White Blood Cells and Inflammation 5.3

Learning Objectives

- List the five types of leukocytes in order of their abundance
- List the polymorphonuclear granulocytes and the agranular leukocytes
- Describe the origin of the platelets and the role of thrombopoietin in their regulation
- List the function of each of the white blood cells
- Describe the difference between the innate and specific or adaptive immune responses
- Describe the reticuloendothelial system
- List the four classical signs of inflammation
- Distinguish between innate and adaptive immunity
- List the events in order in the recruitment of neutrophils and monocytes to the extravascular space
- Describe the function of the complement system

THE WHITE BLOOD CELLS INCLUDE NEUTROPHILS, EOSINOPHILS, BASOPHILS, MONOCYTES, LYMPHOCYTES, AND PLATELETS

The white blood cells are called **leukocytes** (from the Greek "leukos" meaning "white" and "kytos," meaning "cell"). The granular leukocytes (**eosinophils, neutrophils, and basophils**) are named for the granules in their cytoplasm; the agranular leukocytes (**monocytes and lymphocytes**) lack cytoplasmic granules. All of the granular leukocytes have **polymorphic** nuclei that appear as two to five ovate-shaped lobes that are connected by strands. Histological stains help differentiate among the different white blood cells. The microscopic appearance of these cells is shown in Figure 5.3.1.

Each mm^3 of blood contains some $5-5.5 \times 10^6$ red blood cells but only about 4000–11,000 white blood cells. Of these, the neutrophils comprise 50–70%, the lymphocytes 20–40%, the monocytes 2–8%, the eosinophils 1–4%, and the basophils <1%. **Platelets** are cell fragments of **megakaryocytes**. The platelets typically number between 0.2 and 0.5×10^6 particles per mm^3 of blood. The relative fraction of white blood cells contributed by each type is shown in Figure 5.3.2.

WHITE BLOOD CELLS ORIGINATE FROM PLURIPOTENT STEM CELLS

THE PLURIPOTENT STEM CELLS FORM TWO DISTINCT LINEAGES

Pluripotent stem cells in the bone marrow produce **myeloid** and **lymphoid** progenitors. The myeloid progenitor differentiates further into a granulocyte/macrophage progenitor that further differentiates into the granulocytes and the monocytes. **All of these cells are capable of phagocytosis, but it is primarily the neutrophils and the monocytes that defend against bacterial and viral infection by phagocytosis.** The neutrophil is a mature cell that is capable of phagocytosis, whereas the phagocytic abilities of circulating monocytes are small. When the monocytes enter the tissues, they enlarge, develop large numbers of lysosomes, and are then called **macrophages** (see Figure 5.3.3).

The myeloid progenitors also give rise in the marrow to **megakaryocytes** and **erythrocytes**. The megakaryocytes disintegrate in the bone marrow to produce fragments called **platelets**. Lymphocytes originate from various lymphogenous organs, including the lymph nodes or glands, the thymus, tonsils, the bone marrow, and the gut. The lymphoid stem cell further differentiates into the lymphocyte.

HEMATOPOIETIC GROWTH FACTORS STIMULATE FORMATION OF WHITE BLOOD CELLS

Thrombopoietin (**TPO**) is a 332 amino acid glycoprotein made primarily in the liver that stimulates the formation of megakaryocytes from CFU-Meg (colony forming unit, megakaryocyte). TPO binds to receptors that dimerize and activate cytoplasmic kinases. These kinases phosphorylate STAT-1, 3, and 5 and CREB. (See Chapter 2.8—STAT stands for **s**ignal **t**ransduction and **a**ctivator of **t**ranscription; CREB stands for **c**yclic AMP **r**esponse **e**lement **b**inding protein.) Hematopoietic stem cells, megakaryocyte progenitors, megakaryocytes, and platelets have receptors for TPO (Figure 5.3.4). The liver makes TPO at a constant rate, but platelets degrade TPO. Thus, lower platelets results in higher [TPO], which stimulates the differentiation of megakaryocytes into platelets. This regulation of TPO differs significantly from the feedback mechanisms involving erythropoietin and formation of erythrocytes. Other growth factors for differentiation of white blood cells are indicated in Figure 5.3.3.

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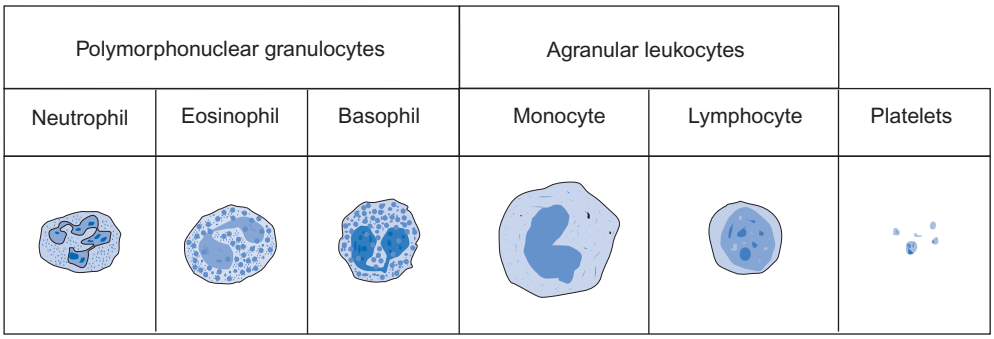

FIGURE 5.3.1 The various types of white blood cells. The polymorphonuclear granulocytes possess lobed nuclei and cytoplasmic granules. Cytoplasmic granules in eosinophils take up eosin, a histological dye; cytoplasmic granules of basophils stain with a basic, blue dye. Neutrophils have granules that do not stain with either eosin or basic dyes. The monocytes are larger than the polymorphonuclear granulocytes. They have a single oval or horseshoe-shaped nucleus and relatively few cytoplasmic granules. Lymphocytes contain a single, oval-shaped nucleus and small amounts of cytoplasm. Platelets are cell fragments.

