Blood is a highly differentiated, complex living tissue that pulsates through the arteries to every part of the body, interacts with individual cells via an extensive capillary network, and returns to the heart through the venous system. Many of the functions of blood are undertaken in the capillaries, where the blood flow slows dramatically, allowing the efficient diffusion and transport of oxygen, glucose, and other molecules across the monolayer of endothelial cells that form the thin capillary walls. In addition to transport, blood and the cells within it mediate other essential aspects of immunity and hemostasis.

The human body is continually invaded by pathogenic microorganisms that enter through skin cuts, mucous membranes, and other sites of infection and tissue disruption. To oppose pathogenic microbes, the body has developed a highly sophisticated immune system. Cells of the immune system, the white blood cells, are derived from bone marrow precursors and are delivered to their sites of action by the blood. These cells, also known as leukocytes, exert their effects in conjunction with antibodies and protein cofactors in blood. In this chapter, we will see how certain leukocytes act without prior sensitization to neutralize offending pathogens, while others require a prior infectious insult to deal with invaders.

In addition to infectious assault, the body is continually threatened by the devastating consequences of vascular leak or hemorrhage as a result of even the most innocuous tissue injury. A highly organized clotting system, consisting of blood platelets that work in conjunction with blood plasma clotting factors, prevents excessive fluid loss by rapidly forming a hemostatic plug. In addition to physically constraining fluids within ruptured vessels, platelets release potent biological cofactors during the development of this hemostatic plug, which promote wound healing, prevent further infection, and promote the development and vascularization of new tissue.
THE COMPONENTS OF BLOOD

Blood is an opaque, red liquid consisting of several types of cells suspended in a complex, amber fluid known as plasma. When blood is allowed to clot or coagulate, the suspending medium is referred to as serum.

Blood Has a Higher Density and Viscosity than Water

Blood is normally confined to the circulation, including the heart and the pulmonary and systemic blood vessels. Blood accounts for 6 to 8% of the body weight of a healthy adult. The blood volume is normally 5.0 to 6.0 L in men and 4.5 to 5.5 L in women.

The density (or specific gravity) of blood is approximately 1.050 g/mL. Density depends on the number of blood cells present and the composition of the plasma. The density of individual blood cells varies according to cell type and ranges from 1.115 g/mL for erythrocytes to 1.070 g/mL for certain leukocytes.

While blood is only slightly heavier than water, it is certainly much thicker. The viscosity of blood, a measure of resistance to flow, is 3.5 to 5.5 times that of water. Blood's viscosity increases as the total number of cells present increases and when the concentration of large molecules (macromolecules) in plasma increases. At pathologically high viscosity, blood flows poorly to the extremities and internal organs.

The Erythrocyte Sedimentation Rate and Hematocrit Are Important Diagnostic Measurements

Erythrocytes are the red cells of blood. Since erythrocytes have only a slightly higher density than the suspending plasma, they normally settle out of whole blood very slowly. To determine the erythrocyte sedimentation rate (ESR), anticoagulated blood is placed in a long, thin, graduated cylinder (Fig. 11.1). As the red cells sink, they leave behind the less dense leukocytes and platelets in the suspending plasma. Erythrocytes in the blood of healthy men sediment at a rate of 2 to 8 mm/hr; those in the blood of healthy women sediment slightly faster (2 to 10 mm/hr).

The ESR can be an important diagnostic index, as values are often significantly elevated during infection, in patients with arthritis, and in patients with inflammatory diseases. In some diseases, such as sickle-cell anemia, polycythemia (abnormal increase in red cell numbers), and hyperglycemia (elevated blood sugar levels), the ESR is slower than normal. The reasons for alterations in the ESR in disease states are not always clear, but the cells tend to sediment faster when the concentration of plasma proteins increases.

Blood cells can be quickly separated from the suspending fluid by simple centrifugation. When anticoagulated blood is placed in a tube that is rotated about a central point, centrifugal forces pull the blood cells from the suspending plasma. The hematocrit is the portion of the total blood volume that is made up of red cells. This value is determined by the centrifugation of small capillary tubes of anticoagulated blood to pack the cells.

Determination of hematocrit values is a simple and important screening diagnostic procedure in the evaluation of hematological disease. Hematocrit values of the blood of healthy adults are 47 ± 5% for men and 42 ± 5% for women. Decreased hematocrit values often reflect blood loss as a result of bleeding or deficiencies in blood cell production. Low hematocrit values indicate the presence of anemia, a reduction in the number of circulating erythrocytes. Increased hematocrit levels may likewise indicate a serious imbalance in the production and destruction of red cells. Increased production (or decreased rate of destruction) of erythrocytes results in polycythemia, as reflected by increased hematocrit values. Dehydration, which decreases the water content and, thus, the volume of plasma, also results in an increase in hematocrit.

Blood Functions as a Dynamic Tissue

While the cellular and plasma components of blood may act alone, they often work in concert to perform their functions. Working together, blood cells and plasma proteins play several important roles, including:

- **Transport** of substances from one area of the body to another
- **Immunity**, the body’s defense against disease
- **Hemostasis**, the arrest of bleeding
- **Homeostasis**, the maintenance of a stable internal environment

**Transport.** Blood carries several important substances from one area of the body to another, including oxygen, carbon dioxide, antibodies, acids and bases, ions, vitamins, cofactors, hormones, nutrients, lipids, gases, pigments, minerals, and water. Transport is one of the primary and most important functions of blood, and blood is the primary means of long-distance transport in the body. Substances can be transported free in plasma, bound to plasma proteins, or within blood cells.
Oxygen and carbon dioxide are two of the more important molecules transported by blood. Oxygen is taken up by the red cells as they pass through capillaries in the lung. In tissue capillaries, red cells release oxygen, which is then used by respiring tissue cells. These cells produce carbon dioxide and other wastes.

The blood also transports heat. By doing so, it maintains the proper temperature in different organs and tissues, and in the body as a whole.

**Immunity.** Blood leukocytes are involved in the body’s battle against infection by microorganisms. While the skin and mucous membranes physically restrict the entry of infectious agents, microbes constantly penetrate these barriers and continuously threaten internal infection. Blood leukocytes, working in conjunction with plasma proteins, continuously patrol for microbial pathogens in the tissues and in the blood. In most cases, penetrating microbes are efficiently eliminated by the sophisticated and elaborate immune systems of the blood.

**Hemostasis.** Bleeding is controlled by the process of hemostasis. Complex and efficient hemostatic mechanisms have evolved to stop hemorrhage after injury, and their failure can quickly lead to fatal blood loss (exsanguination). Both physical and cellular mechanisms participate in hemostasis. These mechanisms, like those of the immune system, are complex, interrelated, and essential for survival.

**Homeostasis.** Homeostasis is a steady state that provides an optimal internal environment for cell function (see Chapter 1). By maintaining pH, ion concentrations, osmolality, temperature, nutrient supply, and vascular integrity, the blood system plays a crucial role in preserving homeostasis. Homeostasis is the result of normal functioning of the blood’s transport, immune, and hemostatic systems.

**Plasma Contains Many Important Solutes**

Plasma is composed mostly of water (93%) with various dissolved solutes, including proteins, lipids (fats), carbohydrates, amino acids, vitamins, minerals, hormones, wastes, cofactors, gases, and electrolytes (Table 11.1). The solutes in plasma play crucial roles in homeostasis, such as maintaining normal plasma pH and osmolality.

**There Are Three Types of Blood Cells**

Blood cells include erythrocytes (red blood cells), leukocytes (white blood cells), and platelets (thrombocytes). Each microliter (a millionth of a liter) of blood contains 4 to 6 million erythrocytes, 4,500 to 10,000 leukocytes, and 150,000 to 400,000 platelets. There are several subtypes of leukocytes, defined by morphological differences (Fig. 11.2), each with vastly different functional characteristics and capabilities. Table 11.2 lists the normal circulating levels of different blood cell types.

Of the total leukocytes, 40 to 75% are neutrophilic, polymorphonuclear (multinucleated) cells, otherwise known as neutrophils. These phagocytic cells actively ingest and destroy invading microorganisms. Eosinophils and basophils are polymorphonuclear cells that are present in low numbers in blood (1 to 6% of total leukocytes) and participate in allergic hypersensitivity reactions. Mononuclear cells, including monocytes and lymphocytes, comprise 20 to 50% of the total leukocytes. These cells generate antibodies and mount cellular immune reactions against invading agents.

The number and relative proportion of the leukocyte subtypes can vary widely in different disease states. For example, the absolute neutrophil count often increases during infection, presumably in response to the infection. Eosinophil counts increase when allergic individuals are exposed to allergens. Lymphocyte counts decrease in AIDS and during some other viral infections. For this reason, in addition to a blood cell count, a differential analysis of leukocyte subtypes, performed by microscopic examination of stained slides, can provide important clues to the diagnosis of disease.

