular contents into lysosomes to form phagolysosomal granules, which generate the release of oxidative metabolites that destroy the engulfed pathogens.[17]

Eosinophils are also granulocytic cells involved in innate immunity and can migrate from the blood into the tissues. They play a less significant role in combating bacterial infections, but eosinophils play a major role against nonphagocytable multicellular pathogens, such as parasites. After activation via high-affinity receptor for IgE (ie, Fcε), eosinophils exocytose their granules causing the release of basic proteins or reactive oxygen species into the microenvironment, causing lysis of the parasite. In addition to Fcε receptors, eosinophils express lower levels of complement receptor 3 and Fcγ for IgG than neutrophils. The high affinity of eosinophils for IgE contributes to their role in the pathogenesis of allergies.[18]

Macrophages and *monocytes* are mononuclear cells capable of phagocytosis. Tissue macrophages arise from the migration of monocytes from the bloodstream into the tissues. Macrophages differ from monocytes in that they possess an increased number of Fc and complement receptors. Macrophages are found within specific tissues and are often called histiocytes. However, they are most often referred to by specialized names depending on the site where they are found (eg, Kupffer cells in the liver, osteoclasts in the bone, and microglial cells in the CNS).[19] The term reticuloendothelial system was commonly used to refer to phagocytic cells of the reticular connective tissue, but the preferred term is now the mononuclear phagocyte system.

2 Despite the first description of Langerhans cells, a type of *dendritic cell* (DC) found in the skin in 1868, our current understanding of the biologic function of DCs did not develop until the last few decades. Before pathogen recognition, most DCs are in an immature/resting state with limited ability to activate T-lymphocytes, but they express numerous receptors (eg, Fc receptors of IgG and IgE, macrophage mannose receptor, and toll-like

receptors) that enable rapid recognition and phagocytosis of multiple antigens. Following antigen recognition and particle engulfment, DCs become activated and greatly increase their expression of the MHC class II, B7-1/B7-2 (L/CD86), CD40, and adhesion molecules. In addition to phagocytosing pathogens in the innate immune system, macrophages and DCs act as APCs to stimulate the adaptive immune system. Macrophages and DCs perform this function by internalizing pathogens, digesting them into small peptide fragments, and then combining these antigenic fragments with MHC molecules, which move to the cell's surface and present peptides to the T-cell receptor (TCR) on the surface of a T-lymphocyte. The recognition of the antigen/MHC complex by the TCR is the first step in the activation of the T-lymphocyte (Fig. e106-3). B-lymphocytes can also act as APCs, which is important to the development of specific antibodies (Fig. e106-4).[19-21]

FIGURE e106-3

Induction of T-helper type 1 (TH1) response. (1) The APC, in this case a DC, engulfs the pathogen by any of numerous cell surface receptors (Fig. e106-2). After phagocytosis of the pathogen by the DC (A), the pathogen is digested into small peptides which becomes associated with major histocompatibility (MHC) class II within the endosome (B). Finally, the MHC class II molecule/peptide complex is expressed on the surface of the DC (C). The activated DC also secretes interleukin (IL)-12. (2) Naïve CD4+ T-lymphocyte activation requires the T-cell receptor (TCR) to recognize the antigenic peptide in association with MHC class II as well as the B7-1 (CD80) binding to CD28. The binding of CD2-CD58 and LFA-1 (CD11a/CD18) allows adherence between the T-lymphocyte and DC. Upon activation, the TH1 CD4+ T-lymphocyte secretes IL-2 and interferon (IFN)-γ and increases the production and expression of the IL-2 receptor (ICAM, intercellular adhesion molecule).

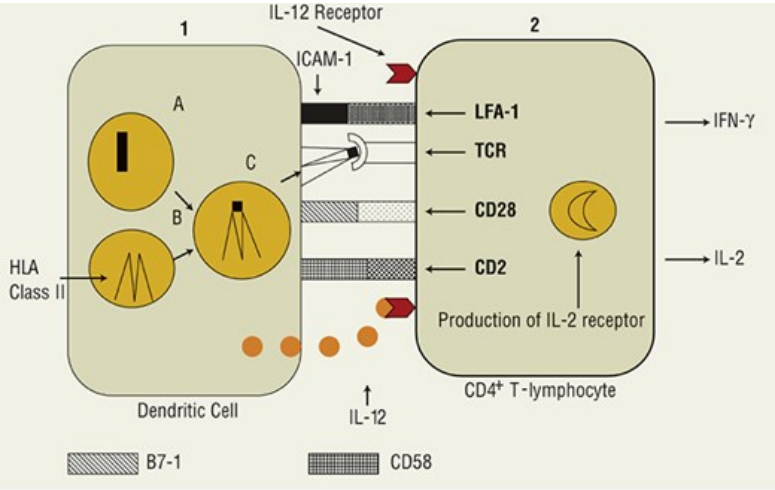
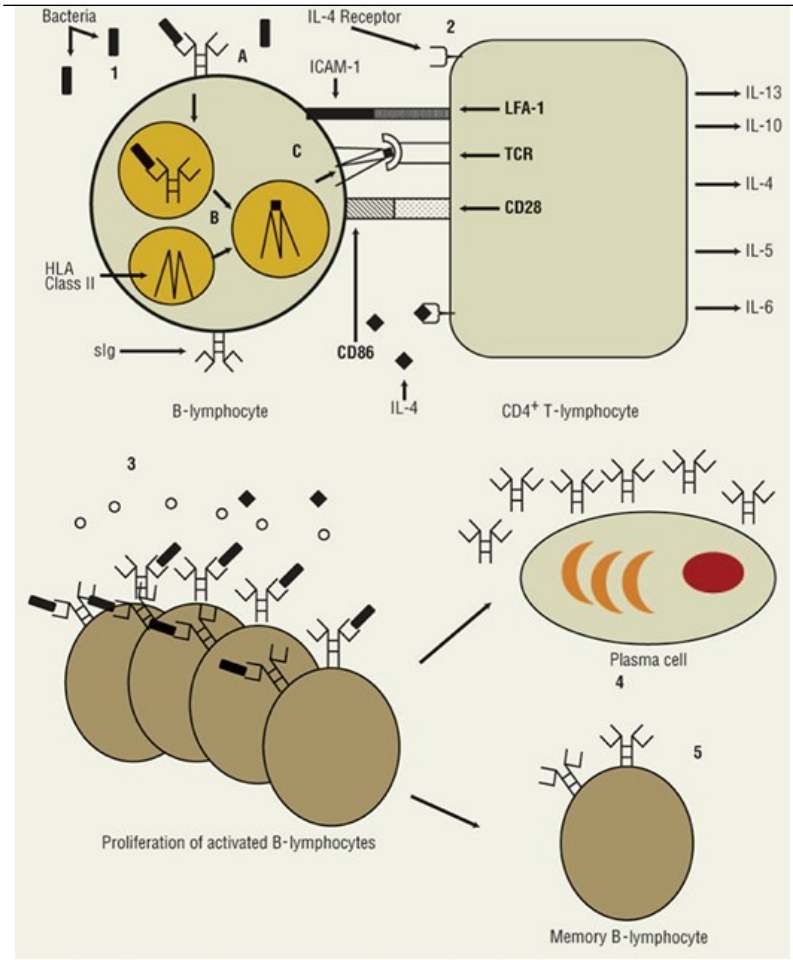


FIGURE e106-4

Induction of T-helper type 2 (TH2) response. (1A) A B-lymphocyte recognizes invading bacteria via its surface immunoglobulin (sIg). (1B) The bound bacteria are phagocytosed into an endosome, where the bacteria are broken down into small peptide fragments. (1C) The small peptide fragments are placed within MHC class II molecules and transported to the surface of the B-lymphocyte for antigen presentation to a CD4+ T-lymphocyte. (2) CD4+ T-lymphocyte recognition requires antigen recognition within the MHC class II peptide groove by the T-cell receptor (TCR) and a secondary signal from B7-2 from the antigen-presenting cell, in this case a B-lymphocyte, binding to CD28 on the T-lymphocyte. When both signals are delivered, the CD4+ T-lymphocyte becomes activated. In the TH2 environment (see the text), the naïve CD4+ T-lymphocyte develops into a TH2 subtype and secretes interleukin (IL)-4, IL-5, IL-6, IL-10, and IL-13, which promotes a TH2 response. (3) In the presence of these cytokines plus antigen binding to the sIg, the B-lymphocyte becomes activated. The activated B-lymphocyte becomes a plasma cell (4), which produces and secretes immunoglobulin or becomes a memory B-lymphocyte (5). A minority of B-lymphocytes become memory B-lymphocytes.



Source: Joseph T. DiPiro, Gary C. Yee, Stuart T. Haines, Thomas D. Nolin, Vicki L. Ellingrod, L. Michael Posey: *DiPiro's Pharmacotherapy: A Pathophysiologic Approach, 12e*

Mast cells and *basophils* act primarily by releasing inflammatory mediators. Mast cells are tissue cells predominately associated with IgE-mediated inflammation. They are especially abundant in the skin, lungs, nasal mucosa, and connective tissue. Granules within the mast cells contain large amounts of preformed mediators that include histamine, heparin, and serotonin. Mast cells can also phagocytize, destroy, and present bacterial antigens to T-lymphocytes.[20] Basophils are similar to mast cells because they contain granules filled with histamine, but they are typically found circulating in the blood and are not found in connective tissue. Like mast cells, basophils also express high-affinity IgE Fc receptors (Fcε). IgE-mediated anaphylaxis (type I hypersensitivity [Chapter e108]) is caused by the degranulation and the release of preformed mediators upon stimulation of mast cells or basophils by an allergen binding to IgE bound to the Fcε receptor on their cell surface.[21]

Soluble Mediators of the Innate Immune System

Soluble mediators of innate immunity involve proteins that include the complement system, mannose-binding lectin, antimicrobial peptides, and C-reactive protein (CRP).[11] The complement system consists of more than 30 proteins in the plasma and on cell surfaces that play a key role in immune defense. The four major functions of the complement system include: (a) lysis of certain microorganisms and cells; (b) stimulation of chemotaxis of phagocytic cells; (c) coating or opsonization of foreign pathogens, which allows phagocytosis of the pathogen by leukocytes expressing complement receptors; and (d) clearance of immune complexes. Complement factors (C3a, C5a) also act as chemotactic factors for phagocytic cells.[22] Two different pathways stimulate the complement cascade. In the *classical pathway*, the antibody binds to its target antigen and activates the first component of complement (C1), thereby initiating the complement cascade. The *alternative complement pathway* relies on the inability of microorganisms to clear spontaneously produced C3b, the active form of the third complement protein, from their surface. Patients with hereditary deficiencies of complement have recurrent bacterial infections or immune complex disease because C3b plays a central role in opsonizing bacteria and clearing immune complexes.

Both mannan-binding lectin and CRP are acute-phase reactants produced by the liver during the early stages of an infection. They act as opsonins by binding to infectious pathogens that serve as an intermediate by binding to their respective receptors on phagocytes. Mannan-binding lectin binds to mannose-rich glycoconjugates on microorganisms, while CRP binds to phosphorylcholine on bacterial surfaces.[11,22]

The chemokine system consists of a group of small polypeptides and their receptors. *Chemokines* play an essential role in linking the innate and adaptive immune response by orchestrating leukocyte trafficking. Chemokines possess four conserved cysteines. Based on the positions of the cysteines, almost all chemokines fall into one of the two categories: (a) CC group in which the conserved cysteines are contiguous or (b) CXC subgroup in which the cysteines are separated by some other amino acid (X). As with all ligand-receptor interactions, a cell can only respond to a chemokine if the cell possesses a receptor that recognizes the chemokine. Chemokine receptors are unique in that they traverse the membrane seven times. CC receptors (CCR) and CXC receptors (CXCR) bind CC ligands (CCL) and CXC ligands (CXCL), respectively (Table e106-4).