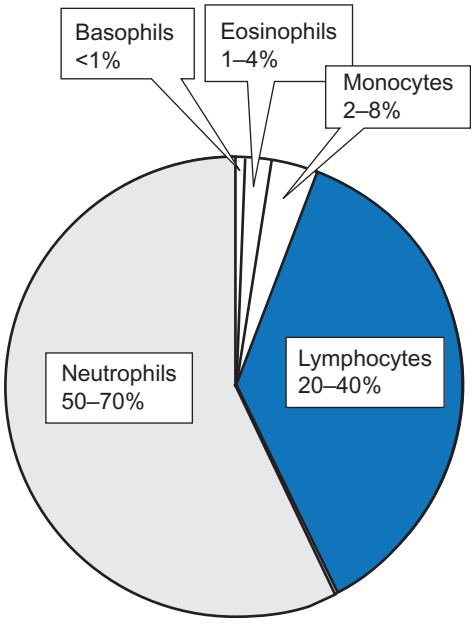

FIGURE 5.3.2 Fraction of leukocytes contributed by each class. The major class of white blood cells is the neutrophil, followed in order by the lymphocytes, monocytes, eosinophils, and basophils.

THE CIRCULATING WHITE BLOOD CELLS ARE IN TRANSIT FROM PRODUCTION SITE TO THE TISSUES

White blood cells are produced in the marrow, but they function in the tissues. Their presence in blood represents transport from the site of production to the site of use. Normally, the marrow stores about three times as many white cells as are present in the blood. Once released into the blood, a typical white cell spends some 4−8 h circulating and about another 4−5 days in the tissues.

NEUTROPHILS ARE *PHAGOCYTES*

The neutrophils are the most numerous of the leukocytes. They leave the circulatory system in response to signals from injured tissues early in the inflammatory response to bacterial invasion. They form part of the nonspecific or innate defense system. An elevated white cell count is often used clinically as an indicator of bacterial versus viral infections. Neutrophils contain a variety of cytoplasmic granules that contain proteins and peptides that kill and digest microbes. Activation of these proteins requires production of **reactive oxygen species (ROS)** including **superoxide**, **hydrogen peroxide, hydroxyl radical,** and **hypochlorous acid**. These ROS are produced by the enzyme **NADPH oxidase** on the plasma membrane that invaginates to form the phagosome, and **myeloperoxidase** that is present in the cytoplasmic granules.

MONOCYTES LEAVE THE CIRCULATORY SYSTEM TO BECOME *TISSUE MACROPHAGES*

Monocytes are the largest of the leukocytes. They are released into the blood from the marrow in an immature form with little phagocytic ability. They circulate in the blood until they find a suitable home in the tissues, where they greatly enlarge to become tissue macrophages. Some of these large phagocytic cells remain mobile, while others attach themselves to tissue components and become **fixed macrophages**. Their life span varies from months to years, depending on their activity.

BASOPHILS RESEMBLE *MAST CELLS*

Basophils are the least numerous of the leukocytes. Structurally and functionally they resemble mast cells. However, basophils originate in the marrow whereas mast cells originate from precursor cells in the connective tissue. Both basophils and mast cells contain secretory granules that store **histamine** and **heparin**, among other chemicals. Histamine release occurs in allergic reactions and in response to inflammation. Histamine profoundly increases capillary permeability and in this way contributes to the inflammatory response by causing tissue **edema**, or swelling. Heparin activates lipoprotein lipase that degrades triglycerides in the blood from dietary sources. Heparin also prevents blood clotting.