**TABLE 11.1 Some Components of Plasma**

<table>
<thead>
<tr>
<th>Class</th>
<th>Substance</th>
<th>Normal Concentration Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cations</td>
<td>Sodium (Na⁺)</td>
<td>136–145 mEq/L</td>
</tr>
<tr>
<td></td>
<td>Potassium (K⁺)</td>
<td>3.5–5.0 mEq/L</td>
</tr>
<tr>
<td></td>
<td>Calcium (Ca²⁺)</td>
<td>4.2–5.2 mEq/L</td>
</tr>
<tr>
<td></td>
<td>Magnesium (Mg²⁺)</td>
<td>1.5–2.0 mEq/L</td>
</tr>
<tr>
<td></td>
<td>Iron (Fe²⁺)</td>
<td>50–170 µg/dL</td>
</tr>
<tr>
<td></td>
<td>Copper (Cu²⁺)</td>
<td>70–155 µg/dL</td>
</tr>
<tr>
<td></td>
<td>Hydrogen (H⁺)</td>
<td>35–45 mmol/L</td>
</tr>
<tr>
<td>Anions</td>
<td>Chloride (Cl⁻)</td>
<td>95–105 mEq/L</td>
</tr>
<tr>
<td></td>
<td>Bicarbonate (HCO₃⁻)</td>
<td>22–26 mEq/L</td>
</tr>
<tr>
<td></td>
<td>Lactate</td>
<td>0.67–1.8 mEq/L</td>
</tr>
<tr>
<td></td>
<td>Sulfate (SO₄²⁻)</td>
<td>0.9–1.1 mEq/L</td>
</tr>
<tr>
<td></td>
<td>Phosphate</td>
<td>3.0–4.5 mg/dL</td>
</tr>
<tr>
<td>Proteins</td>
<td>Total</td>
<td>6–8 g/dL</td>
</tr>
<tr>
<td></td>
<td>Albumin</td>
<td>3.5–5.5 g/dL</td>
</tr>
<tr>
<td></td>
<td>Globulin</td>
<td>2.3–3.5 g/dL</td>
</tr>
<tr>
<td>Fats</td>
<td>Cholesterol</td>
<td>150–200 mg/dL</td>
</tr>
<tr>
<td></td>
<td>Phospholipids</td>
<td>150–220 mg/dL</td>
</tr>
<tr>
<td></td>
<td>Triglycerides</td>
<td>35–160 mg/dl</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>Glucose</td>
<td>70–110 mg/dL</td>
</tr>
<tr>
<td>Vitamins, cofactors, and enzymes</td>
<td>Vitamin B₁₂</td>
<td>200–800 pg/mL</td>
</tr>
<tr>
<td></td>
<td>Vitamin A</td>
<td>0.15–0.6 µg/mL</td>
</tr>
<tr>
<td></td>
<td>Vitamin C</td>
<td>0.4–1.5 mg/dL</td>
</tr>
<tr>
<td></td>
<td>3,3-Diphosphoglycerate (DPG)</td>
<td>3–4 mmol/L</td>
</tr>
<tr>
<td>Other substances</td>
<td>Transaminase (SGOT)</td>
<td>9–40 U/mL</td>
</tr>
<tr>
<td></td>
<td>Alkaline phosphatase</td>
<td>20–70 U/L</td>
</tr>
<tr>
<td></td>
<td>Acid phosphatase</td>
<td>0.5–2 U/L</td>
</tr>
<tr>
<td></td>
<td>Creatinine</td>
<td>0.6–1.2 mg/dL</td>
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<tr>
<td></td>
<td>Uric Acid</td>
<td>0.18–0.49 mmol/L</td>
</tr>
<tr>
<td></td>
<td>Blood urea nitrogen</td>
<td>7–18 mg/dL</td>
</tr>
<tr>
<td></td>
<td>Iodine</td>
<td>3.5–8.0 µg/dL</td>
</tr>
<tr>
<td></td>
<td>CO₂</td>
<td>23–30 mmol/L</td>
</tr>
<tr>
<td></td>
<td>Bilirubin (total)</td>
<td>0.1–1.0 mg/dL</td>
</tr>
<tr>
<td></td>
<td>Aldosterone</td>
<td>3–10 mg/dL</td>
</tr>
<tr>
<td></td>
<td>Cortisol</td>
<td>5–18 µg/dL</td>
</tr>
<tr>
<td></td>
<td>Ketones</td>
<td>0.2–0.2 mg/dL</td>
</tr>
</tbody>
</table>
Erythrocytes Carry Oxygen to Tissues

Erythrocytes are the most numerous cells in blood. These biconcave disks lack a nucleus and have a diameter of about 7 μm and a maximum thickness of 2.5 μm. The shape of the erythrocyte optimizes its surface area, increasing the efficiency of gas exchange.

The erythrocyte maintains its shape by virtue of its complex membrane skeleton, which consists of an insoluble mesh of fibrous proteins attached to the inside of the plasma membrane. This structural arrangement allows the erythrocyte great flexibility as the cell twists and turns through small, curved vessels. In addition to structural proteins of the membrane, several functional proteins are found in the cytoplasm of erythrocytes. These include hemoglobin (the major oxygen-carrying protein), antioxidant enzymes, and glycolytic systems to provide cellular energy (ATP). The plasma membrane possesses ion pumps that maintain a high level of intracellular potassium and a low level of intracellular calcium and sodium.

Hemoglobin, the red, oxygen-transporting protein of erythrocytes, consists of a globin (or protein) portion and four heme groups, the iron-carrying portion. The molecular weight of hemoglobin is about 64,500. This complex protein possesses four polypeptide chains: two α-globin molecules of 141 amino acids each and two molecules of another type of globin chain (β, γ, Δ, or ε), each containing 146 amino acid residues (Fig. 11.3).

Four types of hemoglobin molecules can be found in human erythrocytes: embryonic, fetal, and two different types found in adults (HbA, HbA2). Each hemoglobin molecule is designated by its polypeptide composition. For example, the most prevalent adult hemoglobin, HbA, consists of two α chains and two β chains. Its formula is given as α2β2. HbA2, which makes up about 1.5 to 3% of total hemoglobin in an adult, has the subunit formula α2Δ2. Fetal hemoglobin (α2γ2) is the major hemoglobin component during intrauterine life. Its levels in circulating blood cells decrease rapidly during infancy and reach a concentration of 0.5% in adults. Embryonic hemoglobin is found earlier in development. It consists of two α chains and two ε chains (α2ε2). The production of ε chains ceases at about the third month of fetal development.

The production of each type of globin chain is controlled by an individual structural gene with five different loci. Mutations, which can occur anywhere in these five loci, have resulted in the production of over 550 types of abnormal hemoglobin molecules, most of which have no known clinical significance. Mutations can arise from a single substitution within the nucleic acid of the gene coding for the globin chain, a deletion of the codons, or gene rearrangement as a result of unequal crossing over between homologous chromosomes. Sickle-cell anemia, for example, results from the presence of sickle-cell hemoglobin (HbS), which differs from normal adult hemoglobin A by...
cause of the substitution of a single amino acid in each of the two β chains.

Oxyhemoglobin (HbO₂), the oxygen-saturated form of hemoglobin, transports oxygen from the lungs to tissues, where the oxygen is released. When oxygen is released, HbO₂ becomes reduced hemoglobin (Hb). While oxygen-saturated hemoglobin is bright red, reduced hemoglobin is bluish-red, accounting for the difference in the color of blood in arteries and veins.

Certain chemicals readily block the oxygen-transporting function of hemoglobin. For example, carbon monoxide (CO) rapidly replaces oxygen in HbO₂, resulting in the formation of the stable compound carboxyhemoglobin (HbCO). The formation of HbCO accounts for the asphyxiating properties of CO. Nitrates and certain other chemicals oxidize the iron in Hb from the ferrous to the ferric state, resulting in the formation of methemoglobin (metHb). MetHb contains oxygen bound tightly to ferric iron; as such, it is useless in respiration. Cyanosis, the dark-blue coloration of skin associated with anoxia, becomes evident when the concentration of reduced hemoglobin exceeds 5 g/dL. Cyanosis may be rapidly reversed by oxygen if the condition is caused only by a diminished oxygen supply. However, cyanosis caused by the intestinal absorption of nitrates or other toxins, a condition known as enterogenous cyanosis, is due to the accumulation of stabilized methemoglobin and is not rapidly reversible by the administration of oxygen alone.

Normal Red Cell Values. In evaluating patients for hematological diseases, it is important to determine the hemoglobin concentration in the blood, the total number of circulating erythrocytes (the red cell count), and the hematocrit. From these values several other important blood values can be calculated, including mean cell hemoglobin concentration (MCHC), mean cell hemoglobin (MCH), mean cell volume (MCV), and blood oxygen carrying capacity.