TABLE e106-4

Common Chemokines

Receptor	Cell Expression	Ligand
CCR1	Immature DC	MIP-1α, MIP-1β, MCP-2, RANTES
CCR3	Eosinophils, basophils	Eotaxin-1, eotaxin-2, eotaxin-3, MCP-4
CCR6	Immature DC	Exodus-1
CCR7	Activated DC	CCL21 (SLC), CCL19 (ELC)
CXCR1/2	Neutrophils	IL-8
CXCR3	Natural killer cells, activated T-lymphocytes	IP-10

DC, dendritic cell; ELC, EBI1 ligand chemokine; MCP, monocyte chemoattractant protein; RANTES, regulated upon activation normal T-lymphocyte expressed and secreted; SLC, secondary lymphoid tissue chemokine.

Binding of infectious pathogens to PRRs stimulates the release of chemokines such as macrophage inflammatory protein (MIP)-1α, MIP-1β, MIP-3α, and IP-10 from macrophages and DCs embedded in the tissues. These chemokines attract more immature DCs to the site of inflammation/infection. Immature DCs constitutively express CCR1, CCR5, and CCR6. The interaction between PRRs on the DC to the infectious pathogen causes the activation and maturation of the DC. After activation, DCs downregulate the expression of CCR1, CCR5, and CCR6 and upregulate the expression of CCR7. This switch in chemokine-receptor expression results in the antigen-loaded DC leaving the tissue and migrating toward the lymph nodes.[23]

Naturally occurring antimicrobial peptides include α-defensins, β-defensins, and cathelicidins. These peptides exhibit antibacterial, antifungal, and antiviral activity. Human antimicrobial peptides range from 29 to 37 amino acid residues in length. Neutrophils are rich sources of both α- and β-defensins as well as cathelicidins. Other sources of the human antimicrobial peptides include keratinocytes, Paneth cells of the intestinal and genital tracts, and epithelial cells of the pancreas and the kidney. These peptides can be induced at sites of inflammation or can be constitutively produced. The clinical interest in human antimicrobial peptides centers on their broad-spectrum activity and their rapid onset of killing. They are believed to work by disrupting microbial membranes. An active area of research is how these peptides discriminate between microbial and host membranes.[24]

Adaptive Immune System

The body will generally employ innate and adaptive immune responses to rapidly kill foreign pathogens.[11] The greatest difference between innate and adaptive immune responses is specificity and memory, characterized by antigen-specific receptors located on the surface of B (sIg) and T-lymphocytes (TCR).[13] Cells that mediate the adaptive immune response also secrete cytokines to further amplify the innate immune response. The adaptive immune

response can evolve with each subsequent infection, whereas the innate response stays the same with each infection. During B- and T-lymphocyte development, an individual B- or T-lymphocyte rearranges its immunoglobulin and TCR genes, respectively, to produce a unique immunoglobulin or TCR. This DNA rearrangement generates enough B- or T-lymphocytes to recognize an estimated 1,012 and 1,010 antigens, respectively.

The adaptive immune response can be divided into two major arms: humoral and cellular responses. The humoral response is so named because it was discovered that the factors that provided the immune protection could be found in the “humor” or fluids (eg, serum, plasma, lymph) and generally refers to antibody responses. To generate a good antibody response, T-lymphocytes of the T-helper cell phenotype are necessary. B-lymphocytes activated in this way can differentiate into plasma cells and secrete antibodies or they differentiate into memory B-cells that are specific for the pathogen that reacted with its sIg.

Cells of the Adaptive Immune System

T-lymphocytes constitute the cell-mediated arm of the adaptive system. The immune protection provided by T-lymphocytes cannot be transferred by fluids alone. Rather, it is essential to actually have T-lymphocytes present, thus the term cell-mediated immunity. T-lymphocytes are specially tailored to defend against infections that are intracellular, such as viral infections, whereas B-lymphocytes secrete antibodies that can neutralize pathogens prior to their entry into host cells.

3 The role of the T-lymphocyte is to respond to various pathogens extracellularly (CD4+ T-helper cells and MHC class II) or intracellularly (CD8+ T-cytotoxic cells and MHC class I). T-lymphocytes use a specific antigen receptor, the TCR, to propagate the immune response. The TCR is comprised of two chains, with each chain having a variable and a constant region. The variation of the amino acid sequence within the variable domain of the TCR gives the cell its unique antigen specificity. Linked to the TCR is a complex of single chains known as the CD3 complex.[11,21]

The MHC is a cluster of genes found on chromosome 6 in humans, also known as the human leukocyte antigen (HLA) complex, that is converted to proteins used by the immune system to distinguish self from nonself and provides a so-called immunologic “fingerprint.” The MHC complex is divided into three different classes: I, II, and III. There are six MHC class I genes (A, B, C, E, F, and G) of which only A, B, and C are considered major. These molecules can be found on all nucleated cells within the body and on platelets. MHC class I antigens are not found on mature red blood cells. Molecules encoded by class II MHC genes include DP, DQ, and DR. The expression of these molecules is more restricted and can be found primarily on APCs, such as macrophages, DCs, and B-lymphocytes. The class III HLA antigens encode for soluble factors, complement, and tumor necrosis factors (D-80s).[25] For a CD4+ T-lymphocyte to become activated, it must recognize the antigenic peptide in association with MHC class II (see Figs. e106-3 and e106-4).

CD8+ T-lymphocytes recognize antigenic peptide in association with class I MHC molecules. Class I MHC molecules generally form complexes with endogenous peptides from within the cell, such as those derived from viruses. In contrast, class II MHC molecules generally form complexes with exogenous peptides from antigen that has been phagocytized and digested, such as bacterial peptides (see Fig. e106-3). Thus, the MHC class I and CD8+ T-cell interaction is a sensing system by which the immune system is constantly checking the nucleated cells of the body for what is happening inside the cells of the body.[25,26] DCs, and to a lesser extent macrophages, demonstrate the unique capacity to direct exogenous antigens toward MHC class I molecules, a process termed cross-presentation.[27] In contrast, the MHC class II and CD4+ T-cell interaction is a sensing system by which the immune system is constantly checking for what is happening outside of our cells.

Naïve T-lymphocytes are cells that have not been previously exposed to an antigen specific for their TCR. These cells require two signals for activation. The first signal for activation involves the T-lymphocyte recognizing both the processed antigen and the MHC molecule complex. The second signal involves the interaction of the B7-1 (CD80) or B7-2 (CD86) molecule on the APC with the CD28 molecule on the surface of the T-lymphocyte (see Figs. e106-3 and e106-4). Without the second signal, the naïve T-lymphocyte becomes anergic or inactive. Memory T-lymphocytes are less dependent on the second signal than are naïve T-lymphocytes. CD28 is expressed on both resting and activated T-lymphocytes. After the two activation signals, a message is sent through the TCR to the CD3 complex into the cell. Then calcium influx occurs, resulting in activation of the T-lymphocyte. Activated CD4+ T-lymphocytes begin to express the high-affinity interleukin 2 (IL-2) receptor and release multiple soluble factors (eg, IL-2) to stimulate T-lymphocytes and other cells of the immune system (see Fig. e106-3). Autocrine stimulation by IL-2 leads to the proliferation of the activated T-lymphocyte.

In addition to activation pathways, T-lymphocytes can also express inhibitory receptors on their cell surface. One example is cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), which also binds B7, and is only expressed on activated T-lymphocytes. When B7 binds to CTLA-4 on an activated T-

lymphocyte, an inhibitory signal is sent to the T-lymphocyte, thereby modulating the T-lymphocyte response.[28] The exact mechanism by which CTLA-4 binding inhibits T-lymphocyte activity is not fully understood.

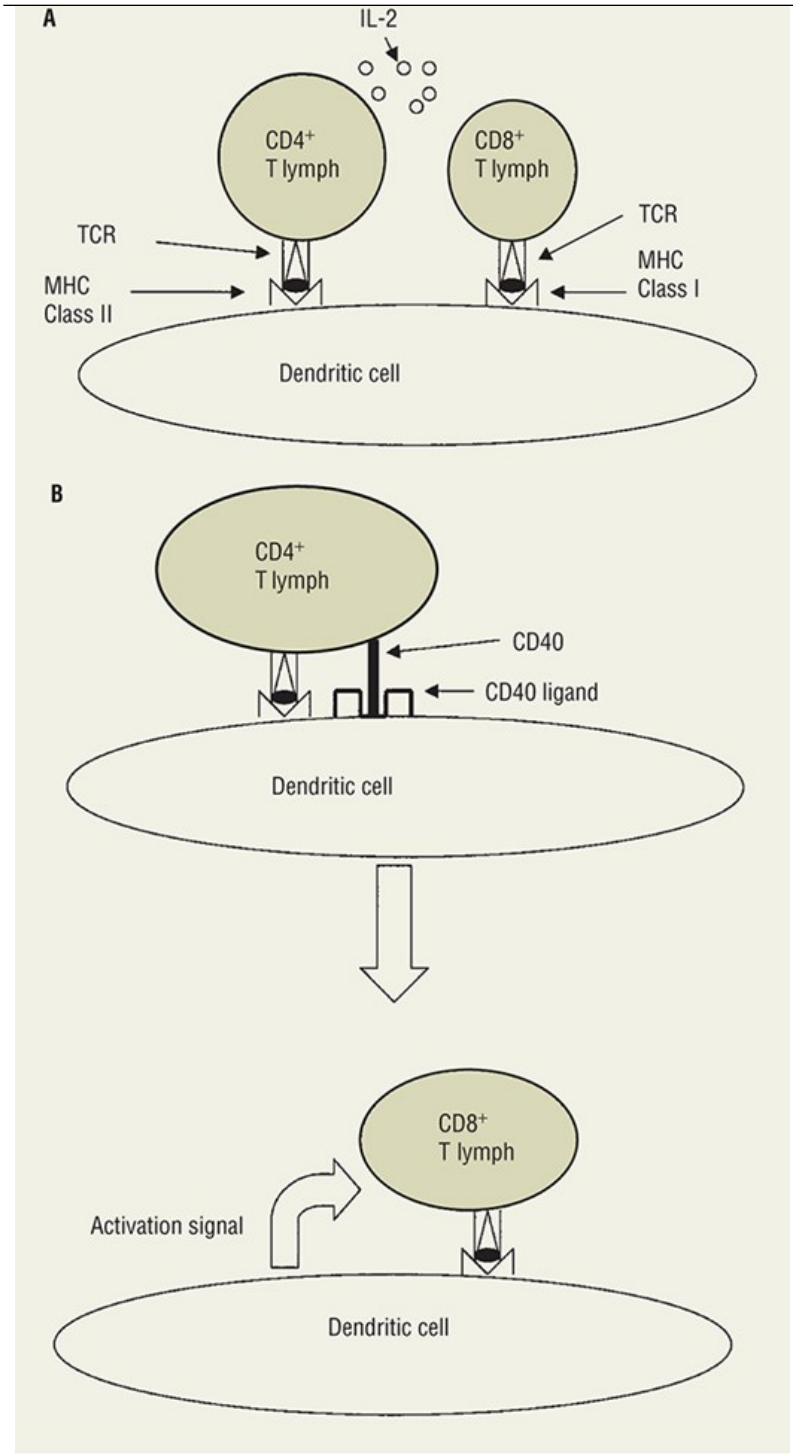
Cell surface markers delineate the functional activity of T-lymphocyte populations. All T-lymphocytes express the CD3 protein. Typically, T-lymphocytes are further divided into helper cells (CD4+), suppressor cells (CD8+), and cytotoxic cells (CD8+). Each subclass plays a distinct role in the cell-mediated immune response. Naïve T-lymphocytes express CD45RA, a high-molecular-weight isoform of CD45, while memory T-lymphocytes express CD45RO, a lower-molecular-weight isoform of CD45.[29] The primary role of CD4+ cells is to stimulate other cells in the immune response. Functionally, CD4+ cells can be divided into T-helper type 1 (TH1), T-helper type 2 (TH2), TH17, T follicular helper (THFH), and T-regulatory (Tregs). This functional system was first described in mice. TH1 cells secrete IL-2 and γ-interferon and stimulate CD8+ cytotoxic cells while TH2 cells secrete IL-4, IL-5, and IL-10 and stimulate B-lymphocyte production of antibodies against extracellular pathogens.[30] Multiple factors determine whether a naïve CD4+ T-lymphocyte develops into a TH1 or a TH2 cell. The cytokine microenvironment plays an important role in this development. IL-12 secreted by the APCs promotes TH1 while IL-4 promotes TH2 development. Other factors that promote TH1 development include B7-1 (CD80), high affinity of the TCR for the antigen, γ- interferon, and α-interferon. Factors that promote TH2 development include B7-2 (CD86), low affinity of the TCR for the antigen, IL-10, and IL-1.[31] THFH also promote B-lymphocyte activation and play a crucial role in the generation of memory B-lymphocytes which leads to long-lived antibody responses.[32] The TH17 subset was discovered because of selective production of IL-17 and plays an important role in immunity in mucosal tissues and in the pathogenesis of multiple inflammatory and autoimmune disorders.[33]