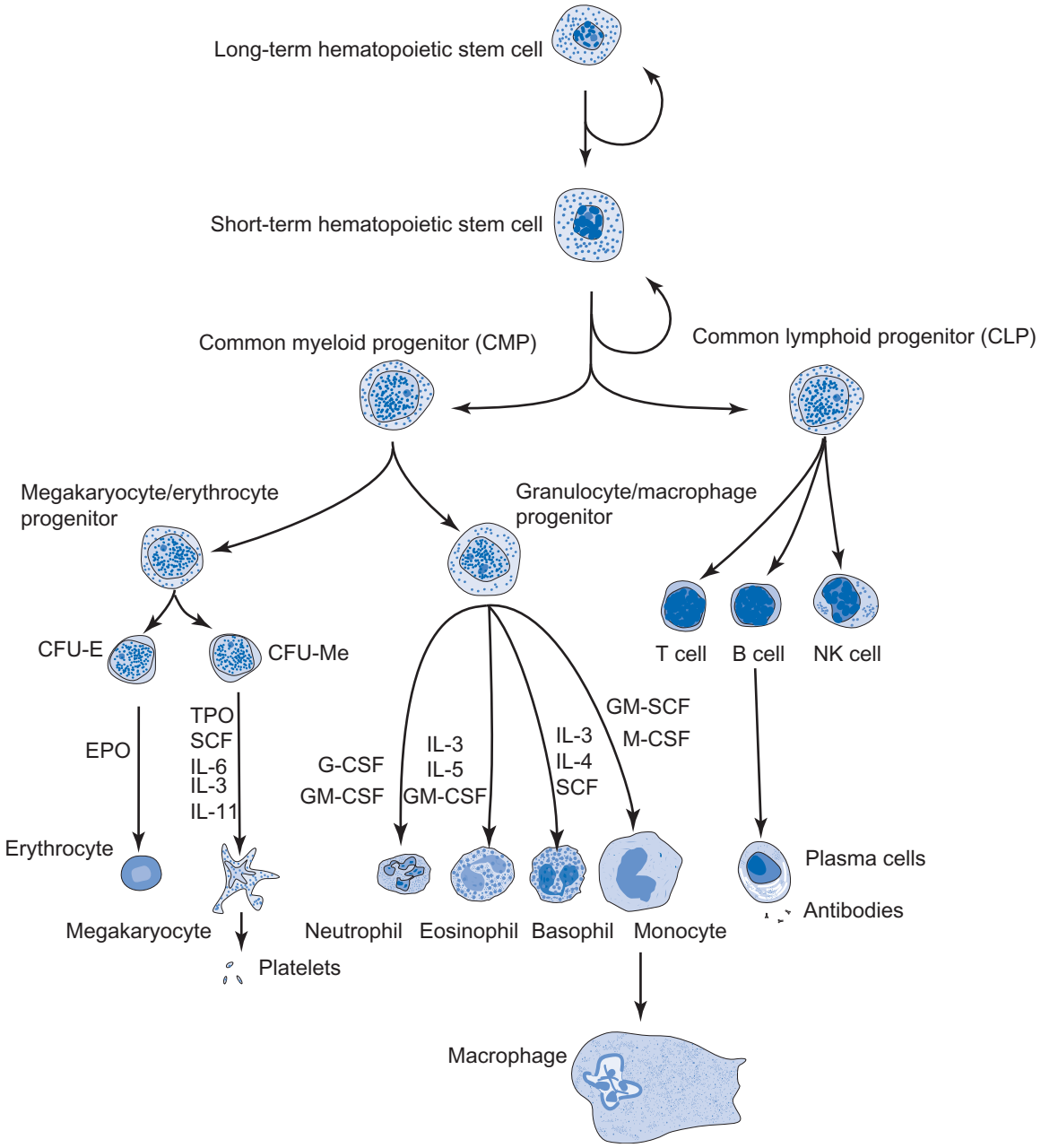

FIGURE 5.3.3 Formation of white blood cells. Long-term hematopoietic stem cells can divide to form new stem cells or to begin differentiation. Differentiating long-term hematopoietic stem cells become short-term hematopoietic stem cells that can also self-renew or begin differentiation by producing multipotent progenitors of two main lines: the lymphoid line and the myeloid line. The lymphoid line gives rise to the T-lymphocytes and B-lymphocytes and natural killer cells (NK cells). The myeloid line gives rise to two further progenitor lines: the megakaryocyte/erythroid progenitor and the granulocyte/macrophage progenitor. These give rise to the erythrocytes, platelets, and the white blood cells. EPO = erythropoietin; TPO = thrombopoietin; IL-3, IL-4, IL-6, IL-11 = interleukins; SCF = stem cell factor (Steel factor); GM-CSF = granulocyte/megakaryocyte colony stimulating factor; G-CSF = granulocyte colony stimulating factor; M-CSF = monocyte colony stimulating factor.

Heparin released from the basophils probably acts primarily as an anticoagulant.

EOSINOPHILS ARE INVOLVED IN DEFENSE OF PARASITIC INFECTIONS AND ALLERGIES

Eosinophils comprise only a few percent of the total number of leukocytes, but are readily identifiable in blood smears because their cytoplasmic granules take on an orange-red to bright yellow color when stained with eosin. Their numbers increase in persons with allergic conditions such as hay fever and asthma and in persons with parasitic infections. Parasites are typically much larger than eosinophils and therefore eosinophils cannot engulf them to destroy them. Eosinophils attach themselves to the juvenile form of parasites and secrete materials that injure or kill the parasite. These materials