The MCHC provides an index of the average hemoglobin content in the mass of circulating red cells. It is calculated as follows:

\[
MCHC = \frac{Hb (g/L)}{hematocrit} \tag{1}
\]

Example: 150 g/L ÷ 0.45 = 333 g/L

Low MCHC values indicate deficient hemoglobin synthesis. High MCHC values do not occur in erythrocyte disorders, because normally the hemoglobin concentration is close to the saturation point in red cells. Note that the MCHC value is easily obtained by a simple calculation from measurements that can be made without sophisticated instrumentation.

The MCH value is an estimate of the average hemoglobin content of each red cell. It is derived as follows:

\[
MCH = \frac{\text{Blood hemoglobin} (g/L)}{\text{Red cell count} (\text{cells/L})} \tag{2}
\]

Example: 150 g/L ÷ (5 × 10¹² cells/L) = 30 × 10⁻¹² g/cell = 30 pg/cell

Since the red cell count is usually related to the hematocrit, the MCH is usually low when the MCHC is low. Exceptions to this rule yield important diagnostic clues.
normal erythrocytes are termed macrocytes; smaller-than-normal erythrocytes are referred to as microcytes. Poikilo- 
cytosis is the presence of irregularly shaped erythrocytes. Burr 
cells are spiked erythrocytes generated by alterations in the plasma environment. Schistocytes are fragments of 
red cells damaged during blood flow through abnormal 
blood vessels or cardiac prostheses.

The hemoglobin content of erythrocytes is also re-
lected in the staining pattern of cells on dried films. Nor-
mal cells appear red-orange throughout, with a very slight 
central pallor as a result of the cell shape. Hypochromic 
cells appear pale with only a ring of deeply colored hemo-
globin on the periphery. Other pathological variations in 
red cell appearance include spherocytes—small, densely 
staining red cells with loss of biconcavity as a result of con-
genital or acquired cell membrane abnormalities; and tar-
gent cells—which have a densely staining central area with 
a pale surrounding area. Target cells are thin but bulge in 
the middle, unlike normal erythrocytes. This alteration is a 
consequence of hemoglobinopathies, mutations in the 
structure of hemoglobin. Target cells are observed in liver 
disease and after splenectomy.

Nucleated red cells are normally not seen in peripheral 
blood because their nuclei are lost before they move from 
the bone marrow into the blood. However, they appear in 
many blood and marrow disorders, and their presence can 
be of diagnostic significance. One type of nucleated red 
cell, the normoblast (see Fig. 11.4), is seen in several types of 
anemias, especially when the marrow is actively re-
sponding to demand for new erythrocytes. In seriously ill 
patients, the appearance of normoblasts in peripheral blood 
is a grave prognostic sign preceding death, often by several 
hours. Another nucleated erythrocyte, the megaloblast, is 
seen in peripheral blood in pernicious anemia and folic acid 
deficiency.

Erythrocyte Destruction. Red cells circulate for about 
120 days after they are released from the marrow. Some of 
the senescent (old) red cells break up (hemolyze) in the 
bloodstream, but the majority are engulfed by macrophages in the monocyte-macrophage system. The hemoglobin released on destruction of red cells is metabolically catabolized and eventually reused in the synthesis of new hemoglobin. Hemoglobin released by red cells that 
lyse in the circulation either binds to haptoglobin, a pro-
tein in plasma, or is broken down to globin and heme. 
Heme binds a second plasma carrier protein, hemopexin, 
which, like haptoglobin, is cleared from the circulation by 
macrophages in the liver. In the macrophage, released he-
moglobin is first broken into globin and heme. The globin
portion is catabolized by proteases into constituent amino acids that are used in protein synthesis. Heme is broken down into free iron (Fe$^{3+}$) and biliverdin, a green substance that is further reduced to bilirubin (see Chapter 27).

**Iron Recycling.** Most of the iron needed for new hemoglobin synthesis is obtained from the heme of senescent red cells. Iron released by macrophages is transported in the ferric state in plasma bound to the iron transporting protein, transferrin. Cells that need iron (e.g., for heme synthesis) possess membrane receptors to which transferrin binds. The receptor-bound transferrin is then internalized. The iron is released, reduced intracellularly to the ferrous state, and either incorporated into heme or stored as ferritin, a complex of protein and ferrous hydroxide. Iron is also stored as ferritin by macrophages in the liver. A portion of the ferritin is catabolized to hemosiderin, an insoluble compound consisting of crystalline aggregates of ferritin. The accumulation of large amounts of hemosiderin formed during periods of massive hemolysis can result in damage to vital organs, including the heart, pancreas, and liver.

The recycling of iron is quite efficient, but small amounts are continuously lost. Iron losses are substantial in women during menstruation. Iron stores must be replenished by dietary uptake. The majority of iron in the diet is derived from heme in meat (“organic iron”), but iron can also be provided by the absorption of inorganic iron by intestinal epithelial cells. In these cells, iron attached to heme is released and reduced to the ferrous form (Fe$^{2+}$) by intracellular flavoprotein. The reduced iron (both released from heme and absorbed as the inorganic iron) is transported through the cytoplasm bound to a transferrin-like protein. When it is released to the plasma, it is oxidized to the ferric state and bound to transferrin for use in heme synthesis.

**Platelets Participate in Clotting**
Platelets are irregularly shaped, disk-like fragments of the membrane of their precursor cell, the megakaryocyte. Megakaryocytes shed platelets in the bone marrow sinusoids. From there the platelets are released to the blood, where they function in hemostasis. Several factors stimulate megakaryocytes to release platelets, including the hormone thrombopoietin, which is generated and released into the bloodstream when the number of circulating platelets drops. Platelets have no defined nucleus. They are one fourth to one third the size of erythrocytes. Platelets possess physiologically important proteins, stored in intracellular granules, which are secreted when the platelets are activated during coagulation. The role of platelets in blood clotting is discussed below.

**Leukocytes Participate in Host Defense**
Each of the three general types of leukocytes—myeloid, lymphoid, and monocytic—follows a separate line of development from primitive cells (see Fig. 11.2). Mature cells of the myeloid series are termed granulocytes, based on their appearance after staining with polychromatic dyes, such as Wright's stain. While monocytes and lymphocytes may also possess cytoplasmic granules, they are not clearly visualized with commonly used stains. Therefore, monocytes and lymphocytes are often referred to as agranular leukocytes.

The nuclei of most mature granulocytes are divided into two to five oval lobes connected by thin strands of chromatin. This nuclear separation imparts a multinuclear appearance to granulocytes, which are, therefore, also known as polymorphonuclear leukocytes. Three distinct types of granulocytes have been identified based on staining reactions of their cytoplasm with polychromatic dyes: neutrophils, eosinophils, and basophils.

**Neutrophils.** Neutrophils are usually the most prevalent leukocyte in peripheral blood. These dynamic cells respond instantly to microbial invasion by detecting foreign proteins or changes in host defense network proteins. Neutrophils provide an efficient defense against pathogens that have gotten past physical barriers such as the skin. Defects in neutrophil function quickly lead to massive infection—and, quite often, death.

Neutrophils are amoeba-like phagocytic cells. Invading bacteria induce neutrophil chemotaxis—migration to the site of infection. Chemotaxis is initiated by the release of chemotactic factors from the bacteria or by chemotactic factor generation in the blood plasma or tissues. Chemotactic factors are generated when bacteria or their products bind to circulating antibodies, by tissue cells when infected with bacteria, and by lymphocytes and platelets after interaction with bacteria.

After neutrophils migrate to the site of infection, they engulf the invading pathogen by the process of phagocytosis. Phagocytosis is facilitated when the bacteria are coated with the host defense proteins known as opsonins.

A burst of metabolic events occurs in the neutrophil after phagocytosis (Fig. 11.6). In the phagocytic vacuole or phagosome, the bacterium is exposed to enzymes that were originally positioned on the cell surface. Thus, phagocytosis involves invagination and then vacuolization of the segment of membrane to which a pathogen is bound. Membrane-bound enzymes; activated when the phagocytic vacuole closes, work in conjunction with enzymes secreted from intracellular granules into the phagocytic vacuole to destroy the invading pathogen efficiently. One important membrane-bound enzyme, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, produces superoxide anion (O$_2^-$). Superoxide is an unstable free radical that kills bacteria directly. Superoxide also participates in secondary free radical reactions to generate other potent antimicrobial agents, such as hydrogen peroxide. Superoxide generation in the phagocytic vacuole proceeds at the expense of reducing agents oxidized in the cytoplasm. The reducing agent, NADPH, is generated from glucose by the activity of the hexose monophosphate shunt. Aerobic cells generate reduced nicotinamide adenine dinucleotide (NADH) and ATP when glucose is oxidized to carbon dioxide. The hexose monophosphate shunt operates in neutrophils and other cells when large
amounts of NADPH are needed to maintain intracellular reducing activity.