CD8+ T-lymphocytes recognize antigen in association with MHC class I. CD8+ cytotoxic cells are instrumental in killing cells recognized as foreign, such as those that have become infected by a virus. CD8+ cytotoxic T-lymphocytes play an important role in the eradication of tumor cells, which often express mutated or altered forms of self-antigens. Cytotoxic T-lymphocytes are also responsible for the rejection of transplanted organs and for the phenomenon known as graft-versus-host disease.[21] Some T-lymphocytes suppress the immune responses and maintain self-tolerance, but it is not clear whether this subset is CD8+. These cells are not CD8+ T-lymphocytes but are CD4+, CD25+ T-lymphocytes. The preferred term for these suppressive T-lymphocytes is regulatory T-lymphocytes.[34]

Our understanding of how CD8+ T-lymphocytes are activated is constantly evolving. The traditional model involves the interaction of a CD8+ T-lymphocyte with an APC, typically a DC. More potent activation of CD8+ T-lymphocyte may result from the interaction of an APC, typically a DC, a CD4+ helper lymphocyte (TH1), and a CD8+ T-lymphocyte (Fig. e106-5A). This model of CD8+ cytotoxic T-lymphocyte activation requires close proximity of two antigen-specific T-lymphocytes (the CD4+ and the CD8+ T-lymphocytes). In addition, CD8+ cytotoxic T-lymphocyte activation can occur in the absence of direct interaction with CD4+ T-lymphocytes. CD4+ T-lymphocytes can activate APCs through CD40; this interaction primes the APC to fully activate CD8+ cytotoxic T-lymphocytes (Fig. e106-5B).[35] It is important to remember that the classification of CD4+ lymphocytes as T-helper lymphocytes and CD8+ lymphocytes as T-cytotoxic lymphocytes is not absolute. Some CD8+ T-lymphocytes secrete cytokines similar to a T-helper lymphocyte and some CD4+ T-lymphocytes can act as cytotoxic cells.

FIGURE e106-5

In the classic model of CD8+ T-lymphocyte activation (A), CD4+ and CD8+ T-lymphocytes recognize antigen on the same DC. In the presence of interleukin (IL)-2 from the activated CD4+ T-lymphocyte and the recognition of antigen in association with major histocompatibility complex (MHC) class I, the CD8+ T-lymphocyte becomes activated. In the new model (B), activated CD4+ T-lymphocytes activate DCs via CD40 ligand binding to CD40. The activated DC then migrates through the tissues to present antigen to CD8+ T-lymphocytes. If recognition via the T-cell receptor (TCR) on the CD8+ T-lymphocyte occurs, the DC can fully activate the CD8+ T-lymphocyte without the presence of CD4+ T-lymphocytes.



Unlike neutrophils and macrophages, cytotoxic T-lymphocytes are unable to ingest their targets. They destroy target cells by two different mechanisms: the *perforin system* and the *Fas ligand pathway*. After recognition by the cytotoxic T-lymphocyte, cytoplasmic granules containing perforins and granzymes are rapidly oriented toward the target cell and the granule contents are released into the intracellular space. Like the membrane attack complex formed after complement activation, perforins form a pore in the target cell membrane. Besides a direct cytotoxic effect on the target cell, the pores allow the granzymes to penetrate into the target cell and induce apoptosis. The second mechanism of cytotoxicity involves the binding of Fas ligand (FasL) on the cytotoxic T-lymphocyte to the Fas receptor on the target cell. The FasL is predominately expressed on CD8+ cytotoxic T-lymphocytes and natural killer (NK) cells, and its expression increases after activation. When the Fas receptor on the target cell is bound by

FasL expressed by the CD8+ cytotoxic T-lymphocyte, the target cell receives a strong signal inducing it to undergo apoptosis (ie, commit suicide).[36] After destroying the target cell by either mechanism, the cytotoxic T-lymphocyte detaches from the target cell and attacks other targets.[37]

A B-lymphocyte recognizes antigen via its antibody or immunoglobulin (sIg) located on its cell surface (see Fig. e106-4). The sIg can recognize an intact pathogen, such as bacteria, and present antigen to T-lymphocytes (ie, acting as APC). However, another major function of B-lymphocytes is to differentiate into a plasma cell to produce antibody specific for the invading pathogen, a process that first requires activation of the B-lymphocyte. The activation of B-lymphocytes also requires two steps: (a) recognition of antigen via the sIg; and (b) the presence of B-lymphocyte growth factors (IL-4, 5, and 6) secreted by activated CD4+ T-lymphocytes. Once activated, the B-lymphocyte becomes a plasma cell, a differentiated cell capable of producing and secreting antibodies and then dying. Some activated B-lymphocytes do not differentiate into plasma cells, but rather form a pool of memory B-cells. The memory B-cells will respond to subsequent encounters with the pathogen, generating a quicker and more vigorous response to the pathogen. Some B-lymphocytes can become activated without help from T-lymphocytes, but these responses are generally weak and do not invoke memory.[11,21]

NK cells, often referred to as large granular lymphocytes, are defined functionally by their ability to lyse target cells without prior sensitization and without restriction by MHC. NK cells recognize target cells by two mechanisms. First, NK cells express an IgG Fc receptor, CD16, that allows recognition of IgG-coated cells. Second, NK cells express killer-activating and killer-inhibiting receptors. The killer-activating receptors recognize multiple targets on normal cells, but the binding of MHC class I to the killer-inhibitor receptor blocks the release of perforins and granzymes. Therefore, cells (eg, tumor cells, virally infected cells) that downregulate MHC class I expression are susceptible to NK cell cytolysis. NK cells play important roles in the surveillance and the destruction of tumors and virally infected host cells, and in the regulation of hematopoiesis.[11,38]

The immune system employs several mechanisms to downregulate responses to prevent autoimmune diseases. Many of these mechanisms are directed at T-lymphocyte activation. After activation (about 2 days), T-lymphocytes express CTLA-4 (a second ligand for B7 [CD152]). As previously discussed, when CTLA-4 binds B7, T-lymphocyte activity is inhibited. Another mechanism of T-lymphocyte inhibition is the programmed cell death 1 (PD-1) system. Once a T-lymphocyte is activated, it expresses the PD-1 receptor. The PD-1 receptor can bind to two separate ligands, known as PD-L1 and PD-L2. When bound to its ligand, PD-1 inhibits antigen receptor signaling in T-lymphocytes, resulting in decreased production of proinflammatory cytokines by the T-lymphocyte.[39] Interestingly, the same Fas/FasL system used by CD8+ cytotoxic T-lymphocytes to destroy their targets is also a mechanism that can inhibit T-lymphocytes. Once T-lymphocytes become activated, they express Fas receptors on their cell surfaces. If the Fas receptor on a T-lymphocyte is bound by FasL, the T-lymphocyte receives a signal inducing it to undergo apoptosis. Certain tissues such as the testis, retina, and some types of cancer cells use the Fas system to protect themselves from harmful immune responses. These tissues constitutively express the FasL, which protects them from activated T-lymphocytes.[40] Another means of modulating T-lymphocyte responses is through a functional subset of CD4+ lymphocytes: Tregs. Tregs are antigen specific and require contact between the Tregs and the target T-lymphocyte to exert their inhibitory effect. Tregs can downregulate T-lymphocyte responses by secreting transforming growth factor-β and IL-10.[34]

Soluble Mediators of the Adaptive Immune Response

Antibodies

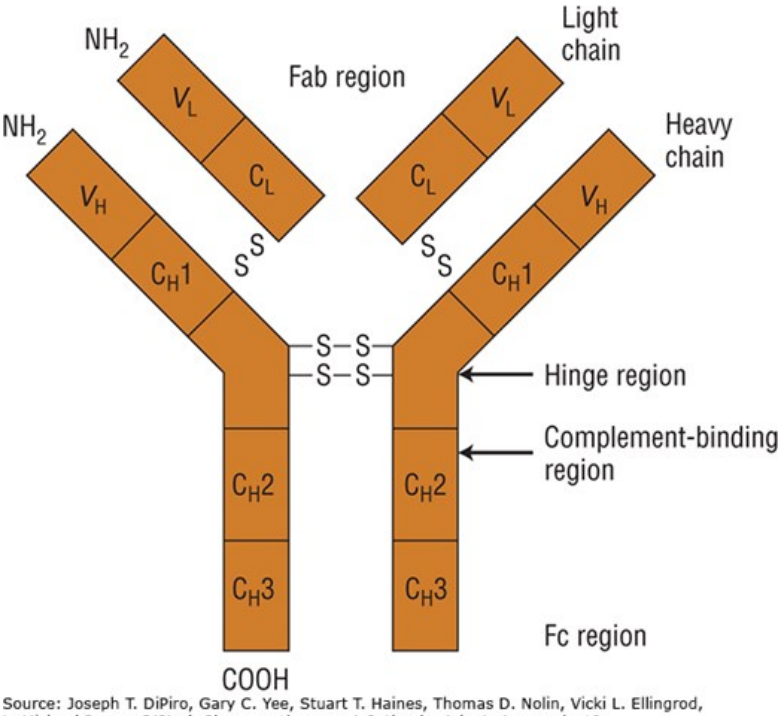
4 When the binding of a specific antigen to the surface immunoglobulin receptor of B-lymphocytes occurs, the B-lymphocyte matures into a plasma cell and produces large quantities of antibodies that have the ability to bind to the inciting antigen. The secreted antibodies may be of five different isotypes: IgA, IgD, IgE, IgG, and IgM. On primary exposure to a given pathogen, the plasma cell will secrete IgM, followed by an eventual switch to predominately IgG. Upon a second exposure to the same antigen, the memory B-lymphocytes will predominately produce IgG. Isotype switching from IgM to IgG, IgA, or IgE is controlled by T-lymphocytes.

An antibody or immunoglobulin is a glycoprotein comprised of two different chains, heavy and light (Fig. e106-6). The basic structure of every immunoglobulin consists of four peptide chains: two identical heavy chains and two identical light chains held together by disulfide bonds. The basic structure of the antibody is a Y-shaped figure. Each arm of the Y is formed by the linkage of the end of the light chain to its heavy chain partner. These arms contain the portions described as the *fragments of antigen binding (Fab fragments)*. The stem of the Y contains the heavy chains, which comprise the *fragment crystallizable (Fc fragment)* portion of the antibody. It is within the Fc portion that complement is activated once the antibody has bound its target. Likewise, it is the Fc portion of the antibody that is recognized by Fc receptors on the surface of phagocytes (see Fig. e106-2). The amino acid composition of the same isotype is homogenous except in the variable regions of the light (V_L) and heavy chains (V_H). The variation in amino acid

composition of the variable region gives the antibody its unique specificity (see Fig. e106-6).

FIGURE e106-6

Schematic diagram of the structure of the IgG molecule. IgG molecule consists of 2 heavy (H) and 2 light (L) chains covalently linked by disulfide bonds. Each chain is composed of variable (*V*) and constant (C) regions. A light chain consists of 1 variable (V_L) and 1 constant (C_L) region. Heavy chains consist of 1 variable (V_H) and 3 or 4 constant (C_H) regions, depending on the isotype. The variable regions (V_L and V_H) compose the antigen-binding region of the IgG molecule, or fragment antigen binding (Fab). The constant regions provide the structure to the IgG molecule and bind to the first component of complement (C_H2) and to Fc receptors via the Fc portion of the molecule (C_H3).