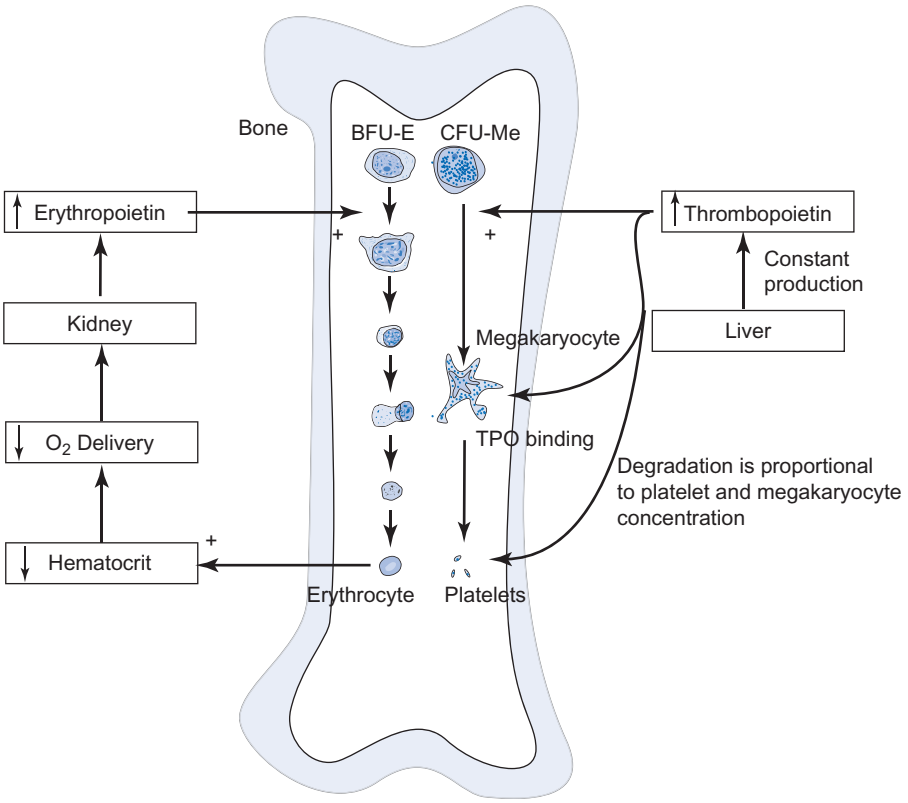

FIGURE 5.3.4 Comparison of EPO and TPO regulation of erythropoiesis and thrombopoiesis. EPO is synthesized mainly in the kidney in response to low O_2 delivery caused by a decreased hematocrit. The increased circulating [EPO] stimulates differentiation of burst-forming units for erythropoiesis BFU-E, so that the production of red blood cells is increased. In the absence of other events, this raises the hematocrit and increases kidney P_{O_2}, decreasing the initial signal for EPO. This control system forms a negative feedback loop. TPO is synthesized at a constant rate by the liver, but it is bound and then degraded by megakaryocytes and thrombocytes (platelets). Thus, when platelets are high, TPO is low and thrombopoiesis is depressed. When platelet counts are low, TPO is high and thrombopoiesis proceeds rapidly.

include hydrolytic enzymes that probably originate from lysosomes, ROS, and a polypeptide that has larvicidal activity.

LYMPHOCYTES FORM A SPECIFIC DEFENSE SYSTEM

The neutrophils, basophils, eosinophils, and monocytes are all part of a ***non-specific*** **defense system**. This means that the cells are capable of destroying a large variety of bacterial or viral invaders or cellular debris resulting from tissue damage, without specific recognition of which bacterium or viral invader. They accomplish this by recognizing common patterns of binding sites on the surfaces of these invaders. Lymphocytes, on the other hand, provide defense against ***specific*** **foreign objects**. Lymphocytes come in a variety of types, broadly classified as **T-lymphocytes**, **B-lymphocytes**, and **natural killer cells**. These descriptors derive from the processing of the lymphocytes during their differentiation. All three cell types derive from a single lymphoid stem cell. The thymus modifies the cells that become T-lymphocytes and these cells promote cell-mediated immunity. The bone marrow influences the cells that become B cells, and these cells differentiate to form **plasma cells** that produce circulating antibodies that comprise humoral immunity. The processing of T-lymphocytes occurs during the perinatal period. Thus, removal of the thymus during late fetal life can seriously interfere with cell-mediated immunity, whereas later ablation of the thymus has little or no effect. The natural killer cells arise in the bone marrow and destroy virus-infected cells and cancer cells. The T-lymphocytes constitute a heterogeneous group of cells that is further broken down into two main subsets: **cytotoxic T cells** and **helper T cells**. Cytotoxic T cells, as their name implies, kill cells. They bind to antigens on the surfaces of target cells and kill them by secreting toxic chemicals. This interaction does not require antibodies. Helper T cells, as their name also implies, do not kill cells on their own. Instead, they help activate both B cells and cytotoxic T cells by secreting **cytokines**. "Cytokine" literally means "causing the cell to move"; in this case it refers to chemicals secreted by one cell that activate other cells. Cytotoxic T cells and helper T cells are distinguishable based on their surface content of specific proteins. Cytotoxic T cells express a protein called CD8 on their surfaces; helper T cells express CD4. Thus, these cells are sometimes referred to as CD8 cells or CD4 cells. Another type of T cell is called the **suppressor T cell** or **regulatory T cell**. These T cells actively suppress other T cells that can produce tissue damage.

TISSUE MACROPHAGES, MONOCYTES, AND SPECIALIZED ENDOTHELIAL CELLS FORM THE *RETICULOENDOTHELIAL SYSTEM*

Monocytes leave the blood and enter tissues where they provide a mobile defense against foreign materials, including bacteria and viruses. The monocytes have little ability to destroy foreign material until they enter the tissues and transform into macrophages. Sometimes tissue macrophages attach to the tissues and remain fixed and inactive for long periods, months to years,

until they become activated. The combination of the circulating monocytes, mobile macrophages within tissues, fixed macrophages, and some specialized cells in the spleen, lymph nodes, and bone marrow constitute the **reticuloendothelial system**. The cells in this system form a network for preventing bacterial or viral invasion of the blood. In addition, almost all of these cells derive from monocytic stem cells and for this reason they are categorized together. This "system" is a phagocytotic defense system that exists in all tissues, but is especially concentrated in those tissues where bacterial invasion is most likely. These are the tissues that interface with the environment:

- **Skin:** Although the skin is typically impervious to bacteria or viruses, breaks in the skin allow their entry. Macrophages in the subcutaneous connective tissue, called **histiocytes**, phagocytose bacteria, viruses, and necrotic tissue.
- **Lymph:** Except during the brief period of bleeding following injury, bacteria and viruses cannot enter the blood because the capillary walls exclude them. However, they can enter the lymphatics that drain the tissues. The lymph vessels drain into lymph nodes located intermittently throughout the lymphatic system. The nodes consist of a meshwork of cavities or **sinuses** that are lined with epithelial cells and large numbers of macrophages. Foreign particles become entrapped in the meshwork and then are engulfed by macrophages.
- **Lung:** Each lobe of the lung contains many millions of **alveoli**, tiny thin-walled sacs in which the blood is separated from the air by a thin layer of endothelial cells and alveolar cells. This short separation is necessary for rapid gas exchange, but it also presents an opportunity for airborne organisms or other materials to enter the blood. To combat this, the alveolar walls also contain large numbers of tissue macrophages that phagocytose materials that stick to the alveoli. If the particles are not digestible by the macrophages, the cells form a giant cell capsule around an offensive particle.
- **Liver:** Bacteria and other materials constantly enter the venous blood that drains the intestine, eventually entering the portal circulation. The portal blood passes through the liver, where macrophages called **Kupffer cells** line the sinuses. These cells effectively scrub the portal blood so that bacteria and other foreign particles that enter the blood through the gut do not pass into the general circulation.
- **Spleen and bone marrow:** If the peripheral defense systems fail and bacteria succeed in entering the general circulation (a condition called **septicemia**), the spleen and bone marrow contain macrophages that can clear the blood. The spleen differs from all other organs in that the red cells leave the capillaries and course through a meshwork of fibers and return to the circulation through the walls of the venous sinuses. The red pulp of the spleen, through which the red cells squeeze, is lined with macrophages that engulf worn and abnormal red blood cells as well as any foreign matter.

INFLAMMATION IS THE NET RESPONSE OF THE BODY TO *TISSUE INJURY*

In the first century, Aulus Celsus in *De Medicina* first described four of the five **cardinal signs of inflammation**. These were

- rubor (redness)
- calor (heat)
- tumor (swelling)
- dolor (pain).

Later, Galen added a fifth sign: impaired function. Tissue injury can be caused by bacteria, trauma, toxic or caustic chemicals, and heat. Regardless of the cause, injured tissue releases a variety of substances that evoke a complex series of changes within the tissue. This entire complex of changes is called **inflammation**. These processes include:

A. **Vasodilation** of the local blood vessels with consequent increased blood flow.
B. **Increased permeability of the capillaries** with consequent leakage of fluid into the interstitial space.
C. **Clotting of the fluid in the interstitial space** due to leakage of clotting proteins from the plasma into the interstitial fluid.

MIGRATION OF GRANULOCYTES AND MONOCYTES FROM THE BLOOD INTO THE TISSUES

The redness and warmth arises from the increased local blood flow. The edema or swelling arises from the increased capillary permeability and subsequent **exudation** of fluid into the tissues. The swelling stretches free nerve endings that can cause pain. The clotting of the fluid in the interstitial space effectively walls off the area so that pathogens cannot easily spread to the rest of the body. The migration of granulocytes and monocytes into the tissues is called **extravasation**. These actions limit the spread of infection and eventually remove it from the body.

INFLAMMATION BEGINS WITH THE RELEASE OF SIGNALING MOLECULES FROM THE DAMAGED TISSUE

Tissue injury caused by trauma, bacterial invasion, chemicals, heat, or other factors releases a set of signaling molecules that initiates the inflammatory response. The nature of the inflammatory response depends on the set of signaling molecules that are released, and this set is determined in part by the kind of injury. A partial list of the signaling molecules and their sources is given in Table 5.3.1.

THE INNATE IMMUNE RESPONSE REQUIRES NO PRIOR EXPOSURE—SPECIFICITY OF THE RESPONSE IS INHERITED IN THE GENOME

The **innate response** to infection requires no prior exposure to the infectious agent. The **adaptive immune**

response occurs only when the innate response is overwhelmed, generally because the pathogens evade recognition by the cells that mediate the innate response. In the adaptive immune response, cells arise that recognize specific parts of the infectious agent. These cells generate antigen-specific cells that target the pathogen, and memory cells that prevent reinfection by the same pathogen.

Microorganisms typically have a cell membrane that is covered on the outside by a cell wall. The cell membrane is a lipid structure similar to the mammalian biological membranes (see Chapter 2.4). The cell wall imparts some structural rigidity to the cell while remaining porous. This cell wall differs among various bacteria, and Gram's stain is used to differentiate many species. **Gram-positive** bacteria have little lipid in their cell walls, whereas **Gram-negative** bacteria are rich in lipid. These walls consist of covalently linked polysaccharide and peptide chains. The wall typically presents repeating patterns of sugar molecules. Phagocytic macrophages within the tissues have surface receptors that recognize common constituents of bacterial surfaces. These receptors include:

- **mannose receptor**, a transmembrane receptor with multiple binding sites for mannose, recognizes the pattern of mannose on the microbes' cell wall.
- **scavenger receptor**, a structurally heterogeneous set of molecules, recognizes anionic polymers and acetylated low-density lipoproteins. These recognize structures on red blood cells that are masked by sialic acid. Old cells that lose their sialic acid are scavenged. Cells with these receptors take up lipoproteins from the blood and are involved in atherogenesis.
- **LPS receptor (CD14).** Gram-negative bacteria produce a component of their cell walls called **lipopolysaccharide** or LPS. The plasma contains a protein that binds LPS, called LPS-binding protein or **LBP**. The complex of LPS and LBP binds to another receptor on the macrophage, CD14.
- **β-glucan receptor** recognizes glucan structures in yeast.

When constituents of bacterial cell walls bind to the receptors, they trigger the macrophage to engulf and destroy the invaders, and simultaneously the macrophage releases **cytokines** and **chemokines**. The cytokines activate cells that have receptors for them; the chemokines attract cells with receptors for them. The cytokines include interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-12 (IL-12), and tumor necrosis factor-α (TNF-α). The chemokine interleukin-8 attracts neutrophils, basophils, and T cells to the site of infection. The process of macrophage recognition, engulfment, and release of cytokines and chemokines is illustrated in Figure 5.3.5.