Oxygen reduction by the NADPH oxidase that generates superoxide in neutrophils is driven by an increased availability of NADPH after phagocytosis:

$$\text{NADPH} + 2\text{O}_2 \rightarrow 2\text{O}_2^- + \text{NADP}^+ + \text{H}^+ \quad (4)$$

A complex set of biochemical events unfolds after phagocytosis to activate the neutrophil NADPH oxidase, which is dormant in resting cells. The oxidase is activated by its interaction with an activated G protein and cytosolic molecules that are generated during phagocytosis. The NADPH oxidase is activated in a manner that allows the enzyme to secrete the toxic free radical, superoxide, into the phagocytic vacuole while oxidizing NADPH in the cell’s cytoplasm. This explosion of metabolic activity, collectively termed the respiratory burst, leads to the generation of potent, reactive agents not otherwise generated in biological systems. These agents are so reactive that they actually generate light (biological chemiluminescence) when they oxidize components in the bacterial cell wall.

Other bactericidal agents and processes operate in neutrophils to ensure efficient bacterial killing. Phagocytized bacteria encounter intracellular defensins, cationic proteins that bind to and inhibit the replication of bacteria. Defensins and other antibacterial agents pour into the phagocytic vacuole after phagocytosis. Agents stored in neutrophil granules include lysozyme, a bacteriolytic enzyme, and myeloperoxidase, which reacts with hydrogen peroxide to generate potent, bacteria-killing oxidants. One of the oxidants generated by the myeloperoxidase reaction is hypochlorous acid (HOCl), the killing agent found in household bleach. Granules also contain collagenase and other proteases.

**Eosinophils.** Eosinophils are rare in the circulation but are easily identified on stained blood films. As the name implies, the eosinophil takes on a deep eosin color during polychromatric staining; the large, refractile cytoplasmic granules of these cells stain orange-red to bright yellow. Like neutrophils, eosinophils migrate to sites where they are needed and exhibit a metabolic burst when activated. Eosinophils participate in defense against certain parasites, and they are involved in allergic reactions. The exposure of allergic individuals to an allergen often results in a transient increase in eosinophil count known as eosinophilia. Infection with parasites often results in a sustained overproduction of eosinophils.

**Basophils.** Basophils are polymorphonuclear leukocytes with multiple pleomorphic, coarse, deep-staining metachromatic granules throughout their cytoplasm. These granules contain heparin and histamine, which have anticoagulant and vasodilating properties, respectively. The release of these and other mediators by basophils increases regional blood flow, facilitating the transport of other leukocytes to areas of infection and allergic reactivity or other forms of hypersensitivity.

**Monocytes and Lymphocytes.** In contrast to granulocytes, monocytes and lymphocytes are mononuclear cells. Monocytes are phagocytic cells but lymphocytes are not; both participate in multiple aspects of immunity. Monocytes were originally differentiated from lymphocytes based on morphological characteristics. The cytoplasm of monocytes appears pale blue or blue-gray with Wright’s stain. The cytoplasm contains multiple fine reddish-blue granules. The monocyte nucleus may be shaped like a kidney bean, indented, or shaped like a horseshoe. Frequently, however, it is rounded or ovoid. Upon activation, monocytes transform into macrophages—large, active mononuclear phagocytes.

Morphologically, circulating lymphocytes have been assigned to two broad categories: large and small lymphocytes. In blood, small lymphocytes are more numerous than larger ones; the latter closely resemble monocytes. Small lymphocytes possess a deeply stained, coarse nucleus that is large in relation to the remainder of the
activities ranging from tumor destruction, a function of the immune response. Cytokine subtype diversity and potency of the cytokines has opened the door to the development of a variety of new pharmacological agents that have proved useful in the treatment of cancer, immune disorders, and other diseases.

**Blood Cells Are Born in the Bone Marrow**

Mature cells are transient residents of blood. Erythrocytes survive in the circulation for about 120 days, after which they are broken down and their components recycled, as discussed above. Platelets have an average lifespan of 15 to 45 days in the circulation, many, if not most, of these cells are consumed as they continuously participate in day-to-day hemostasis. The rate of platelet consumption accelerates rapidly during the repair of bleeding caused by trauma. Leukocytes have a variable lifespan. Some lymphocytes circulate for 1 year or longer after production. Neutrophils, constantly guarding body fluids and tissues against infection, have a circulating half-life of only a few hours. Neutrophils and other blood cells must, therefore, be continuously replenished.

As mentioned earlier, the process of blood cell generation, hematopoiesis, occurs in healthy adults only in the bone marrow. Extramedullary hematopoiesis (e.g., the generation of blood cells in the spleen) is observed only in some disease states, such as leukemia. Hematopoietic cells are found in high levels in the liver, spleen, and blood of the developing fetus. Shortly before birth, blood cell production gradually begins to shift to the marrow. In newborns, the hematopoietic cell content of the circulating blood is relatively high; hematopoietic cells are also found in the blood of adults, but in extremely low numbers. Large numbers of hematopoietic cells can be recovered from aspirates of the iliac crest, sternum, pelvic bones, long bones, and ribs of adults. Within the bones, hematopoietic cells germinate in extravascular sinuses, called marrow stroma. Circulating factors and factors released from capillary endothelial cells, stromal fibroblasts, and mature blood cells regulate the generation of immature blood cells from hematopoietic cells and the subsequent differentiation of newly formed immature cells.

Blood cell production begins with the proliferation of pluripotent (uncommitted) stem cells. Depending on the stimulating factors, the progeny of pluripotent stem cells may be other uncommitted stem cells or stem cells committed to development along a certain lineage. The committed stem cells include myeloblasts, which form cells of the myeloid series (neutrophils, basophils, and eosinophils), erythroblasts, lymphoblasts, and monoblasts (Fig. 11.7; see also Fig. 11.2). Promoted by hematopoietins and other cytokines, each of these blast cells differentiates further, a process that ultimately results in the formation of mature blood cells. This is a dynamic process; the hematopoietic cells of the bone marrow are among the most actively reproducing cells of the body. Interruption of hematopoiesis (e.g., by cancer treatment) results in the eventual disappearance of granulocytes from the blood, a condition known as granulocytopenia, or, when specific to neutrophils, neutropenia, in a matter of hours. Platelets disappear next—thrombocytopenia—followed by erythrocytes, a sequence that reflects...
Hematopoiesis. All circulating blood cells are believed to be derived from a common, uncommitted bone marrow progenitor, the pluripotent stem cell. This cell differentiates along different lineages, depending on the conditions it encounters and the levels of individual hematopoietins available. CFU-GEMM, granulocyte-erythrocyte-macrophage-megakaryocyte colony-forming unit; CFU-GM, granulocyte-macrophage colony-forming unit; BFU-E, erythroid burst forming unit; CFU-MEG, megakaryocyte colony-forming unit; CFU-M, macrophage colony-forming unit; CFU-G, granulocyte colony-forming unit; CFU-Eo, eosinophil colony-forming unit; CFU-Bas, basophil colony-forming unit.

The Innate Immune System Consists of Nonspecific Defenses

Infectious agents cannot easily penetrate intact skin, the first line of defense against infection. Infection is a major complication when the intact skin barrier is compromised, such as by burns or trauma. Even a small needle prick can result in a fatal infection.

Natural openings to body cavities and glands are an effective entry point of infectious agents. Usually, however, these openings are protected from invasion by pathogens in at least two ways. First, they are coated with mucus and other secretions that contain secretory immunoglobulins as well as antibacterial enzymes, such as lysozyme. Second, organisms that invade these openings cannot easily reach the blood but, instead, lodge in an organ that communicates with both the exterior and the interior of the body, such as a lung or the stomach. Many pathogens cannot survive the low pH of stomach acid. In the lungs, organisms face the efficient phagocytic activity of alveolar macrophages. These cells, derived from blood monocytes, are mobile but confined to the pulmonary capillary net-
delivery of immune system elements to the site. The result is the contact invaders in a tissue. The response results in increased proliferation triggered by circulating proteins and blood cells when they confront invasion by pathogens. The inflammatory response is initiated by inflammation providing a multifaceted defense against tissue injury.

**Inflammation Is a Multifaceted Process**

Microbes that successfully break through these physical barriers face destruction by the fixed macrophages of the monocyte-macrophage system. These cells line the sinusoids and vasculature of many organs, including the liver, spleen, and bone marrow. The nonmobile, fixed phagocytic macrophages efficiently remove foreign particles, including bacteria, from the circulation.