Source: Joseph T. DiPiro, Gary C. Yee, Stuart T. Haines, Thomas D. Nolin, Vicki L. Ellingrod, L. Michael Posey: *DiPiro's Pharmacotherapy: A Pathophysiologic Approach, 12e*

IgG, the most prevalent antibody class, comprises about 80% of serum immunoglobulins. IgG is usually the second isotype of antibody produced during an initial humoral immune response. IgG is the only isotype of antibody that can cross the placenta. Therefore, early maternal humoral protection of neonates is primarily due to maternal IgG that has crossed the placenta in utero.

Four different subclasses of IgG have been described: IgG1, IgG2, IgG3, and IgG4. These subclasses differ slightly in their constant amino acid sequences. IgG1 constitutes the majority (60%) of the subclasses. Different subclasses recognize different types of antigens. IgG1 and IgG3 primarily recognize protein antigens, while IgG2 and IgG4 commonly bind to carbohydrate antigens. Another difference in the subclasses is their ability to activate complement with IgG3 and IgG1 being the most efficient, while IgG4 is unable to activate the complement system.

IgM can be found on the surface of B-lymphocytes (sIg) as a monomeric Y-shaped structure. In contrast, secreted IgM is a pentamer in which five of the monomers are joined together by a joining chain (J-chain). IgM is the first class of antibodies produced following initial exposure to an antigen. Because the pentameric form of IgM has no Fc portions exposed, phagocytic cells cannot bind pathogens opsonized by IgM. However, IgM is an excellent activator of the complement cascade by the classic pathway.

IgA is found primarily in the fluid secretions of the body: tears, saliva, nasal fluids; and also in the GI, genitourinary, and respiratory tracts. IgA functions by preventing pathogens from adhering to and infecting the epithelial cells at these sites. IgA is also secreted in a nursing mother's breast milk; IgG and IgM are also secreted but in lower concentrations. In bodily secretions, IgA is in a dimeric form in which a J-chain and a secretory chain hold two monomers together. The dimeric form is resistant to proteolysis in mucosal secretions.

IgD is the least understood isotype. IgD is found on the surface of B-lymphocytes at different stages of maturation and may be involved in the differentiation of these cells. The main function of circulating IgD has not yet been determined. However, mice treated with exogenous anti-IgD antibodies display a marked increase in immunoreactivity and secretion of all types of immunoglobulins and several T-cell specific cytokines. High levels of anti-IgD autoantibodies of various subtypes have also been observed in most autoimmune diseases with frequencies of more than 50%, suggesting that IgD may play an important role in the etiology of these diseases.[41]

IgE is the least common of the serum antibody isotypes. Most of the IgE in the body is bound to the IgE Fc receptors on mast cells. When the IgE on the surface of mast cells binds antigen, it causes the mast cell to release various inflammatory substances (eg, histamine). The overall effect is stimulation of inflammation. The major function of IgE antibody is to eliminate parasites, but because developed countries of the world have few, if any parasites, the response has shifted and it now plays an important role in allergies. Hay fever is an example of allergic reactions primarily due to antigen binding to IgE.

Cytokines

Cytokines are soluble factors released or secreted by cells. These proteins affect the activity of other cells (paracrine) or the secreting cell itself (autocrine). For example, activated CD4+ T-lymphocytes secrete IL-2, which further activates the secreting cells, CD8+ T-lymphocytes, and NK cells. Research has shown that many cytokines have a broad spectrum of effects depending on their concentration, the presence of other factors, and the target cell (Table e106-5). New cytokine families and their roles in disease processes are being discovered regularly. Cytokines provide communication between the divisions of the immune system. Cytokines produced from APCs generally promote chemotaxis of other cells and induce a state of inflammation.[38] Cytokines can also prevent the activation or response of immunologic cells. For example, IL-10 is an anti-inflammatory cytokine produced in the respiratory tract to prevent IgE synthesis and activation of eosinophils when exposed to benign inhaled particles.[38] Cytokines do not act alone in vivo, but in combination with other cytokines. For example, activated CD4+ T-lymphocytes secrete both IL-2 and interferon-γ, which are synergistic in activating NK cells. As shown in Tables e106-1 and e106-5, cytokines are broadly classified as regulatory or hematopoietic growth factors.[21,42-47] This classification does not describe all their activities. For example, GM-CSF released by activated T-lymphocytes not only acts as a hematopoietic growth factor, but it also activates circulating granulocytes and APCs to phagocytize foreign pathogens.

TABLE e106-5

Regulatory Cytokines

Cytokines	Sources	Principal Effects
IL-1	Macrophages, fibroblasts, endothelial cells	Activation of T- and B-lymphocytes, hematopoietic growth factor, and induction of inflammatory events
IL-2	CD4+ T-lymphocytes (TH1 subset)	Activation of T-lymphocytes, B-lymphocytes, and NK cells
IL-4	CD4+ T-lymphocytes (TH2 subset), mast cells, basophils, eosinophil s	B- and T-lymphocytes growth factor, activation of macrophages, promotes IgE production, proliferation of bone marrow precursors
IL-5	CD4+ T-lymphocytes (TH2 subset), mast cells	Activation of B-lymphocytes and eosinophils, promotes IgE production
IL-6	CD4+ T-lymphocytes (TH2 subset), macrophages, mast cells, fibroblasts	T- and B-lymphocytes growth factor, hematopoietic growth factor, augments inflammation
IL-8	T-lymphocytes, monocytes, endothelial cells, fibroblasts	Neutrophil, basophil, and T-lymphocytes chemotaxis
IL-10	T- and B-lymphocytes, macrophages	Cytokine synthesis inhibitory factor, growth of mast cells
IL-12	Macrophages, neutrophils, dendritic cells	Induce TH1 cells, ↑ NK cell activity, ↑ generation of cytotoxic T-lymphocytes

IL-13	Activated T-lymphocytes	Proliferation of B-lymphocytes, suppression of proinflammatory cytokines, directs IgE isotype switching
IL-14	T-lymphocytes	Induces B-lymphocytes proliferation, inhibits secretion of Igs
IL-15	Macrophages, fibroblasts, dendritic cells, epithelial cells	T-lymphocytes proliferation and activation of NK cells
IL-16	$CD8^+$ T-lymphocytes, epithelial cells	Chemoattractant for CD4+ T-lymphocytes and eosinophils; stimulation of secondary cytokine secretion from and proliferation of CD4+ T-lymphocytes
IL-17	CD4+ T-lymphocytes (TH17 subset)	Proinflammatory cytokine that promotes the neutrophil expansion and accumulation in the tissues
IL-18	Macrophages	Induces γ-interferon production
IL-23	Macrophages	Inflammatory macrophages express IL-23R and are activated by IL-23 to produce IL-1, TNF-α, and IL-23 itself
IL-28 and -29[a]	Antigen-presenting cells, but proposed that all nucleated cells may produce	Alternative to α/β interferons to provide immunity against viral infections by inhibiting viral replication
IL-31	Activated T-lymphocytes	Involved in the recruitment of PMNs, monocytes, and T-cells to the site of inflammation
IL-32	NK-cells, T-lymphocytes, epithelial cells	Induces proinflammatory cytokines including TNF-α and IL-8
IL-35	CD4+ T-lymphocytes (Treg subset)	T-cell suppression
TNF-α	Macrophages, NK cells, T-lymphocytes, B-lymphocytes, mast cells	Activation of neutrophils, endothelial cells, lymphocytes, and liver cells to produce acute phase proteins
TNF-β	T-lymphocytes	Tumoricidal
IFN-α	Monocytes, other cells	Antiviral, activation of NK cells and macrophages, upregulation MHC class I
IFN-γ	T-lymphocytes, NK cells	Activation of macrophages, NK cells, upregulation of MHC class I and II

[a]Also known as the new type III IFN-γ family.

The division of the immune system into the two functional groups does not imply that the divisions do not interact with each other. Both soluble mediators (eg, complement, antibody, and cytokines) and cells (eg, neutrophils, macrophages, DCs, T-lymphocytes, and B-lymphocytes) are needed to generate a vigorous immune response. The innate system will usually respond first. DCs, macrophages, and neutrophils in the tissues will recognize pathogens via surface receptors (see Fig. e106-2). To amplify the immune response, the APCs will present antigen to CD4+ T-lymphocytes (see Figs. e106-3 and e106-4). The activated CD4+ T-lymphocytes will secrete cytokines to activate B-lymphocytes, CD8+ T-lymphocytes, NK cells, macrophages, and neutrophils. The next section of the chapter discusses the evaluation of the immune system.

DISEASES OF THE IMMUNE SYSTEM

Although this chapter is not intended to detail the diseases of the immune system, it is necessary to review the terminology and provide specific

examples of diseases of the immune system to understand the role of monitoring and possible intervention with pharmacotherapy. Diseases that impair the physical defense component of the immune system are often not considered as diseases of the immune system, but loss of normal physical defenses can compromise the immune response and result in infectious sequelae. For example, in cystic fibrosis, altered chloride transport results in thick respiratory secretions, which leads to colonization of the airways by pathogens and frequent respiratory infections. Primary immunodeficiency diseases are those characterized by either a congenital inability to produce components of the immune system (ie, severe combined immunodeficiency or hypogammaglobulinemia) or acquired, as seen with HIV infection. Autoimmune diseases result from a dysregulation of a component or a combination of components of the immune system (eg, rheumatoid arthritis, systemic lupus erythematosus [SLE]).[48] Autoimmune diseases are often characterized by the production of autoantibodies against a particular host structure that is critical for normal function, the loss of self-tolerance to otherwise healthy tissues, or the loss of tolerance or anergy to a ubiquitous antigen (ie, gluten in celiac sprue).[44] Often medications that suppress the immune system are necessary to control symptoms and halt autoimmune disease progression. Administration of immunosuppressive medications in the setting of autoimmune diseases or organ transplantation may reduce disease symptoms, but may also reduce the host's ability to fight off infection or cancer. Exogenous regulation of the immune system must be done carefully, and we must continue to discover new methods for the appropriate evaluation of immune responses.

EVALUATION OF IMMUNE FUNCTION

Assessment of a patient's immune function requires knowledge and understanding of multiple components including mechanical defenses, cell phenotypes and cell numbers, and soluble components. Recent developments have allowed immunologists to better understand the role of the immune response in many diseases. This is important because the upregulation and downregulation of immune responses are necessary to treat various disease states. Therefore, pharmacotherapeutic considerations must balance the risk of disrupting normal immunologic homeostasis. Improvements in immune monitoring are necessary for the goal of patient-specific immunologic pharmacotherapy. Despite technological advances, careful patient evaluations are required to accurately assess the structure and function of the immune system. Specific methods for assessment of patient immune status are discussed later.

Innate Immunity: Evaluation of Mechanical Immunodefenses

As discussed earlier, the mechanical aspects of host defense are extremely important in protection from infection; therefore, assessment of mechanical defenses is critical. Much of the assessment of mechanical immunodefense is accomplished by recognition of situations where it may be compromised. Careful patient examination usually reveals the extent of compromise and laboratory tests are generally not necessary for evaluation of this component. To assess the extent of compromise in mechanical immunodefenses, the clinician should carefully examine the patient and identify the specific types of risks present. Specific examples of altered mechanical defenses are listed in Table e106-6.