TABLE 5.3.1 Sources of Selected Inflammatory Mediators Produced During Inflammation

Mast Cells	Platelets	Macrophages	Eosinophils	Complement	Kinins	Nerves
Histamine	5-HT	TNF-α	PAF	C3a	Bradykinin	ATP
ATP	ADP	IL-1		C5a		SP
Prostaglandins		IL-6				NKA
Leukotrienes		IL-8				CGRP
TXA_2		IL-12				

TXA_2, thromboxane A_2; 5-HT, 5-hydroxy tryptamine; TNF-α, tumor necrosis factor-α; IL-1, interleukin 1; IL-6, interleukin 6; IL-8, interleukin 8; IL-12, interleukin 12; PAF, platelet activating factor; SP, substance P; NKA, neurokinin A; CGRP, calcitonin-gene related peptide.
Source: *Adapted from J. Linden, Blood flow regulation in inflammation, in K. Ley, ed.,* Physiology of Inflammation*, Oxford University Press, New York, NY, 2001.*

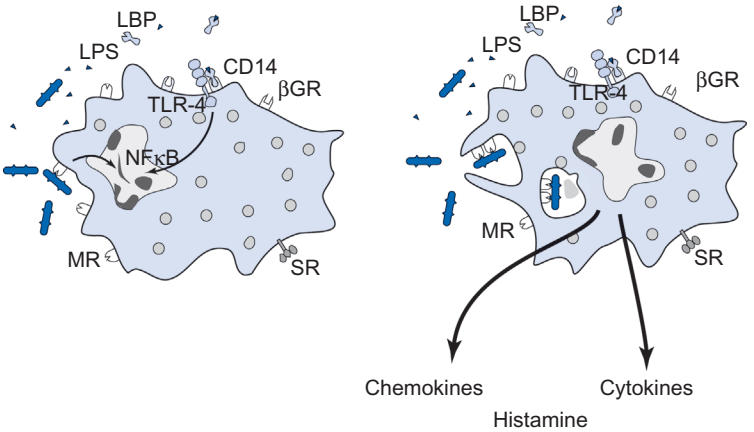

FIGURE 5.3.5 Macrophage phagocytosis and secretion of chemokines and cytokines. Macrophages have a variety of surface receptors that recognize bacterial invaders without prior exposure. Examples include a mannose receptor (MR), β-glucan receptor (βGR), and scavenger receptor (SR). Bacterial LPS binds to a plasma LPS binding protein (LPB) that then binds to a surface receptor, CD14, that is linked to toll-like receptor 4 (TLR-4). This binding initiates a cascade of events that activate a series of protein kinases that eventually activates NF-κB by phosphorylating its inhibitor. The NF-κB then activates the transcription of genes that promote the inflammatory response, the release of chemokines such as interleukin-8, and cytokines such as IL-1, IL-6, IL-12, and TNF-α. These trigger part of the inflammatory response and begin recruitment of leukocytes to the site of infection.

NEUTROPHILS AND MONOCYTES LEAVE THE CIRCULATORY SYSTEM BY *DIAPEDESIS* IN RESPONSE TO CHEMOTAXIC COMPOUNDS

Tissues react to bacterial or viral invasion by producing a variety of chemotaxic compounds that attract circulating neutrophils and monocytes. These include chemicals produced by the bacteria or by damaged tissue or by the reaction of cells to the foreign invaders.

Chemotaxis refers to the migration of cells in response to a chemical signal (see Figure 5.3.6).

- **Leukocytes are "captured":** Leukocytes first move toward the outer edge of the circulation ("marginate"), allowing the leukocytes to closely approach the endothelial surface. The endothelial cells expose a protein called **P-selectin** in response to inflammatory mediators such as histamine. P-selectin makes the surface of the capillary sticky to leukocytes. It captures the leukocytes by binding to PSGL-1