**Bone Marrow Transplantation**

When a patient has a terminal bone marrow disease, such as leukemia or aplastic anemia, often the only possibility for a cure is a bone marrow transplant. In this procedure, healthy bone marrow cells are used to replace the patient’s diseased hematopoietic system. These cells are obtained from a donor who is usually a close relative of the patient. To identify a suitable donor, relatives’ blood leukocytes are screened to determine whether their antigenic pattern matches that of the patient. The antigenic composition of leukocytes in bone marrow and peripheral blood are identical, so analysis of blood leukocytes usually provides enough information to determine whether the transplanted cells will engraft successfully. If significantly different from the recipient’s tissue type, transplanted leukocytes may be recognized as foreign by the patient’s immune system and, therefore, rejected.

More commonly, sufficient differences between the engrafted cells and the host’s own tissue lead to debilitating consequences as a result of graft-versus-host disease (GVHD). In GVHD, functional T cells in the proliferating graft recognize host tissue as foreign and mount an immune response. The disease often begins with a skin rash, as transplanted lymphocytes invade the dermis, and ends in death as lymphocytes destroy every organ system in the marrow recipient.

Recent discoveries have led to useful ways to limit or prevent GVHD. These advances have decreased the morbidity of marrow transplants and have substantially increased the potential pool of bone marrow donors for a given patient. Immunosuppressive agents, including steroids, cyclosporin, and anti-T cell antiserum, effectively decrease the immune function of the transplanted lymphocytes. Another useful approach involves “purging”—the physical removal of T cells from bone marrow prior to transplantation. T cell-depleted bone marrow is much less capable of causing acute GVHD than untreated marrow. These techniques have enabled the successful transplantation of bone marrow obtained from unrelated donors. Unrelated transplants were never possible before these advances because GVHD would almost certainly develop, even when the antigenic type of the donor’s leukocytes closely matched that of the recipient’s. Thus, many patients died for lack of a related donor. Today, transplants of unrelated marrow are common.

Many problems remain, however. One of the most serious, and the most common, is donor identification. An unrelated transplant is successful only if the donor’s leukocyte antigens closely match those of the recipient. Since there are several antigenic determinants and each can be occupied by any one of several genes, there are thousands of possible combinations of leukocyte antigens. The chance that any individual’s cells will randomly match those of another is less than one in a million. Therefore, the identification of a suitable donor is a little more complicated than finding a needle in a haystack. On the other hand, these odds virtually guarantee that suitable donors are not only available but, in all probability, plentiful in the general population. Finding them is a formidable problem that often generates intense frustration when donors for terminally ill transplant candidates are not quickly identified.

To address this problem, bone marrow transplant registries have been established. In these registries, the results of extensive leukocyte antigen typing are stored in a computer. Typing is performed on leukocytes isolated from a small sample of blood, so the procedure does not significantly inconvenience prospective donors. For some registries, potential donors of a specific ethnic background are targeted; in others, blood samples are obtained from as many healthy individuals as possible, regardless of their heritage. The database is searched when an individual in need of a transplant cannot identify a suitable relative. In conjunction with continued development of methods to reduce or eliminate GVHD, the expanding bone marrow transplant registries may someday allow identification of a donor for anyone who needs a bone marrow transplant.

**CLINICAL FOCUS BOX 11.1**

Bone Marrow Transplantation

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work. As efficient phagocytic cells, they continuously patrol the pulmonary vasculature to remove inhaled microbes. Microbes that successfully break through these physical barriers face destruction by the fixed macrophages of the monocyte-macrophage system. These cells line the sinusoids and vasculature of many organs, including the liver, spleen, and bone marrow. The nonmobile, fixed phagocytic macrophages efficiently remove foreign particles, including bacteria, from the circulation.

**Inflammation Is a Multifaceted Process**

Microbial invaders that lodge in body tissues and begin to proliferate trigger an inflammatory response (Fig. 11.8). Inflammation provides a multifaceted defense against tissue invasion by pathogens. The inflammatory response is initiated by circulating proteins and blood cells when they contact invaders in a tissue. The response results in increased blood flow to the affected tissues, which accelerates the delivery of immune system elements to the site. The result is redness, heat, and swelling (edema) of the affected tissue. Blood cells participating in the inflammatory response release a variety of inflammatory mediators that perpetuate the response. If the pathogens persist, the inflammatory response may become chronic and may itself cause substantial tissue damage. Not only microbes, but also proteins, chemicals, and toxins the body recognized as foreign, can induce an inflammatory response.

Certain inflammatory mediators increase blood flow to the inflamed area. Other mediators increase capillary permeability, allowing diffusion of large molecules across the endothelium and into the infected site. These molecules may be plasma proteins, or they may be generated by plasma proteins or substances released by blood leukocytes. They often play important roles in eliminating the pathogenic agent or enhancing the inflammatory response. Finally, chemotactic factors produced by cells that arrived early in the inflammatory cascade cause polymorphonuclear leukocytes to migrate from the blood to the affected area. Neutrophils are an important participant in the in-
Hematotherapy and Stem Cell Research: Clinical Tools of the Future

Many diseases result from a specific defect in the immune or hematopoietic system. These diseases may be effectively treated by infusion of specific precursors of the defective cells, a process termed hematotherapy. In a typical bone marrow transplant, the entire hematopoietic system (and, consequently, the immune system) of the recipient is ablated and restored with cells from the donor. In this situation, the most primitive stem cells of the immune or hematopoietic system are eliminated and replaced. In situations such as AIDS, thrombocytopenia, certain anemias, and genetic immunodeficiency, however, only specific committed progenitor cells of the hematopoietic or immune system are affected. We may soon be able to replace these and keep the healthy portion of the patient’s hematopoietic system intact.

In recent years, much interest has focused on the isolation, identification, and propagation of the stem cells of various tissues. Hematopoietic stem cells have recently been grown in culture and may soon be used for therapeutic purposes. Hematopoietic stem cells are either committed or pluripotent. As such, they either are destined to generate a specific lineage of cells or are capable of generating further developed stem cells that can commit to development along any one of several lineages. Pluripotent stem cells are needed to reconstitute hematopoiesis after the complete disruption that occurs during whole-body irradiation or after the infusion of chemotherapy agents to treat leukemia and solid tumors.

Committed stem cells may be used for specific defects. For example, in AIDS, virus-laden T cells are rapidly eliminated, resulting in low circulating levels. Although pharmaceutical progress has resulted in extended survival for these patients, they are at high risk for life-threatening infection resulting from low T cell levels. It may be possible to support patients by periodic infusions of T cell precursors, generated in efficient bioreactors from the patient’s own primitive stem cells. These bioreactors would be fueled by specific cytokines that direct the stem cells to specifically generate committed T cell progenitors. Stem cells used to initiate the culture would be obtained from the patient’s marrow and grown under virus-free conditions. After sufficient T cell progenitors were generated, the cultures would be processed to isolate and concentrate the cells. Patients would receive an infusion whenever their T cell counts plummeted, protecting them against infection and allowing sustained survival.

In addition to AIDS, hematotherapy holds promise for several other diseases and conditions as well. Infusions of neutrophil progenitors may be useful for cancer patients during aggressive therapy. Red cell progenitors may be successfully cultivated and infused for those with certain anemias. Platelet progenitors may be used in patients with one of the many forms of inborn or acquired thrombocytopenia. In addition, this emerging therapeutic approach may soon be enhanced by genetic engineering. In this process, newly or modified genes are inserted into the growing stem cells to replace defective or missing ones. For example, a patient may be unable to mount an appropriate immune response because of the lack of a specific enzyme secreted by healthy leukocytes. Stem cells of these patients may be modified in culture to eliminate this defect and infused back into the patient. If the infused cells take hold and generate sufficient progeny, the patient’s immune defect may be reversed, resulting in a cure of a once fatal disease.

By far, the major clinical use of stem cells to date has been to restore the hematopoietic system of patients treated with radiation or chemotherapy for cancer. Less frequently, hematopoietic stem cells have been used to augment the defective immune system of patients born with genetic defects. New uses of stem cells appear to be on the horizon. In recent years, several groups have announced the successful isolation and culture of primitive, nonhematopoietic stem cells from human embryos and fetal tissue. In addition, current reports indicate that these primitive stem cells, cells that can be induced to differentiate into any type of cell in the body, can be successfully isolated from adult tissues, including tissues that would otherwise be discarded, such as fat obtained during liposuction. Stem cells could be potentially used for the regeneration and reconstruction of all types of damaged tissues.

References
Noble M. Can neural stem cells be used to track down and destroy migratory brain tumor cells while also providing a means of repairing tumor-associated damage? Proc Natl Acad Sci U S A 2000;97:12393–12395.

Inflammatory response. They can exert potent antimicrobial effects, as well as release a variety of agents that can further amplify and perpetuate the response.

The remarkable ability of the inflammatory response to sustain itself while it generates potent cytolytic agents can result in many undesirable effects, including extensive tissue damage and pain. A variety of antinflammatory agents control some of these undesirable effects. These agents are designed to block some of the consequences of the inflammatory response without compromising its antimicrobial efficiency. They do this by neutralizing inflammatory mediators or by preventing inflammatory cells from releasing or responding to inflammatory mediators.