TABLE e106-6

Examples of Alteration in Mechanical Immunodefenses That Result in Impaired Immune Status

Reduced gastric pH
Achlorhydria
Use of histamine-2 blockers and proton pump inhibitors
Patients with acquired immunodeficiency syndrome
Break in skin barrier
Burns
Surgical incision
Penetrating trauma
Vascular access devices
Impaired mucociliary function of the lungs
Smoking
Impaired esophageal or epiglottal function
Endotracheal intubation
Stroke
Recumbent position
Altered urine flow
Urinary stones
Anatomic deformities obstructing flow
Bladder catheter
Anatomic alterations of the heart resulting in turbulent blood flow and endocarditis

Innate and Adaptive Immunity: Gross Evaluation of Cellular Components

A major aspect of the assessment of immune function relates to the cells of the immune system. Assessment of cells in the clinical setting includes the determination of cell type and cell number or function. Determination of the cell types and numbers is generally performed first because of the ease of obtaining these results and the common correlation with the clinical situation.

To quickly screen cell numbers, a *white blood cell* (*WBC*) *count* with differential is performed. Normal cell counts are shown in Table e106-7.[49] This simple test often steers the differential diagnosis. In interpreting a WBC with differential, the clinician must consider several factors. A normal cell count does not mean that a leukocyte disorder does not exist. For example, in chronic granulomatous disease, a child may have a normal neutrophil count, but the neutrophils are unable to destroy bacteria. Second, a differential is reported as a percentage of the WBCs. Therefore, one must also assess both the absolute number and the percentage of white cell subtypes. For example, a patient admitted to the hospital with pneumonia has an elevated WBC (15,000 cells/mm^3 [15×10^9/L]) with a manual differential of 70% segs, 10% bands, 15% lymphocytes, and 5% monocytes. The WBC is predominately neutrophils; segs or mature neutrophils (70%); and bands or immature neutrophils (10%). The percentage of lymphocytes is low at 15% (see Table e106-7), but the absolute number of lymphocytes is actually normal, 2,250 cells/mm^3 (15,000 cells/$mm^3 \times 0.15$) (2.25×10^9/L [or 15×10^9/L $\times$ 0.15]). A third factor to consider is that most lymphocytes are in secondary lymphoid organs (eg, lymph nodes and spleen), and changes in peripheral blood lymphocytes do not always mirror changes in the secondary lymphoid organs. Additionally, most granulocytes, macrophages, and mast cells are also in the tissues, not the bloodstream.

TABLE e106-7

Leukocyte Counts in Adults

Cell	Absolute Count (Range)[a]	Percentage (Range)[b]
White blood cells	7,500 (4,500-11,000)	100
Neutrophils	4,500 (2,300-7,700)	60 (50-70)
Eosinophils	20 (0-45)	3 (0-5)
Basophils	4 (0-20)	1 (0-2)
Monocytes	30 (0-80)	4 (0-10)
Lymphocytes	210 (160-240)	32 (28-39)
T-lymphocytes	140 (110-170)	72 (67-76)[b]
CD4[a]	80 (70-110)	42 (38-46)[b]
CD8[a]	70 (50-90)	35 (31-40)[b]
B-lymphocytes	30 (20-40)	13 (11-16)[b]
Natural killer cells	30 (20-40)	14 (10-19)[b]
CD4/CD8 ratio	1.2 (1-1.5)	

[a]Cell counts are expressed as cells/mm^3 or $\times 10^6$/L.

[b]Percentage of lymphocyte subpopulations expressed as percentage of total lymphocyte population. For expression in SI units multiply each number by 0.01 to give the corresponding fraction.

To assess the numbers of granulocytes (neutrophils, basophils, and eosinophils) and monocytes, one uses a WBC with differential. An increased WBC count with immature neutrophils (eg, bands, metamyelocytes, myelocytes, and promyelocytes) in the peripheral blood is called a "shift to the left," and is abnormal. It most often indicates a bacterial infection, but can also indicate trauma or leukemia. It has long been recognized that the lower the absolute neutrophil count, the greater the risk of infection. Drugs (eg, chemotherapy) and diseases (eg, collagen vascular disorders) may lower the neutrophil count and make the patient more susceptible to infections. Patients with a neutrophil count of less than 1,500 cells/mm^3 (1.5×10^9/L) are considered to have neutropenia. Functional analysis of these cell types is rarely done in routine clinical practice. Patients with suspected functional deficits in these cell types are generally referred to tertiary medical centers for evaluation and treatment.

A routine WBC with differential can determine the total lymphocyte count. Lymphocyte populations with different functions or in various stages of activation can be characterized based on their cell surface markers. These cell surface markers are known as clusters of differentiation (CD). The CD is usually a protein or glycoprotein on the cell surface. CD followed by a number designates the marker. Hundreds of monoclonal antibodies have been designed to recognize these cell surface markers. Monoclonal antibodies can be labeled with a fluorescent marker. The labeled monoclonal antibodies are then incubated with the patient's cells. The antibodies will recognize and bind to the cells expressing the CD of interest, and the cells are then counted with flow cytometry. For flow cytometry, the cell suspension is put under pressure such that the cells flow past a laser in a stream of single cells. The laser will excite the fluorescently labeled antibodies bound to the lymphocytes. A light detector counts the labeled cell as the fluorescent tag

emits light and determines the cell size based on its light scatter characteristics. These evaluations are valuable for the assessment of patients with immune deficiency states such as AIDS or leukemias, and for patients who have received hematopoietic stem cell or organ transplants. For example, the number of CD4+ cells in HIV-positive patients correlates with the risk of opportunistic infection and delineates the time to initiate antiviral therapy. Some of the more common CD antigens and their respective cellular distribution are listed in Table e106-8.[50] Flow cytometry can be used for leukocyte phenotyping, tumor cell phenotyping, and some types of DNA analysis.

TABLE e106-8

Cluster of Differentiation (CD) Guide: Characterization of Human Leukocyte Antigens

CD	Predominant Cellular Distribution
CD3	All T-lymphocytes
CD4	Helper T-lymphocytes, either TH1 or TH2
CD5	T-lymphocytes, B-lymphocyte subset
CD8	Cytotoxic/suppressor T-lymphocytes
CD14	Monocytes, neutrophils
CD20	B-lymphocytes
CD25	Activated T-lymphocytes, B-lymphocytes, interleukin-2 receptor α-chain (Tac)
CD33	Committed myeloid progenitor cells
CD34	Hematopoietic progenitor cells that include the stem cell
CD56	Natural killer cells
CD83	Dendritic cells

Innate and Adaptive Immunity: Functional Evaluation

Several disease states are characterized by an adequate number of cells but the cells are nonfunctional or they do not produce cytokines to communicate effectively. Although no single test can predict the function of the immune system, available tests can measure the viability of certain cell lines and communication between cells. Historically, the most common in vivo assay of lymphocyte function is the delayed hypersensitivity skin test. This test specifically evaluates the presence of delayed-type hypersensitivity or the presence of memory T-lymphocytes. Specifically, a small amount of antigen, of which the patient is known to have been previously exposed, is administered. Under normal immunologic host conditions, exposure to this amount of antigen in the skin should produce lymphocytic infiltrate into the area within a few hours, followed by additional lymphocyte recruitment and phagocytes (eg, macrophages and neutrophils) translocation. The maximal intensity of the inflammatory reaction occurs by 24 to 72 hours. This reaction is often referred to as type IV hypersensitivity (ie, cell mediated; see Chapter e108). A delayed-type hypersensitivity reaction is a test of cell-mediated immunity used to assess immunocompetency. The most common method to assess delayed-type hypersensitivity is to administer intradermally a panel of recall antigens. Commonly used antigens include *Candida albicans*, mumps, Trichophyton, tetanus toxoid, and purified protein derivative of tuberculin.[51] Measurements in millimeters of induration at the site of injection should be taken 48 to 72 hours after the placement of the antigens. A reaction is considered positive if the diameter of the induration is 2 mm or greater. The degree of sensitivity correlates with the area of induration.[50] Reaction to even a single antigen indicates a functioning cell-mediated immune response. Most immunocompetent individuals will

show a positive reaction to at least one of these antigens. Possible reasons for not mounting a response to these antigens include congenital T-lymphocyte deficiency, cancer, HIV, or immunosuppressive drug therapy.[51] No response is sometimes mounted because the individual being tested has not been previously exposed to a particular test antigen, although this is rare.

Global assessment of the in vivo immunologic response is also commonly used in solid organ transplantation during the diagnosis and assessment of acute rejection. For example, pathologists can detect cellular rejection on gross tissue biopsy by counting the number of lymphocytes present in the tissue and correlating their presence with other clinical findings, such as increased serum creatinine in kidney transplant recipients.

In vivo assessment of B-lymphocyte function involves immunizing the patient with a protein (eg, tetanus toxoid) and a polysaccharide (eg, pneumococcal polysaccharide vaccine) antigen to elicit and measure antibody responses after immunization. Two to three weeks after immunization, the patient's serum is tested for antibodies specific for the immunized antigen. This test measures B-lymphocyte responsiveness to the inoculated antigens but is reserved for patients who are suspected to have impaired B-lymphocyte function.[49]

A number of specific in vitro lymphocyte functional assays are used in the research setting and a few assays are performed at specialized clinical laboratories. One of these tests is the lymphocyte proliferation assay. In this assay, lymphocytes are obtained from a patient's peripheral blood and cultured in vitro. The cells are exposed to nonspecific mitogens, such as pokeweed mitogen, phytohemagglutinin, or concanavalin A. Then the cells are incubated in growth media containing tritium-labeled (^{3}H) thymidine, a nucleotide used in the synthesis of DNA. Normally in the presence of the mitogens, lymphocytes will be stimulated to proliferate. Proliferating lymphocytes will incorporate ^{3}H thymidine as they replicate DNA. The level of radioactivity of the cells can be measured on a β-scintillation counter and is proportional to the degree of proliferation. The patient sample needs to be compared to lymphocytes from normal, healthy subjects. Patients with immune deficiencies (eg, AIDS and cancer) have fewer or less-active lymphocytes, as detected by this test.

A modification of the lymphocyte proliferation assay can be used in allogeneic hematopoietic stem cell transplantation to evaluate how closely a donor and host are "matched" to predict a patient's risk for developing graft-versus-host disease. A mixed lymphocyte culture (MLC) can be used to assess the potential of the donor cells to attack the host cells, graft-versus-host disease (see Chapter e163). In this test, donor cells and host cells are incubated in vitro. The host lymphocytes are irradiated prior to the incubation so that they cannot proliferate. In vitro, ^{3}H thymidine is provided to the cells and uptake is measured. The degree of uptake correlates to the level of proliferation of donor lymphocytes. If the cells are well matched, proliferation is minimal. If the cells are mismatched, proliferation will be noted with the level of proliferation predictive of the risk and severity of graft-versus-host disease. With the introduction of DNA-based, "high resolution" molecular typing of HLA antigens, the MLC is rarely used today.[51]

The Cylex Immune Cell Function Assay is an FDA-approved test used to determine the magnitude of suppression of CD4+ cells.[52] Briefly, CD4+ cell activity is measured by quantification of the amount of ATP produced and characterized as high, medium, or low.[53] Initial retrospective experience has been reported in the solid organ transplant population. This assay is one of the first functional assays aimed at assessing individual patient response to immunosuppressive therapy and may allow for tailoring of immunosuppression.

Evaluation of immune cell activity such as factor forkhead box P3 (FOXP3) Treg-cell activity has been used to evaluate the incidence and severity of acute rejection based on elevated FOXP3 mRNA expression in urine and tissue cell samples. Since Tregs control autoimmune reactions, the presence of these cells in an allograft may indicate a level of "tolerance" to the donor tissue. Tolerance is a state in which the body knows that foreign tissue (eg, kidney transplant) exists but does not attack it. Initial studies have evaluated biopsy samples from organ transplant recipients and correlated them with levels of FOXP3. FOXP3 is present only during periods of inflammation, such as rejection, and potentially projects the allograft tissue.[54] In addition to the tests described earlier, several other tests and assays have been devised to evaluate the function of CD8+ T-lymphocytes, NK cells, and monocytes/macrophages. Although these evaluations are not commonly performed, they may be helpful in some specific diseases and may eventually be the way in which we monitor and detect immunologic events in the future. A thorough discussion of these tests is available.[55]

Immunoglobulins

The measurement of immunoglobulins is a direct measure of B-cell function. The most common evaluation of immunoglobulins is the measurement of individual isotypes by immunoturbidity or immunonephelometry techniques. Although serum protein electrophoresis (SPEP) provides an estimate of the total immunoglobulin concentration, this technique is primarily used to investigate plasma cell dyscrasias and quantitate specific monoclonal protein peaks (eg, multiple myeloma and Waldenstrom macroglobulinemia). Depending on the specific SPEP technique, five or six separate zones are

detected by this method: albumin, α1-globulin, α2-globulin, β-globulin (or β1 and β2 by some techniques), and γ-globulin.