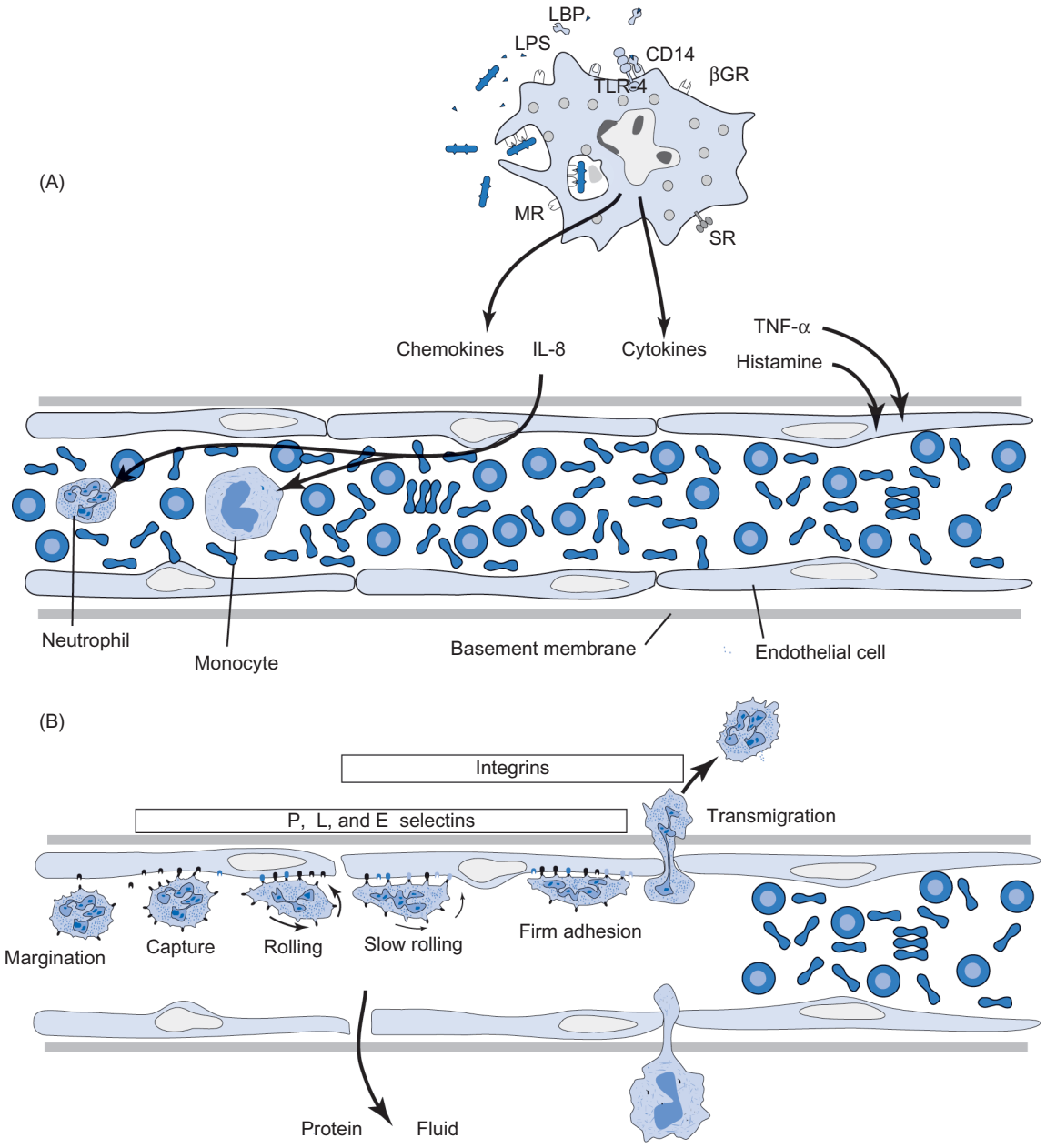

FIGURE 5.3.6 Recruitment of leukocytes to the site of infection. Macrophages release chemokines and cytokines in response to phagocytosis of foreign organisms. The chemokines attract the leukocytes. Cytokines released by the macrophages elicit proteins on the surface of the endothelial cells in two phases: a rapid phase involving proteins already present, and a delayed phase corresponding to new synthesis of membrane proteins. P-selectins bind to PSGL-1 on the leukocytes, capturing them. These cells first rapidly roll along the endothelial surface, mediated by binding to P- and L-selectin on the endothelial cells. Binding to E-selectin on the endothelium slows the rolling. Eventually, the leukocytes adhere firmly to the endothelium and begin transmigration across the capillary wall.

(**P**-selectin **g**lycoprotein **l**igand-1) on the surface of the leukocytes. P-selectin exposure is rapid and short-lived, reaching a peak within 10 min after stimulation by cytokines.

- **Leukocytes roll along the endothelial surface:** The transient association of P-selectin with its ligand on the leukocyte allows the leukocyte to roll along the endothelial cell surface. Other proteins involved in the rolling include **E-selectin** and **L-selectin**. E-selectin is on the surface of the endothelium and L-selectin is on the leukocyte.
- **The rolling slows:** All three selectins will support leukocyte rolling, but at different velocities. E-selectin slows the rolling to $5-10\ \mu m\ s^{-1}$. The slowing is due in part to increased densities of E-selectin, which are increased 1–2 h after the onset of inflammation.
- **The leukocytes firmly adhere:** The binding of E-selectin is believed to begin the firm attachment of leukocytes to the endothelial surface. Other proteins involved include **ICAM-1** (**i**ntercellular **a**dhesion **m**olecule) on the endothelial cell that binds to an integrin, **LFA-1** (**l**eukocyte **f**unctional **a**ntigen) on the leukocyte.
- **The leukocytes transmigrate by ameboid motion through the capillary wall:** After adhering, leukocytes further interact with other proteins on the endothelial surface, particularly **PECAM-1** (**p**latelet-**e**ndothelial **c**ell **a**dhesion **m**olecule) and other leukocyte integrins. Both neutrophils and monocytes cross through the capillary wall by **ameboid motion**. This process consists of the extension of **pseudopodia** (literally, "false feet") by the cell, by deformation of the cell's cytoskeleton. Since the cytoskeleton is attached to the cell membrane, its deformation deforms the entire cell. Retraction of the opposite end of the cell results in a net forward movement of the cell. This **transmigration** is also called **diapedesis**.

THE COMPLEMENT SYSTEM DESTROYS MICROBES THAT HAVE ATTACHED ANTIBODIES

The complement system consists of about 12 soluble plasma proteins that are made mainly in the liver and circulate in the blood. They are inactive until activated by an infection. They are called "complement" because of their ability to amplify and complement the activity of antibodies. The complement proteins form a proteolytic cascade in which successive members cleave the next member, forming an active serine protease and releasing peptide fragments that can be used in recruiting phagocytes and lymphocytes. The final result of complement activation is the formation of a large transmembrane channel from aggregation of several units of C9.

IgG OR IgM ACTIVATES COMPLEMENT IN THE CLASSICAL PATHWAY

IgG or IgM antibodies bound to the surface of a microbe activates C1, a six-headed molecule that binds to a polymeric antigen–antibody aggregate. Binding of C1 activates a protease in a subunit of C1, which then activates a serine protease in another subunit of C1. The activated C1 then sequentially activates C4 to C4b, which activates C2 to C2b. The complex C4b2b cleaves C3.