Defensive Mechanisms Are Integrated Systems

As discussed above, the innate and adaptive immune systems work together in ways that obscure their differences.
Indeed, consideration of these two systems as distinct, individual entities is neither justified nor correct, owing to their extensive interdependence. They are described individually only as an aid to their presentation. In this respect, it is important to define the characteristics that differentiate each system (Table 11.3). In general, responses of the innate immune system are neither specific nor inducible, that is, the response is not programmed by or directed against a specific pathogen and is not amplified as a result of previous encounters with the pathogen. The adaptive response, in contrast, is both specific and inducible; the response is set in motion by a particular pathogen and develops against that specific pathogen.

### TABLE 11.3 Characteristics of the Innate and Adaptive Immune Systems

<table>
<thead>
<tr>
<th></th>
<th>Innate</th>
<th>Adaptive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistance</td>
<td>Not improved by repeat infection</td>
<td>Improved by previous infection</td>
</tr>
<tr>
<td>Specificity</td>
<td>Not directed toward specific pathogen</td>
<td>Targeted response directed by specific</td>
</tr>
<tr>
<td></td>
<td></td>
<td>elements of immune system</td>
</tr>
<tr>
<td>Soluble factors</td>
<td>Lysozyme, complement, acute phase proteins,</td>
<td>Antibodies</td>
</tr>
<tr>
<td></td>
<td>interferon, cytokines</td>
<td></td>
</tr>
<tr>
<td>Cells</td>
<td>Phagocytic leukocytes, NK Cells</td>
<td>T cells, B cells</td>
</tr>
</tbody>
</table>

While characteristics of the innate and adaptive immune system differ, each system depends on elements of the other for optimal functioning. The initiation of responses by the innate system, as well as efficient phagocytosis by neutrophils in the tissues, often depends on the presence of a small amount of specific antibody in blood plasma. Antibody is generated by cells of the adaptive immune system in response to specific foreign molecules called antigens. In turn, the effective functioning of antibodies and other mediators of the adaptive immune system depends on neutrophils and other effector agents usually associated with the innate immune system. Thus, the innate and the adaptive systems depend on highly evolved, interactive, defensive mechanisms to kill and remove microbial intruders.

### Adaptive Immunity Is Specific and Acquired

The adaptive immune system can be considered at three levels:
- The **afferent arm**, which gives the system its remarkable ability to recognize specific antigenic determinants of a wide range of infectious agents
- The **efferent arm**, which supplies a cellular and molecular assault on the invading pathogens
- **Immunological memory**, which specifically accelerates and potentiates subsequent responses to the same activating agent or antigen

The specificity of the recognition, effector, and memory aspects of the adaptive immune system derives from the specificity of antibody molecules as well as that of receptors on T cells and B cells. The lymphocytes of the immune system are capable of recognizing and specifically responding to hundreds of thousands of potential antigens, which may be presented, for example, as glycoproteins on the surface of bacteria, the coat protein of viruses, microbial toxins, or membranes of infected cells. Only a few circulating lymphocytes need to recognize an individual antigen initially. This initial recognition induces proliferation of the responsive cell, a process known as **clonal selection** (Fig. 11.9). Clonal selection amplifies the number of specific T cells or B cells (i.e., T or B lymphocytes programmed to respond to the inciting stimulus).

While all of the cells generated after a single clone has expanded are specific for the inducing antigen, they may not all possess the same functional characteristics. Some of the daughter lymphocytes may be effector cells. For example, when B cells are activated, their progeny **plasma cells** are capable of generating antibodies. Other progeny in the expanded clone may play an afferent recognition role and, thereby, function as **memory cells**. The increased number of these cells, which mimic the reactive specificity of the original lymphocytes that responded to the antigen, accelerate responsiveness when the antigen is encountered again. Memory cells thus account for one of the primary tenets of immunity: Resistance is increased after initial exposure to the infectious agent. Long-term immunity to many viruses—such as influenza, measles, smallpox, and polio—can be induced by **vaccination** with a killed or mutant form of the pathogen.
The Adaptive Immune Response Involves Cellular and Humoral Components

Depending on the nature of the stimulus, its mode of presentation, and prior challenges to the immune system, an antigen may elicit either a cellular or humoral immune response. Both are ultimately mediated by lymphocytes, the cellular response by T cells and humoral response by B cells. As discussed above, stimulated B cells differentiate into plasma cells, which secrete antibody specific for the inciting stimulus. Cells then proliferate and differentiate into either memory cells, which potentiate subsequent responses to the inciting antigen, or plasma cells, which secrete antibody.

Cell-Mediated Immunity. Cell-mediated immunity (or cellular immunity) is accomplished by activated T cells. The effector cells of this response do not secrete antibody but exert their influence by a variety of cellular mechanisms. These effector processes include direct cytotoxicity mediated by cytotoxic T cells, the suppression or activation of immune mechanisms in other cells—suppressor T cells or helper T cells, respectively; and the secretion of cytotoxic or immunomodulating cytokines, such as tumor necrosis factor and interleukin-2. T cells and their products may act directly or exert their effects in concert with other effector cells, such as neutrophils and macrophages.

The immune responses mediated by antibodies and T lymphocytes differ in several important respects. In general, antibodies are known to induce immediate responses to antigens and, thereby, provoke immediate hypersensitivity reactions. For example, allergy or anaphylactic hypersensitivity results when a certain type of antibody on the surface of fixed mast cells binds to its specific antigen. Antibody binding leads to the release of histamine and other mediators of the allergic response from intracellular granules.

Immediate hypersensitivity reactions also occur when circulating antibodies bind antigen in the tissues, thereby forming immune complexes that activate the complement system, a group of at least nine distinct proteins that circulate in plasma. A cascade of events occurs when the first protein recognizes preformed immune complexes, a large cross-linked mesh of antigen molecules bound to antibodies. In addition, complement can be activated when one of the proteins is exposed to the cell wall of certain bacteria. Initiation of this system results in edema, an influx of activated phagocytic cells (chemotaxis), and local inflammatory changes.

In contrast to the rapid onset of biological responses when antigen binds antibody, the consequences of T cell activation are not noticeable until 24 to 48 hours after antigen challenge. During this time, the T cells that initially recognize the antigen secrete factors that recruit and activate other cells (e.g., macrophages) and release factors that damage the antigen, cells possessing the antigen, or the surrounding tissue. A common example is the delayed-type hypersensitivity reaction to purified protein derivative (PPD), a response used to assess prior exposure to the bacteria that cause tuberculosis. Injected under the skin of sensitive individuals, PPD elicits the familiar inflammatory reaction characterized by local erythema and edema 1 to 2 days later.

Cell-mediated immune responses, while slow to develop, are potent and versatile. These delayed responses provide for defense against many pathogens, including viruses, fungi, and bacteria. T cells are responsible for the rejection of transplanted tissue grafts and containment of the growth of neoplastic cells. A deficiency in T cell immunity, such as that associated with AIDS, predisposes the affected patient to a wide array of serious, life-threatening infections.

Humoral Immunity. Humoral immunity consists of defense mechanisms carried out by soluble mediators in the blood plasma. Antibodies (also called immunoglobulins) are glycoproteins secreted by plasma cells. Antibodies are found in high levels in plasma and other body fluids. They have the ability to bind specifically to the antigenic determinant that induced their secretion.

Antibodies Bind Antigens

The primary structure of an antibody is illustrated in Figure 11.10. Each antibody molecule consists of four polypeptide...
chains (two heavy chains and two light chains) held together as a Y-shaped molecule by disulfide bridges. Each heavy chain and light chain possesses a constant region (where the amino acid sequence of individual molecules is similar) and a variable region, where alterations in the amino acid sequence convey to the antibody its individual antigen specificity.

The amino terminal portions of the variable regions, the antigen-binding sites, are known as the Fab regions. Each antibody unit possesses two identical antigen-binding sites, one at each end of the “Y.” The carboxy terminal end of the heavy chain is termed the Fc region. Polypeptide fragments consisting of Fc and Fab regions of antibody molecules can be generated by protease digestion and separated by chromatography. Fc fragments can bind to cells such as neutrophils, monocytes, and mast cells through their Fc receptors. Fc receptor binding amplifies the biological activity of antigen-bound antibody. In addition to the ability to bind antigen, the antibody molecule may have a variety of other important biological functions, depending on its class.