5 The γ-globulin fraction contains the five isotypes of immunoglobulin (IgG, IgA, IgM, IgE, and IgD). A normal total immunoglobulin or γ-globulin concentration ranges from 0.8 to 1.6 g/dL (8-16 g/L). Total immunoglobulin or γ-globulin concentrations cannot be used to measure antigen-specific antibodies or specific isotypes, although they can be measured with other laboratory tests. In a patient suspected of having humoral immune deficiency or B-lymphocyte failure (primary and secondary hypogammaglobulinemia), specific immunoglobulin isotypes in the plasma should be measured.

There are many indications for the measurement of antigen-specific antibodies. Some common indications are listed in Table e106-9. More contemporary methods to perform these measurements include enzyme-linked immunosorbent assay (ELISA) and a variety of other immunoassay techniques. The most common reason to measure antigen-specific antibody is to determine whether or not a patient has been exposed to an infectious agent. Generally, IgM antibodies directed against the pathogen indicate an active or recent infection while IgG antibodies directed against the pathogen indicate prior exposure. This observation correlates with our understanding of B-lymphocyte responses in which plasma cells produce IgM initially in response to an infection, but later switches to IgG. Therefore, IgM antibodies will be present during an active infection and shortly after recovery from the infection. IgG concentrations will increase at the end of the primary exposure but predominate after a second exposure. IgG predominates after a second exposure because memory B-lymphocytes predominately secrete IgG in the serum. Other uses of antigen-specific antibody include determining if a patient has had exposure and is likely to be protected from infection (eg, hepatitis A virus) or to determine adequate response to vaccination (eg, hepatitis B vaccine). Measurement of antihuman IgG antibodies is used before and after solid organ transplant to detect and potentially predict allograft compatibility and treat antibody-mediated rejection.

TABLE e106-9

Potential Indications for Measurement of Antigen-Specific Antibody

Environmental or drug allergy
Exposure to or infection with bacteria
Streptococci (ASO titer)
Staphylococcus aureus (teichoic acid antibody)
Neisseria gonorrhoeae
Legionella pneumophila
Exposure to or infection with viruses
Human immunodeficiency virus
Cytomegalovirus
Epstein–Barr virus
Hepatitis A, B, or C
Rubella
Exposure to or infection with other pathogens
Syphilis
Lyme disease
Typhoid
Chlamydia
Immune disorders
Rheumatoid factor antibody, rheumatoid arthritis
Antinuclear antibodies, systemic lupus erythematosus
Platelet-associated immunoglobulin G, idiopathic thrombocytopenic purpura
Blood typing and crossmatching
Transplantation
Human leukocyte antigen (HLA) antibodies

Antigen-specific IgE is not commonly measured in patients with allergies. Because the presence of antigen-specific IgE is related to clinical allergy, measurement of these antibodies can help to diagnose allergies and determine offending substances, but the circulating levels are so low they do not normally lead to interpretable results. Contemporary laboratories use enzyme-labeled anti-IgE antibody as a conjugate, with the use of an appropriate substrate for chemiluminescent, fluorescent, or colorimetric detection. The basic technique involves adding the antigen of interest, which is typically bound to beads or disks, to the patient's serum. After incubation and several washings, an enzyme-labeled antibody to IgE is added to bind to any IgE antibody bound to the antigen. After further washings, the enzyme-antibody conjugate bound to IgE, which is bound to the antigen on the bead or disk, is measured by an appropriate substrate that generates the measurement signal and is quantified.

Antigen skin testing is the preferred method to determine the presence of allergen-specific IgE. When produced, IgE binds to high-affinity IgE Fc receptors on basophils or mast cells. Contact of an allergen with the specific IgE on the basophil or mast cell surface causes activation of these cells and the release of inflammatory mediators (eg, histamine). When this occurs systemically, it can cause anaphylaxis. When it occurs in a confined area, such as the skin, erythema and induration are observed within a few minutes of allergen injection. This is the method used to detect penicillin allergy and environmental or food allergies. A positive skin reaction (≥5 mm of induration) within 15 to 20 minutes indicates the presence of allergen-specific IgE.

The four subclasses of IgG: IgG1, IgG2, IgG3, and IgG4 make up 65%, 20%, 10%, and 5% of total plasma IgG, respectively. Concentrations of the subclasses are often measured in patients with suspected primary and secondary hypogammaglobulinemia. IgG2 and IgG4 deficiencies are associated with chronic infections. IgG4 deficiencies are also associated with autoimmune disorders.

Complement System

The complement system consists of over 30 different proteins that lyse and opsonize invading pathogens and serve as chemotactic factors. Numbers following the letter C (eg, C1 and C2) refer to the various proteins of the complement system. Assessment of the complement system is important in patients suspected of having humoral immune deficiencies (ie, recurrent infections).[50] A test for the global assessment of the complement system is the CH50, the total hemolytic complement test. This test is based on the premise that complement is needed for a rabbit anti-sheep antibody to lyse sheep red blood cells. The source of the complement is the patient's serum. The normal range varies because each laboratory standardizes the test, but a standard curve is developed by adding titrated amounts of sera and measuring the amount of hemolysis. Hemolysis is determined with a spectrophotometer based on the amount of hemoglobin released. The patient's serum is then tested, and the amount of serum needed to lyse 50% of the red blood cells is reported as the CH50. Many laboratories assess total complement activity with semiquantitative enzyme immunoassay methods based on enzyme-conjugated monoclonal antibodies that bind to newly expressed antigens of the terminal complement proteins or methodology based on lysis of antibody sensitized dinitrophenyl-labeled liposomes with trapped glucose-6-dehydrogenase. These tests do not measure the function of any specific complement component but are used as a screening test for any complement system defects. If a defect is found, individual complement proteins can then be evaluated by either functional or immunochemical methods.

Several disease states can alter complement concentrations. Low complement concentrations are frequently found during states of acute inflammation (eg, systemic lupus erythematosus, rheumatoid arthritis, collagen vascular disorders, poststreptococcal glomerulonephritis, and subacute bacterial endocarditis). These states of low complement concentrations are generally related to high rates of complement utilization or consumption that cannot be compensated for by increased complement synthesis.[22]

Since the liver is the primary source of several components of the complement system (ie, C2, C3, C4, factors B and D), a global decrease in complement factors can occur in severe liver failure. Inherited complement deficiencies have been described in patients with systemic lupus erythematosus, autoimmune diseases, recurrent gonococcal and meningococcal infections, membranoproliferative glomerulonephritis, and hereditary angioedema.[22]

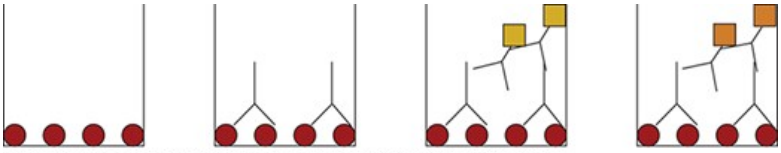
The complement system has been used to diagnose and treat solid organ rejection. Antibody-mediated or humoral rejection is evaluated by measuring the amount of donor MHC-specific antibody present in the recipient's serum. The presence of donor-specific antibodies is correlated with evidence of antibody-mediated rejection on tissue samples. This is characterized by the presence of complement split products, namely C4d, which is present after complement-dependent antibody-mediated rejection. C4d covalently binds to the allograft tissue and can be stained for biopsy samples. Unfortunately, unless biopsy findings can be correlated with a clinical finding consistent with rejection, the presence of C4d and its prognosis on long-term allograft function are unknown.

Cytokines

Disease states involving the loss or upregulation of cytokines are sometimes overlooked as diseases of the immune system. However, as we have just discussed, cytokines are essential components of both the innate and adaptive immune systems and provide the communication linking them together. Multiple cytokines with overlapping and redundant functions have been identified. Methods to detect and measure cytokines in biological samples have been developed. For nearly all the identified cytokines, commercial kits are available to measure endogenous and exogenously administered cytokines. The most common and preferable methods to measure cytokines are ELISAs (enzyme-linked immunosorbent assay) and RIAs (radioimmunoassay). ELISAs, RIAs, or enzyme immunoassays are easy to perform and can identify and quantify the presence of cytokines, but they do not intrinsically measure biologic activity (see Fig. e106-7). Bioassays measure biologic activity, but are cumbersome and extremely variable. ELISA is only able to measure how much cytokine is produced by the cells in the culture. An ELISPOT is an enzyme-linked assay for detecting and enumerating cytokine-producing leukocytes.[56] In contrast to conventional ELISA, ELISPOT allows the user to detect absolute numbers and frequencies of cytokine-secreting leukocytes.

FIGURE e106-7

Enzyme-linked immunosorbent assay (ELISA). ELISA is a commonly used method for measuring concentrations of a wide variety of substances. To measure the concentration of antibodies to a particular antigen, the antigen is coated onto a solid phase, such as a microtiter plate or beads. If the purpose of the assay is to measure the concentration of antigen in solution, an antibody to the antigen is coated on the solid phase. The biologic fluid, often sera, is added to the wells. An enzyme-labeled antihuman antibody is added next. Finally, the chromogenic substrate for the enzyme is added. The intensity of the color as measured spectrophotometrically is proportional to the concentration of the antibody in the biologic fluid.



The clinical relevance of endogenous cytokine concentrations is not clear. Not only is the immune system affected by cytokines, such as IL-1, IL-6, and TNF-α, but other systems (skeletal, endocrine, and CNS) are also affected. Therefore, measurement of cytokine concentrations may be important to evaluate other systems in addition to the immune system.

Administration of recombinant cytokines in clinical practice may change not only the concentration of that particular cytokine but also the resultant concentration of other cytokines. For example, systemic administration of GM-CSF to patients not only increases concentrations of GM-CSF but also of TNF-α, IL-6, IL-8, macrophage CSF, and EPO.[57,58] Secondary endogenous cytokine release should be taken into account when considering the therapeutic effects of these agents and when monitoring cytokine concentrations.

In the future, blood and tissue cytokine concentrations may be measured. For example, while many centers measure blood cyclosporine or tacrolimus concentrations to ensure adequate immunosuppression, it may be advantageous to monitor IL-2 concentrations because one of the primary actions of calcineurin inhibitors is to inhibit IL-2 production. Furthermore, perhaps it would be beneficial to measure tissue IL-2 concentrations in the transplanted organ to get a better estimate of the extent of immunosuppression.

Soluble Receptors and Receptor Antagonists

The inflammatory response is highly regulated. The activity of cytokines, their receptors, and their antagonists are in a delicate balance. Although cytokine receptors are usually found on the target cell, soluble cytokine receptors can modulate cytokine activity in at least two ways: (a) acting as anti-inflammatory agents by binding cytokines with high affinity, but without biological activity; and (b) augmenting cytokine activity by prolonging the cytokines plasma half-life and even maintaining agonist activity on cells that do not inherently respond to the cytokine.[59,60] Finally, antagonists to cytokine receptors have been identified.

TNF-α plays a central role in the inflammatory response by increasing the expression of adhesion molecules in the tissues and by stimulating the production of proinflammatory cytokines (eg, IL-2 and IL-8), prostaglandins, and nitric oxide. Soluble tumor necrosis factor receptors (sTNFRs) act primarily as inhibitors of TNF by preventing TNF from binding to the membrane-bound TNFRs, or by causing the cells to shed the receptor from the surface of the cell so that it can no longer serve as a signaling molecule.[61] Both monoclonal antibodies against TNF (eg, infliximab and adalimumab)

and sTNFRs (eg, etanercept) have been shown to modulate the activity of TNF and are used clinically for the treatment of autoimmune diseases.