A MANNAN-BINDING PROTEIN ACTIVATES C3

In a separate pathway, a plasma protein called mannan-binding lectin (MBL) forms a cluster of six mannose-binding heads around a central stalk. This protein specifically binds to mannose and fucose residues on the surface of bacterial cell walls that have the proper spacing and orientation. This complex is associated with a MBL-associated serine protease or MASP. These cleave C4 and C2 as in the classical pathway, resulting in the proteolytic cleavage of C3.

PROTEOLYTIC CLEAVAGE OF C3 IS THE CENTRAL EVENT

Proteolytic cleavage of C3 releases a large fragment, C3b, and a smaller fragment, C3a. The smaller fragment as well as fragments of C4 and C5 recruit phagocytes from the circulation. C3b binds covalently to the surface of the pathogen where it activates the remainder of the complement cascade, and where it can be recognized by phagocytic cells to enhance phagocytosis.

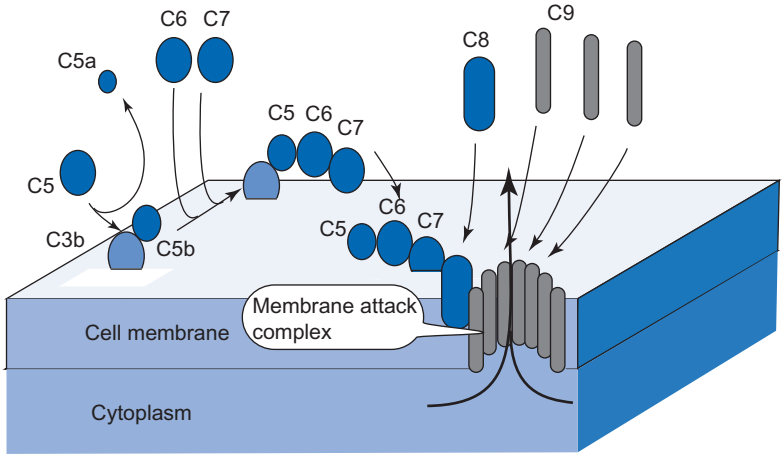

FIGURE 5.3.7 Mechanism of cell lysis by complement. See text for detailed explanation. (Source: *Modified from Alberts et al.*, Molecular Biology of the Cell, *Garland Science, New York, NY, 2002.*)

C9 UNITS AGGREGATE TO FORM A PORE THAT LYSES THE CELL

The membrane-immobilized C3b triggers a cascade of events beginning with the cleavage of C5 to C5a and C5b. C5b remains loosely bound to C3b and rapidly binds C6 and C7 to form C567. This firmly attaches to the pathogen membrane via C7. A molecule of C8 then binds to form C5678. This complex then binds C9, which subsequently undergoes a conformational change that exposes a hydrophobic region on C9. It inserts into the membrane and binds other units of C9, forming a large aqueous channel. This is called the **membrane attack complex**. This large channel causes the cytoplasm of the pathogen to leak out, killing the cell. The process is illustrated in Figure 5.3.7.

SUMMARY

The white blood cells include the polymorphonuclear granulocytes (neutrophils, eosinophils, and basophils) and the agranular leukocytes (monocytes and lymphocytes). Platelets are particles of megakaryocytes. The white cells are formed in bone marrow from pluripotent stem cells that form two lines: the myeloid line and the lymphoid line. The neutrophils are the most numerous white cell that phagocytose bacteria and other materials. Monocytes leave the circulatory system to become tissue macrophages that may live for long times in the tissues and have phagocytotic capability. Basophils resemble mast cells except that basophils originate in the marrow. They secrete histamine and heparin. Eosinophils are believed to defend against parasitic infections. Neutrophils, basophils, and eosinophils form a nonspecific defense system. The lymphocytes form a specific defense by recognizing a specific antigen. Lymphocytes come in a variety of types including T-lymphocytes, B-lymphocytes, and natural killer cells. The T-lymphocytes are further classified into cytotoxic T cells, helper T cells, and suppressor T cells. T cells provide cell-mediated immunity whereas B cells confer humoral immunity. The phagocytic cells occupy all regions of the body that may provide a route for bacterial infection. Together these phagocytic cells comprise the reticuloendothelial system.

Inflammation is the response of the body to tissue injury. Its five cardinal signs are: redness, increased temperature, edema, pain, and loss of function. Inflammation begins with the release of one or more of a host of signaling molecules by cells in the damaged area. Tissue macrophages, for example, have receptors that recognize bacterial cell wall constituents or LPS released from gram-negative bacteria. Binding of these ligands causes the macrophage to release cytokines and chemokines to both activate and attract white blood cells to the site of damage. Neutrophils and monocytes can cross the capillary wall by diapedesis to enter the infected or damaged area. Recruitment of neutrophils requires the expression of endothelial surface proteins called P-, L-, and E-selectins, and intercellular adhesion molecules. Receptors for these proteins reside on the leukocyte cell membrane.

Complement destroys pathogens by punching holes in their cell membranes. In the classic pathway, complement is activated by immunoglobulins IgG or IgM bound to the pathogen. This begins a cascade of proteolytic events and recruitment of complement factors until, at last, a series of complement proteins called C9 aggregate to form a water-filled channel across the membrane of the cell.

REVIEW QUESTIONS

1. What is the most common leukocyte? The least common?
2. How does TPO regulate the number of platelets?
3. What do platelets do?
4. How does the innate immune system differ from the adaptive immune system?
5. What cells constitute the reticuloendothelial system?
6. What cells do monocytes become in the tissues?
7. What are the steps involved in recruitment of neutrophils outside of the vascular system?
8. How does complement kill bacteria?