Table 11.4 summarizes some characteristics and functions of the five major classes of antibodies; these classes are grouped based on differences in the amino acid composition of the constant region of the heavy chains. IgG is the most prevalent antibody in serum and is responsible for adaptive immunity to bacteria and other microorganisms. Bound to antigen, IgG can activate serum complement, which releases several inflammatory and bactericidal mediators. At the surface of bacteria, exposed Fc portions of IgG molecules facilitate the phagocytosis of bacteria by blood phagocytes, a process called opsonization. IgG exists in serum as a monomer. It can cross the placenta and is secreted into colostrum, protecting the fetus as well as the newborn from infection.

Unlike IgG, both IgM and IgA usually exist as polymers of the fundamental Y-shaped antibody unit. In most IgA molecules, two antibody units are held together by a secretory piece (J chain), a protein synthesized by epithelial cells. In this conformation, IgA is actively secreted into saliva, tears, colostrum, and mucus. IgA is thus known as secretory immunoglobulin. IgM is the first antibody secreted after an initial immune challenge and provides resistance early in the course of infection. IgM consists of five Y units. Its size and large number of antigen-binding sites provide the molecule with an excellent capacity for agglutination, the ability to clump particulate antigens, such as bacteria and blood cells. Clumped antigens are efficiently and quickly removed by fixed phagocytic cells of the monocyte-macrophage system.

IgE, a monomeric antibody slightly larger than IgG, avidly binds cells that store and release mediators of allergy and anaphylaxis, including mast cells and basophils. These cells are heavily granulated. The granules contain histamine, leukotrienes, and other biologically active agents that increase vascular permeability, dilate blood vessels (and, thereby, reduce blood pressure), and contract smooth muscle cells in lung airways. The granules are released when IgE, bound to mast cells at the Fc region, binds its specific anti-
HEMOSTASIS

Circulating in a high-pressure, closed system that communicates with all tissues and cells in the body, blood exchanges oxygen, nutrients, and wastes and provides necessary components for host defense. This communication takes place largely in the complex and dynamic networks of capillary beds that provide oxygen to almost every cell in the body (only the cornea and intervertebral disks are avascular, these tissues receive oxygen by diffusion). Disruption of the integrity of the fragile capillaries may result from minor tissue injury associated with normal physical activity or from massive tissue trauma as a result of serious injury or infection, and may quickly lead to death. Any opening in the vascular network may lead to massive bruising or blood loss if left unrepaired.

To minimize bleeding and prevent blood loss after tissue injury, components of the hemostatic system are activated. The components of this dynamic, integrated system include blood platelets, endothelial cells, and plasma coagulation factors. They may be activated on exposure to foreign surfaces during bleeding, or by torn tissue at the site of injury, or by products released from the interior of damaged cells. Hemostasis can be viewed as four separate but interrelated events:

- Compression and vasoconstriction, which act immediately to stop the flow of blood
- Formation of a platelet plug
- Blood coagulation
- Clot retraction

Physical Factors Immediately Act to Constrain Bleeding

Immediately after tissue injury, blood flow through the disrupted vessel is slowed by the interplay of several important physical factors, including compression or back-pressure exerted by the tissue around the injured area, and vasoconstriction. The degree of compression varies in different tissues; for example, bleeding below the eye is not readily deterred because the skin in this area is easily distensible. Back-pressure increases as blood which leaks out of the disrupted capillaries accumulates. In some tissues, notably the uterus after childbirth, contraction of underlying muscles compresses blood vessels supplying the tissue and minimizes blood loss. Damaged cells at the site of tissue injury release potent substances that directly cause blood vessels to constrict, including serotonin, thromboxane A₂, epinephrine, and fibrinopeptide B.

Platelets Form a Hemostatic Plug

Platelets regulate bleeding in three stages. First, they form multicellular aggregates linked by protein strands at sites of openings in blood vessels. The aggregates form a physical barrier that begins to limit blood loss soon after the opening occurs. Second, phospholipids on the platelet plasma membrane activate the enzyme thrombin, which initiates a cascade of events ending in clot formation. Finally, platelets possess multiple storage granules, which they discharge (secrete) to enhance coagulation.

Platelet activation results in the sequential responses of adherence, aggregation, and secretion. Adherence is initiated when one or more substances, released from cells or activated in plasma at the site of hemorrhage, bind to receptors in the platelet plasma membrane. Receptor binding results, via second messengers, in adherence (to other platelets and the inner, endothelial surface of blood vessels) and secretion.

Disruption of the endothelium at sites of tissue injury exposes a variety of proteins in the subendothelial matrix, such as collagen and laminin, which either induce or support platelet adherence. Endothelial cells also rapidly deploy cellular adherence antigens known as integrins on the outer surface of their plasma membranes during wound healing. These adherence antigens are deployed to the cell membrane by cellular processes set in motion by factors generated during coagulation or by factors released from platelets during clotting. In turn, activated endothelial cells release substances that participate in hemostasis. von Willebrand factor, a protein synthesized by endothelial cells and megakaryocytes, enhances platelet adherence by forming a bridge between cell surface receptors and collagen in the subendothelial matrix. The protein thrombin, which is generated by the plasma coagulation cascade, is a potent activator of platelet adherence and secretion. Ruptured cells at the site of tissue injury release adenosine diphosphate (ADP), which causes platelets to aggregate at the damaged site. These aggregates effectively stop the flow of blood from the ruptured vessels.

Blood Coagulation Results in the Production of Fibrin

Platelet aggregates are trapped in a highly organized, firm, and degradable network of fibrin, an insoluble protein generated in plasma as a consequence of activation of either the intrinsic or extrinsic clotting cascades, discussed below. The fibrin network traps red cells, leukocytes, platelets, and serum at sites of vascular damage, thereby forming a blood clot. The stable, fibrin-based blood clot eventually replaces the unstable platelet aggregate formed immediately after tissue injury. Fibrin is an insoluble polymer of proteolytic products of the plasma protein fibrinogen. Fibrin molecules are cleaved from fibrinogen by thrombin, which is generated in plasma during clotting. In the initial step of fibrin formation, thrombin cleaves four small peptides (fibrinopeptides) from each molecule of fibrinogen. The fibrinogen molecule devoid of these fibrinopeptides is called fibrin monomer. The fibrin monomers spontaneously assemble into ordered fibrous arrays of fibrin, resulting in an insoluble matrix of fibrous strands. At this stage, the clot is held together by noncovalent forces. A plasma enzyme, fibrin stabilizing factor (Factor XIII), catalyzes the formation of covalent bonds between...
strands of polymerized fibrin, stabilizing and tightening the blood clot.

**The Coagulation Cascade.** Blood clotting is mediated by the sequential activation of a series of coagulation factors, proteins synthesized in the liver that circulate in the plasma in an inactive state. They are referred to by number (designated by a Roman numeral) in a sequence based on the order of the discovery of each factor. The plasma coagulation factors and their common names are listed in Table 11.5.

The sequential activation of a series of inactive molecules resulting in a biological response is called a metabolic cascade. The sequential activation of coagulation factors resulting in the conversion of fibrinogen to fibrin (and, hence, clotting) is called the coagulation cascade. The deficiency or deletion of any one factor of the cascade has severe consequences. Individuals deficient in factor VIII (antihemophilic factor), for example, display prolonged bleeding time on tissue injury, as a result of delayed clotting. Those who lack factor VIII have hemophilia, a condition resulting in severe coagulation defects.

Two separate coagulation cascades result in blood clotting in different circumstances. The two systems are the **intrinsic coagulation pathway** and the **extrinsic coagulation pathway** (Fig. 11.11). The final steps in fibrin formation are common to both pathways. In the intrinsic pathway, all the factors required for coagulation are present in the circulation. For initiation of the extrinsic pathway, a factor extrinsic to blood but released from injured tissue, called tissue thromboplastin or tissue factor (factor III), is required. Phospholipids are required for activation of both coagulation pathways. Phospholipids provide a surface for the efficient interaction of several factors. A component of tissue factor provides the necessary phospholipid for the extrinsic pathway. Phospholipids required for the activation of the intrinsic pathway are found on platelet membranes.

![FIGURE 11.11 Steps in the coagulation cascade.](image)

The final events leading to fibrin formation by both pathways result from the activation of the common pathway. The common pathway is initiated by the conversion of inactive clotting factor X to its active form, factor Xa (see Fig. 11.11) and results in the conversion of prothrombin to thrombin, thereby catalyzing the generation of fibrin. Thrombin also enhances the activity of clotting factors V and VIII, accelerating “upstream” events in the coagulation pathway. Finally, thrombin is a potent platelet and endothelial cell stimulus and enhances the participation of these cells in coagulation.

Factor X is activated during both the extrinsic and the intrinsic pathways. In the extrinsic pathway, factor X is activated by a complex consisting of activated factor VII, Ca$^{2+}$, and factor III (tissue factor). Activation of this complex by tissue factor bypasses the requirement for coagulation factors VIII, IX, XI, and XII used in the intrinsic pathway. In the intrinsic pathway, clotting is initiated by the activation of factor XII by contact to exposed surfaces, such as subendothelial matrix. Factor XIIa initiates a cascade of events, including activation of factor X, subsequent conversion of prothrombin to thrombin, and, finally, fibrin formation.