The best-characterized receptor-binding antagonist is the interleukin-1 receptor antagonist (IL-1RA). IL-1RA blocks the binding of IL-1 to its receptor by competing for the same binding site, but IL-1RA does not possess agonist activity.[62] A recombinant IL-1RA, anakinra, is used clinically for the treatment of severe rheumatoid arthritis.[63]

Our developing understanding of soluble receptors and receptor antagonists allows us to better mimic natural mechanisms for minimizing the toxicity of exogenously administered cytokines (eg, IL-1, IL-2, and TNF-α) and to immunomodulate various diseases (eg, solid-organ transplant rejection, collagen vascular disorders, inflammatory bowel disorders, autoimmune dermatologic conditions, and sepsis).

MODULATION OF THE IMMUNE RESPONSE

6 Modulation of the immune response through the administration of pharmacological agents or with blood product components comes with both risks and benefits. One example is the administration of recombinant activated protein C (drotrecogin alfa) to patients in septic shock. Administration of recombinant activated protein C reduced levels of IL-6, a potent proinflammatory cytokine that is thought to contribute to many of the clinical manifestations of septic shock. Unfortunately, protein C also possesses anticoagulant and fibrinolytic properties, leading to a significantly increased risk of bleeding. As a result, no survival benefit was observed when drotrecogin alfa was given to patients in septic shock. This ultimately led to the withdrawal of drotrecogin alfa from the market.[64] Another example of drugs with both risks and benefits is TNF inhibitors. While TNF inhibitors suppress the immune system to halt the damage of autoimmune disorders and alleviate symptoms, they also increase the risk of opportunistic viral infections. Many of our newer biological agents directed at immune pathways are derived from animals and are subsequently humanized with various genetic engineering techniques to increase their biological effectiveness and decrease their antigenicity. Despite these efforts, these agents can serve as antigens and elicit an immune response, which may have a variety of consequences such as decreased efficacy over time. For example, an agent commonly used in solid organ transplantation is rabbit antithymocyte globulin (rATG), a polyclonal antibody derived from rabbits that have been immunized against human lymphocytes. rATG is sometimes given to prevent or treat graft rejection in patients undergoing solid organ transplantation. Patients with previous exposure to rATG can develop antibodies against the drug because the rabbit antibodies can be antigenic in humans. This can result in decreased effectiveness of rATG, because the rabbit antibodies are neutralized by human antirabbit antibodies (HARAs). Once rATG is bound by HARAs, rATG is no longer able to bind to its target (ie, human lymphocytes). Another potential consequence of HARAs is the deposition of the HARA/rATG complex in the kidneys and joints, producing high fevers and renal failure. Based on these examples, one can understand why manipulation of the immune system must be carefully assessed and appropriate patient instruction given.

Immunosuppression

Immunosuppression was first developed and used to allow transplantation of foreign tissues or to treat malignancies of the immune system. These medications are usually expensive and associated with potentially serious adverse drug reactions. Immunosuppressants block critical steps of the immune response, and patients must be counseled on their risk of infection and the plan to monitor the effectiveness of the immunosuppressant. As we learn more about the function of the immune system, various immunosuppressive medications targeting a growing number of immunoregulatory pathways are being used to treat autoimmune diseases, including irritable bowel and Crohn's disease, dermatologic conditions such as psoriasis, multiple sclerosis, as well as rheumatoid arthritis and psoriatic arthritis. Current pharmacologic targets that result in immunosuppression include calcineurin (cyclosporine, voclosporin), FKBP12/calcineurin (tacrolimus, sirolimus, everolimus), inosine monophosphate dehydrogenase (IMDPH) (mycophenolate), TNF-α (adalimumab, certolizumab, etanercept, golimumab, infliximab), IL-1 (anakinra), IL-2 (basiliximab), IL-6 (sarilumab, tocilizumab, satralizumab), IL-12 (ustekinumab), IL-13 (tralokinumab), IL-17 (secukinumab, brodalumab, ixekizumab), IL-23 (tildrakizumab, ustekinumab, risankizumab, guselkumab), the Janus Associated Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) pathway (baricitinib, tofacitinib, upadacitinib), CD80/CD86 (abatacept, belatacept), and the cellular adhesion pathways that lymophcytes use to navigate into various tissues (natalizumab, vedolizumab). Sphingosine 1 plays an important role in lymphocytes' ability to exit lymph nodes, and several sphingosine 1-phosphate receptor modulators reduce immune-mediated damage in multiple sclerosis (ozaminod, ponesimod, fingolimod, siponimod). In 2021, several drugs targeting various elements of the complement system were granted approval for various autoimmune disorders: complement C3 (pegcetacoplan) and complement C5a receptor (avacopan). Other new targets for immunosuppressive drugs that have emerged include Rho-associated coiled-coil-containing kinase 2 (ROCK2) inhibitors (belumosudil), type-1 interferon receptor antagonists (anifrolumab), and neonatal Fc receptor antagonists (efgartigimod). As noted above, most, if not all of these medications increase the risk of various types of infections.

Several also increase the risk of certain types of cancers derived from immune cells, as well as unusual immune-related adverse events. It is critically important for clinicians to weigh the risks and benefits of these medications. Several key concepts and questions can be used to help clinicians discern the potential benefits and harms of administering any immunosuppressant. These include: (a) what is its mechanism of action, (b) what arm of the immune system does it effect, (c) when is its onset of action, (d) how was this compound derived and does it have the potential to stimulate antibody production if the patient is reexposed, (e) is this compound's effect dose- or duration-related, (f) what type of infection is the patient at risk for and is infection prophylaxis required, and (g) how do I monitor the biological effect of this compound?

Immunopotentiation

Immunopotentiators can be used to restore normal immune system function or to activate the immune system. The best example of immunopotentiation of the immune system is the practice of immunizations. Active immunization with a vaccine or toxoid induces the host's immune system to confer protection against a pathogen (eg, hepatitis A, hepatitis B, and diphtheria toxoid). This process requires the uptake of the immunogenic epitope by APCs followed by presentation to CD4+ T-lymphocytes and the subsequent development of either a cellular or humoral immune response. Another example of immunopotentiation is the administration of IL-2 to patients with malignant melanoma. As previously discussed, IL-2 is a potent activator of T-lymphocyte and NK cell activity, which can be sufficient to break immune tolerance and result in immune-mediated tumor destruction.[65]

In contrast to active immunization, passive immunity involves the administration of human immunoglobulin to provide short-term protection to individuals who will be or have been exposed to a pathogen. IV immunoglobulin (IVIG) consists of more than 90% polyclonal IgG prepared from donated plasma. In patients with primary or secondary hypogammaglobulinemia, IVIG restores circulating IgG concentrations and decreases the risk of infections. In addition to restoring IgG concentrations, IVIG can potentially modulate the immune response. For example, in immune thrombocytopenic purpura, an autoantibody directed against the platelets leads to the destruction of the platelets by antibody-dependent cellular cytotoxicity. IVIG saturates the Fc receptors on phagocytic cells, thereby preventing the engulfment of autoantibody-opsonized platelets. IVIG can also contain anti-idiotype antibodies to modulate an immune response. Anti-idiotype antibodies are directed against the idiotype or hypervariable region of a native antibody. After administration of IVIG, the anti-idiotypes bind to the hypervariable region of the autoantibody and prevent the autoantibody from opsonizing circulating platelets. In addition, the anti-idiotypes directed against the autoantibody can bind to the surface immunoglobulin on the B-lymphocyte producing the autoantibody that leads to the destruction of the B-lymphocyte.[66]

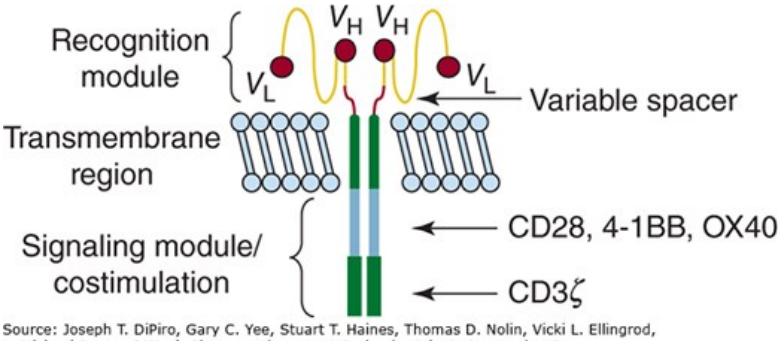
Through our increased understanding of critical steps in T-lymphocyte responses, several monoclonal antibodies that enhance T-lymphocyte activity have been developed to treat cancer. These drugs are classified as immune checkpoint inhibitors. Immune checkpoints are inhibitory pathways that decrease T-lymphocyte activity. Cancer cells can use immune checkpoints to evade recognition by the immune system. When these inhibitory checkpoint pathways are blocked with monoclonal antibodies, T-lymphocyte activity is increased. The first checkpoint inhibitor to be approved was ipilimumab, a humanized monoclonal antibody that binds to CTLA-4 on activated T-lymphocytes. Blockade of CTLA-4 prevents the inhibition of T-lymphocytes mediated by CTLA-4 signaling and thus releases them to attack their target. Single-agent ipilimumab improves overall survival in patients with unresectable or metastatic melanoma.[67] Four additional agents, pembrolizumab, nivolumab, cemiplimab, and dostarlimab bind to PD-1 on activated T-lymphocytes, preventing PD-L1 or PD-L2 expressed on tumor cells from inhibiting activated T-lymphocytes. In addition to targeting PD-1, it is also possible to target PD-L1, achieving a similar effect. Three drugs, atezolizumab, avelumab, and durvalumab, all target PD-L1, and prevent the ligand from binding to the PD-1 receptor on T-lymphocytes. Targeting two immune checkpoint pathways, CTLA-4 and PD-1, with the combination of nivolumab and ipilimumab has even greater antitumor activity than either agent alone in patients with advanced melanoma.[68-70] It is important to note that the inhibition of these immune checkpoint pathways can result in increased activity directed at self-antigens. This phenomenon is, unfortunately, fairly common in patients receiving immune checkpoint inhibitor-based therapies, and is known as an immune-mediated adverse event. These immune-mediated adverse events most commonly manifest as colitis, hepatitis, dermatitis, pneumonitis, or endocrinopathies, although experience has shown that virtually any tissue type or organ system can be affected.[71-77] It is also important to note that the combination of ipilimumab and nivolumab results in a higher incidence and severity of immune-mediated adverse events.[67-70] Despite these toxicities, immune checkpoint inhibition is one of the fastest growing areas of cancer treatment and research. In fact, since ipilimumab was first approved by the FDA in 2011, immune checkpoint inhibitors have been approved by the FDA for the treatment of at least 15 different cancers. These cancers include colorectal cancer (for patients with microsatellite instability-high or mismatch repair deficient tumors), cervical cancer, cutaneous squamous cell carcinoma, gastric cancer, hepatocellular carcinoma, Hodgkin lymphoma, melanoma, Merkel cell carcinoma, nonsmall cell lung cancer, primary mediastinal large B-cell lymphoma, renal cell carcinoma, small cell lung cancer, squamous cell cancer of the head and neck, urothelial carcinoma, and any solid tumor that

expresses a high level of microsatellite instability.

Sipuleucel-T immunotherapy shows significant activity in castration-resistant prostate cancer. Sipuleucel-T takes advantage of our increased knowledge of APCs. Sipuleucel-T involves isolation of APCs from a patient by leukapheresis followed by in vitro activation of the APCs with a recombinant fusion protein, PA2024, which contains GM-CSF to activate the APCs and a tumor antigen, prostatic acid phosphatase (PAP), common to prostate cancer. Finally, the activated APCs are reinfused into the patient every 2 weeks. The activated APCs then present PAP to the patient's T-lymphocytes, which then leads to increased numbers of T-lymphocytes attacking the prostate cancer (Fig. e106-8).[78]

FIGURE e106-8

Chimeric Antigen Receptors are comprised of an extracellular domain consisting of the single chain variable fragment (ScFv) portion of an antibody, a transmembrane domain, and an intracellular domain, which usually contains one or more costimulatory domains. The number and type of costimulatory domains may affect the cytotoxicity, cell growth, and survival characteristics of the T-cell.



Source: Joseph T. DiPiro, Gary C. Yee, Stuart T. Haines, Thomas D. Nolin, Vicki L. Ellingrod, L. Michael Posey: *DiPiro's Pharmacotherapy: A Pathophysiologic Approach, 12e*

Adoptive cell transfer (ACT) is an active area of cancer research that shows great promise. ACT uses a patient's own T-lymphocytes, derived either from a surgically resected tumor specimen or from peripheral blood and genetically engineered to express antitumor TCRs, to treat cancer.[79] In one study of patients with advanced melanoma, 40% of patients achieved a complete response that lasted at least 2.5 years.[80] As our understanding of the immune system has expanded, more advanced genetic engineering techniques have allowed a wider variety of cancers to be targeted through ACT. One particular type of ACT involves creating a highly modified version of a TCR, known as a Chimeric Antigen Receptor (CAR), in which the extracellular domain of the TCR is replaced with a portion of an antibody, while the intracellular signaling domain is either left alone or modified to include various costimulation domains (see Fig. e106-8). In patients with chemotherapy-refractory B-cell malignancies, CAR T-cell targeting CD19 resulted in a 53% complete response rate, with some responses lasting almost 2 years.[81] This early research led to the development of two FDA-approved CAR T-cell therapies, both of which target CD19. The first FDA-approved CD19 CAR T-cell therapy was tisagenlecleucel, which is approved to treat refractory or relapsed B-cell precursor acute lymphoblastic leukemia, and B-cell lymphomas.[82,83] The second FDA-approved CD19 CAR T-cell therapy was axicabtagene ciloleucel.[84] Five CAR T-cell therapies have been FDA-approved for hematological malignancies. There is tremendous interest in applying this approach to other cancers, and beyond. For example, some experts believe that CAR T-cell technology can be used to treat infectious diseases that are historically difficult to treat such as drug-resistant bacterial infections or HIV.[85,86] As we continue to expand our knowledge of the immune system and its complex interactions, we will develop new therapeutic approaches to modulate immune responses in the treatment of human diseases.

CONCLUSION

As our understanding of the immune system and its function has continued to expand, so too has our ability to specifically target and modulate the immune response for therapeutic purposes. New technologies have allowed us to suppress specific immune functions or to upregulate immune activity either broadly or specifically by targeting specific subsets of the immune system. These new therapeutic options have wide-ranging implications, from the treatment of various autoimmune disorders to new therapeutic options for cancer and possibly for infectious diseases. Equally important is an understanding of the implications of suppressing or upregulating immune function so that immune-related adverse events or opportunistic infections can quickly be identified and treated when they occur. A solid understanding of the structure and function of the immune system will become increasingly important for clinicians as we continue to harness, modulate, and assess the immune system to treat disease.

ABBREVIATIONS

APC	antigen-presenting cell
CAR	chimeric antigen receptor
CD	clusters of differentiation
CD4	T-helper cells
CD8	T-cytotoxic cells
CRP	C-reactive protein
CSF	colony-stimulating factor
DC	dendritic cell
ELISA	enzyme-linked immunosorbent assay
EPO	erythropoietin
FasL	Fas ligand
Fc	fragment crystallizable
FOXP3	factor forkhead box P3
GM-CSF	granulocyte-macrophage colony-stimulating factor
HLA	human leukocyte antigen
IgG	immunoglobulin G
IL	interleukin
IL-1RA	interleukin-1 receptor antagonist
IVIG	intravenous immunoglobulin
MALT	mucosa-associated lymphoid tissue
MHC	major histocompatibility complex
MIP	macrophage inflammatory protein
NK	natural killer
PAMPs	pathogen-associated molecular patterns
PD1	programmed cell death 1

PRRs	pattern recognition receptors
RIA	radioimmunoassay
sIg	surface immunoglobulin
SPEP	serum protein electrophoresis
TCR	T-cell receptor
TH1	T-helper type 1
TH2	T-helper type 2
TH17	T-helper 17 cells
Tregs	T-regulatory cells
TNF-α	tumor necrosis factor-α
WBC	white blood cell

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SELF-ASSESSMENT QUESTIONS

1. Which of the following receptors does not inhibit T-cell activity?

   A. PD-1

   B. CD28

C. CTLA-4

D. Fas

2. Which of the following is a part of the adaptive immune system?

A. Macrophage

B. Eosinophil

C. B-cell

D. Neutrophil

3. Which antibody type is usually produced when the immune system first encounters a pathogen?

A. IgA

B. IgD

C. IgG

D. IgM

4. Activation of the complement system can result in all of the following, except:

A. The stimulation of plasma cells to secrete antibodies

B. Opsonization of foreign pathogens

C. Lysis of microorganisms

D. Stimulation of chemotaxis of phagocytes

5. Which of the following treatments depends on passive immunity?

A. Tetanus vaccine

B. Cyclosporine to prevent transplant rejection

C. Antivenin to treat a rattlesnake bite

D. Nivolumab to treat metastatic melanoma

6. Which of the following is not an example of physical or chemical defense against pathogens?

A. Stomach acid

B. Lysozymes in tears

C. Cilia on epithelial cells in the lung

D. Langerhans cells in the skin

7. Which of the following is a pattern recognition receptor?

A. Toll-like receptor on macrophages

B. Surface immunoglobulin on B-cells

C. T-cell receptor on T-cells

D. Killer-activating receptors on NK cells

8. Which of the following is an example of a humoral immune response?

A. A neutrophil phagocytosing a pathogen

B. An IgG molecule binding an antigen on a viral pathogen

C. An NK cell destroying a virally infected cell

D. A CD4+ T-cell secreting IL-2 to activate CD8+ T-cells

9. T-cells mature in which of the following organs?

A. Bone Marrow

B. Lymph Nodes

C. Spleen

D. Thymus

10. Which of the following medications stimulates immune activity?

A. Pembrolizumab

B. Infliximab

C. Adalimumab

D. Vedolizumab

11. Which of the following is a CAR T-Cell–based treatment?

A. Sipuleucel-T

B. Axicabtagene ciloleucel

C. Talimogene laherparepvec

D. Trastuzumab emtansine

12. All of the following have been shown to result in decreased concentrations of complement except:

A. Liver failure

B. Systemic lupus erythematosus

C. Hereditary angioedema

D. Type 2 diabetes

13. Which of the following is the most common immunoglobulin found in human serum?

A. IgA

B. IgE

C. IgG

D. IgM

14. Which of the following cell markers is present on all T-cells?

A. CD3

B. CD4

C. CD8

D. CD25

15. Which of the following is a mechanism used by cytotoxic T-cells to destroy their targets?

A. Phagocytosis

B. Opsonization

C. Histamine release

D. Perforin release

SELF-ASSESSMENT QUESTION-ANSWERS

1. **B.** PD-1, CTLA-4, and Fas receptors send inhibitory signals to T-cells once they bind to their ligands. CD28 binds to CD80 or CD86 and is part of the signaling pathway that results in T-cell activation.

2. **C.** Macrophages, eosinophils, and neutrophils are components of the innate immune system. Since B-cells are able to recognize new antigens and develop into memory cells that allow for a more rapid and potent immune response upon additional exposure to an antigen, they are classified as a component of the adaptive immune system.

3. **D.** Upon exposure to a given antigen, plasma cells secret IgM initially. Over time, plasma cells eventually switch over to the secretion of IgG.

4. **A.** The complement system does not signal plasma cells to secrete antibodies. The complement system can opsonize foreign pathogens, preventing the pathogen from interacting with or infecting cells or structures in the body. Additionally, opsonization may signal granulocytes, such as neutrophils to phagocytose and destroy the pathogen. Additionally, the binding of complement has been shown to result in the lysis of certain microorganisms and cells. Finally, breakdown products of complement (C3a and C5a) can serve as chemoattractants which attract neutrophils to infected tissues.

5. **C.** When we administer antivenin, we are administering exogenously produced antibodies to a patient to neutralize a venom with which the patient has been envenomated. This is an example of passive immunity because the patient is not producing the antibodies themself. Following a tetanus vaccine, the individual's B-cells recognize the antigens in the tetanus vaccine and develop a memory against these antigens, an example of active immunity. Cyclosporine is a potent inhibitor of T-cell activation and it is given to inhibit an active immune response (eg, to prevent transplant rejection or graft-versus-host disease). Finally, nivolumab, a PD-1 inhibitor, prevents the inhibition of T-cells. When we administer nivolumab, we are increasing the activity of the immune system by stimulating an active immune response against cancer cells.

6. **D.** Stomach acid, lysozyme in tears, and cilia on epithelial cells in the lungs are examples of physical or chemical defenses against invading pathogens. Langerhans cells are a type of dendritic cell found in the skin. Dendritic cells serve as antigen-presenting cells, which play an important role in the activation of T-cells and B-cells.

7. **A.** Toll-like receptors are pattern-recognition receptors that bind to highly conserved structures present on a large number of different

microorganisms. When a pattern recognition receptor (which is expressed on phagocytes) binds to its target, the cell expressing the target is immediately phagocytosed. In contrast, surface immunoglobulins, T-cell receptors, and killer-activating receptors recognize specific targets and require multiple interactions with the target and other cells to initiate an immune response.

8. **B.** Examples of cellular immunity include neutrophils phagocytizing a pathogen, NK cells destroying a virally infected cell, and CD4+ T-cells secreting IL-2 to activate CD8+ T-cells. IgG binding an antigen on a viral pathogen is an example of humoral immunity, which generally refers to antibody-based responses.

9. **D.** While all of the options listed represent the major tissues and organs of the immune system, the correct answer is that T-cells mature in the thymus. In fact, T-cells are named T-cells because they mature in the thymus.

10. **A.** Pembrolizumab stimulates immune activity by blocking T-cell inhibition. Infliximab and adalimumab are both monoclonal antibodies that bind to TNF-α, preventing it from binding to TNF receptors. By inhibiting the activity of TNF-α, a pro-inflammatory cytokine, infliximab, and adalimumab decrease immune activity. Vedolizumab is a monoclonal antibody that binds to a specific type of integrin, a molecule that T-cells use to migrate from the bloodstream into inflamed tissues. By preventing T-cells from being able to contribute to an ongoing inflammatory response, the drug decreases immune activity.

11. **B.** Axicabtagene ciloleucel is a CAR T-cell that targets the CD19 receptor on B-cells and is used to treat B-cell malignancies. Sipuleucel-T is a cell-based therapy; APCs are collected from the patient and are exogenously primed with an antigen found on prostate cancer before being reinfused into the patient. Talimogene laherparpvec is a genetically modified herpes simplex virus that is injected directly into melanoma tumors and increases immune response at the site of injection. Trastuzumab emtancine is an antibody-drug conjugate that targets the HER-2 molecule commonly overexpressed on different cancers and delivers a cytotoxic microtubule inhibitor (the emtansine molecule) to the cell.

12. **D.** Decreased complement levels are associated with liver failure, systemic lupus erythematosus, and hereditary angioedema. Type-2 diabetes has not been associated with decreased complement levels.

13. **C.** IgG comprises about 80% of serum immunoglobulins, while IgA makes up about 15%, IgM represents about 5%, IgD is about 0.2%, and only trace amounts of IgE are detected.

14. **A.** CD3 is expressed on all T-cells. CD4 is expressed on a subset of T-cells called Helper T-cells. CD8 is expressed on a subset of T-cells known as cytotoxic T-cells. Finally, CD25 is expressed on activated T-cells and is not present on inactivated T-cells.

15. **D.** T-cells release perforin which forms pores in the membrane of the target cell. These pores allow granzymes, which are also released by the T-cell to enter the target cell and initiate apoptosis in the target cell. T-cells do not phagocytize or opsonize their targets. T-cells also do not produce or release histamine.