<table>
<thead>
<tr>
<th>Scientific Name</th>
<th>Common Name</th>
<th>Other Names</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor I</td>
<td>Fibrinogen</td>
<td></td>
</tr>
<tr>
<td>Factor II</td>
<td>Prothrombin</td>
<td></td>
</tr>
<tr>
<td>Factor III</td>
<td>Tissue thromboplastin</td>
<td>Tissue factor</td>
</tr>
<tr>
<td>Factor IV</td>
<td>Calcium</td>
<td></td>
</tr>
<tr>
<td>Factor V</td>
<td>Proaccelerin</td>
<td>Labile factor</td>
</tr>
<tr>
<td>Factor VII</td>
<td>Proconvertin</td>
<td>Serum prothrombin conversion accelerator (SPCA)</td>
</tr>
<tr>
<td>Factor VIII</td>
<td>Antihemophilic factor</td>
<td>Platelet cofactor 1</td>
</tr>
<tr>
<td>Factor IX</td>
<td>Christmas factor</td>
<td>Platelet thromboplastin component</td>
</tr>
<tr>
<td>Factor X</td>
<td>Stuart factor</td>
<td></td>
</tr>
<tr>
<td>Factor XI</td>
<td>Plasma thromboplastin</td>
<td>antecedent</td>
</tr>
<tr>
<td>Factor XII</td>
<td>Hageman factor</td>
<td>Contact factor</td>
</tr>
<tr>
<td>Factor XIII</td>
<td>Fibrin stabilizing factor</td>
<td></td>
</tr>
</tbody>
</table>
Any attempt to describe a distinct division of coagulation into two separate pathways is an oversimplification, and the cascade theory has been extensively modified. There are many points of interaction between the two pathways, and no one pathway will account for hemostasis. For example, thrombin generated during activation of the extrinsic pathway is an essential cofactor for factor VIII of the intrinsic pathway. Factor VIIa of the extrinsic pathway directly activates factor IX of the intrinsic system. Factor VII can be activated by factors IXa, Xa, and Xlla and thrombin. The many additional points of interaction are beyond the scope of this discussion, but the concept of independently acting intrinsic versus extrinsic coagulation pathways has been abandoned. However, the activity of the intrinsic system and the extrinsic system are monitored individually in clinical coagulation tests for diagnostic purposes. The test used to monitor activity of the intrinsic system is the partial thromboplastin time (PTT). The extrinsic system is evaluated by determination of the prothrombin time (PT).

To a large extent, the interaction of coagulation factors occurs on the surfaces of platelets and endothelial cells. While plasma can eventually clot in the absence of surface contact, localization and assembly of coagulation factors on cell surfaces amplifies reaction rates by several orders of magnitude.

Clot retraction is a phenomenon that usually occurs within minutes or hours after clot formation. The clot draws together, extruding a very large fraction of the serum. The retraction requires platelets. Clot retraction decreases the breakdown of the clot and enhances wound healing.

Fibrinolysis and Wound Healing. Several important mechanisms exist to regulate and eventually reverse the final consequence of coagulation in order to allow healing to proceed. Platelet function is strongly inhibited, for example, by the endothelial cell metabolite prostacyclin (PGI₂), which is generated from arachidonic acid during cellular activation. Activated endothelial cells also release tissue plasminogen activator (TPA), which converts plasminogen to plasmin, a protein that hydrolyzes fibrin, resulting in dissolution of the fibrin clot in a process called fibrinolysis. Thrombin bound to thrombomodulin on the surface of endothelial cells converts protein C to an active protease. Activated protein C and its cofactor, protein S, restrain further coagulation by proteolysis of factors Va and VIIIa. Furthermore, activated protein C augments fibrinolysis by blocking an inhibitor of TPA. Finally, antithrombin III is a potent inhibitor of proteases involved in the coagulation cascade, such as thrombin. The activity of antithrombin III is accelerated by small amounts of heparin, a mucopolysaccharide present in the cells of many tissues. Deficiencies or abnormalities in proteins that regulate or constrain coagulation may result in thrombotic disorders, in which intravascular clot formation leads to severe problems, including embolism and stroke. Such disorders have been associated with abnormalities in protein C, protein S, antithrombin III, and plasminogen.

While the blood clot resolves, multiple factors participate in wound healing. Optimal wound healing requires the recruitment or generation of new tissue cells as well as new blood vessels to nourish the repairing tissue. Thus, secreted proteins and lipids that attract cells (chemoattractants), induce cells to proliferate (mitogens), and induce primitive cells to differentiate (growth factors) are called into play. These agents act in concert to induce the formation of new tissue and repair the injured area. The healing area is vascularized by a process known as angiogenesis, the formation of new blood vessels from preexisting ones. Platelets, activated during clotting, play an important role in the angiogenic response because they secrete factors that induce proliferation, migration, and differentiation of two of the major components of blood vessels, endothelial cells, and smooth muscle cells.

Of the factors released from platelets involved in the angiogenic response, a novel lipid—sphingosine 1-phosphate—plays an important role in wound healing and angiogenesis. Released during clotting and acting in conjunction with protein growth factors, this lipid induces the proliferation of new tissue cells to replace damaged ones and drives the formation of new blood vessels until the healing process is complete. It does so by inducing the migration, proliferation, and differentiation of fibroblasts, smooth muscle cells, and endothelial cells at the site of tissue repair. Sphingosine 1-phosphate exerts its effects optimally when acting in conjunction with protein growth factors that possess angiogenic capabilities, including vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF). Recent research has been undertaken to define, in detail, the biochemical events that drive the angiogenic response because directed regulation of angiogenesis has profound clinical implications. For example, exogenously applied angiogenic factors may prove useful in accelerating repair of tissue damaged by thrombi in the pulmonary, cerebral, or cardiac circulation. In addition, angiogenic factors may assist in the repair of lesions that normally repair slowly—or not at all—such as skin ulcers in patients who are bedridden or diabetic.

Inhibition of angiogenesis may have profound clinical implications also, since unwanted tissues, such as growing tumors, require the development of blood vessels to survive. Therefore, agents which interfere with the angiogenic response, either by acting on the factors involved or the cells that respond to them, may prove particularly useful in the treatment of patients with cancer. Several novel pharmaceuticals are currently being evaluated for their use as regulators of angiogenesis, including thrombospondin, angiostatin, and endostatin, which block neovascularization in tumors and have shown great promise in laboratory testing. Further research will determine if these agents are effective in patients and will identify new, specific regulators of this fundamental process.
**REVIEW QUESTIONS**

**DIRECTIONS:** Each of the numbered items or incomplete statements in this section is followed by answers or by completion of the statement. Select the ONE lettered answer or completion that is BEST in each case.

1. Which type of hemoglobin is not normally found within human erythrocytes?
   - (A) HbA
   - (B) HbA2
   - (C) HbCO
   - (D) HbO2
   - (E) Reduced hemoglobin (Hb)

2. A reactant generated by neutrophils that plays an important role in bacterial killing is
   - (A) NADPH oxidase
   - (B) Hexose monophosphate shunt
   - (C) G proteins
   - (D) Superoxide anion
   - (E) Myeloperoxidase

3. Which cell type is defective in patients with AIDS?
   - (A) T cells
   - (B) B cells
   - (C) Neutrophils
   - (D) Monocytes
   - (E) Basophils

4. Which of the following would be expected to contain relatively high numbers of functional hematopoietic cells?
   - (A) Adult liver
   - (B) Umbilical cord blood
   - (C) Adult circulating blood
   - (D) Adult spleen

5. What is the process that amplifies the number of T cells or B cells programmed to respond to a specific infectious stimulus?
   - (A) Hematopoiesis
   - (B) Hematotherapy
   - (C) Inflammation
   - (D) Innate immunity
   - (E) Clonal selection

6. The response to the antigen used in the tuberculosis skin test, PPD, is not noticeable until 24 to 48 hours after injection because
   - (A) It takes that long for B cells to respond
   - (B) It takes that long for T cells to respond
   - (C) It takes that long for neutrophils to arrive at the site
   - (D) It takes that long for eosinophils to respond
   - (E) The skin test antigen is slowly converted to a more reactive antigen that quickly initiates the skin response

7. Antibody specificity is determined by the amino acid sequence within the
   - (A) Fc region
   - (B) Constant region
   - (C) Variable region
   - (D) Fc receptors
   - (E) J chain

8. The first step in the extrinsic coagulation pathway is
   - (A) Activation of factor X
   - (B) Activation of factor XII
   - (C) Conversion of prothrombin to thrombin
   - (D) Release of tissue thromboplastin
   - (E) Conversion of fibrinogen to fibrin

**SUGGESTED READING**