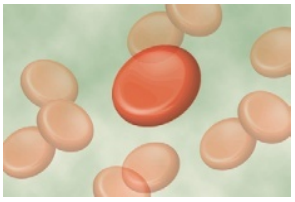
Red Blood Cells, Anemia, and Polycythemia



With this chapter we begin discussing the *blood cells* and cells of the *macrophage system* and *lymphatic system.* We first present the functions of red blood cells, which are the most abundant cells of the blood and are necessary for the delivery of oxygen to the tissues.

Red Blood Cells (Erythrocytes)

A major function of red blood cells, also known as *erythrocytes,* is to transport *hemoglobin,* which in turn carries oxygen from the lungs to the tissues. In some lower animals, hemoglobin circulates as free protein in the plasma, not enclosed in red blood cells. When it is free in the plasma of the human being, about 3 percent of it leaks through the capillary membrane into the tissue spaces or through the glomerular membrane of the kidney into the glomerular filtrate each time the blood passes through the capillaries. Therefore, hemoglobin must remain inside red blood cells to effectively perform its functions in humans.

The red blood cells have other functions besides transport of hemoglobin. For instance, they contain a large quantity of *carbonic anhydrase,* an enzyme that catalyzes the reversible reaction between carbon dioxide (CO_2) and water to form carbonic acid (H_2CO_3), increasing the rate of this reaction several thousandfold. The rapidity of this reaction makes it possible for the water of the blood to transport enormous quantities of CO_2 in the form of bicarbonate ion (HCO_3^-) from the tissues to the lungs, where it is reconverted to CO_2 and expelled into the atmosphere as a body waste product. The hemoglobin in the cells is an excellent *acid-base buffer* (as is true of most proteins), so the red blood cells are responsible for most of the acid-base buffering power of whole blood.

Shape and Size of Red Blood Cells. Normal red blood cells, shown in Figure 32-3, are biconcave discs having a mean diameter of about 7.8 micrometers and a thickness of 2.5 micrometers at the thickest point and 1 micrometer or less in the center. The average volume of the red blood cell is 90 to 95 cubic micrometers.

The shapes of red blood cells can change remarkably as the cells squeeze through capillaries. Actually, the red blood cell is a "bag" that can be deformed into almost any shape. Furthermore, because the normal cell has a great excess of cell membrane for the quantity of material inside, deformation does not stretch the membrane greatly and, consequently, does not rupture the cell, as would be the case with many other cells.

Concentration of Red Blood Cells in the Blood. In healthy men, the average number of red blood cells per cubic millimeter is 5,200,000 (±300,000); in women, it is 4,700,000 (±300,000). Persons living at high altitudes have greater numbers of red blood cells, as discussed later.

Quantity of Hemoglobin in the Cells. Red blood cells have the ability to concentrate hemoglobin in the cell fluid up to about 34 grams in each 100 milliliters of cells. The concentration does not rise above this value because this is the metabolic limit of the cell's hemoglobin-forming mechanism. Furthermore, in normal people, the percentage of hemoglobin is almost always near the maximum in each cell. However, when hemoglobin formation is deficient, the percentage of hemoglobin in the cells may fall considerably below this value and the volume of the red cell may also decrease because of diminished hemoglobin to fill the cell.

When the hematocrit (the percentage of blood that is in cells—normally, 40 to 45 percent) and the quantity of hemoglobin in each respective cell are normal, the whole blood of men contains an average of 15 grams of hemoglobin per 100 milliliters of cells; for women, it contains an average of 14 grams per 100 milliliters.

As discussed in connection with blood transport of oxygen in Chapter 40, each gram of pure hemoglobin is capable of combining with 1.34 ml of oxygen. Therefore, in a normal man a maximum of about 20 milliliters of oxygen can be carried in combination with hemoglobin in each 100 milliliters of blood, and in a normal woman 19 milliliters of oxygen can be carried.

Production of Red Blood Cells

Areas of the Body That Produce Red Blood Cells.

In the early weeks of embryonic life, primitive, nucleated red blood cells are produced in the *yolk sac.* During the middle trimester of gestation, the *liver* is the main organ for production of red blood cells but reasonable numbers are also produced in the *spleen* and *lymph nodes.* Then, during the last month or so of gestation and after birth, red blood cells are produced exclusively in the *bone marrow.*

As demonstrated in Figure 32-1, the bone marrow of essentially all bones produces red blood cells until a person is 5 years old. The marrow of the long bones, except for the proximal portions of the humeri and tibiae, becomes quite fatty and produces no more red blood cells after about age 20 years. Beyond this age, most red cells continue to be produced in the marrow of the membranous bones, such as the vertebrae, sternum, ribs, and ilia. Even in these bones, the marrow becomes less productive as age increases.

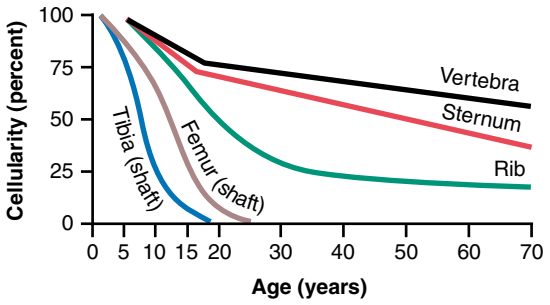


Figure 32-1 Relative rates of red blood cell production in the bone marrow of different bones at different ages.

Genesis of Blood Cells

Pluripotential Hematopoietic Stem Cells, Growth Inducers, and Differentiation Inducers. The blood cells begin their lives in the bone marrow from a single type of cell called the *pluripotential hematopoietic stem cell,* from which all the cells of the circulating blood are eventually derived. Figure 32-2 shows the successive divisions of the pluripotential cells to form the different circulating blood cells. As these cells reproduce, a small portion of them remains exactly like the original pluripotential cells and is retained in the bone marrow to maintain a supply of these, although their numbers diminish with age. Most of the reproduced cells, however, differentiate to form the other cell types shown to the right in Figure 32-2. The intermediate-stage cells are very much like the pluripotential stem cells, even though they have already become committed to a particular line of cells and are called *committed stem cells.*

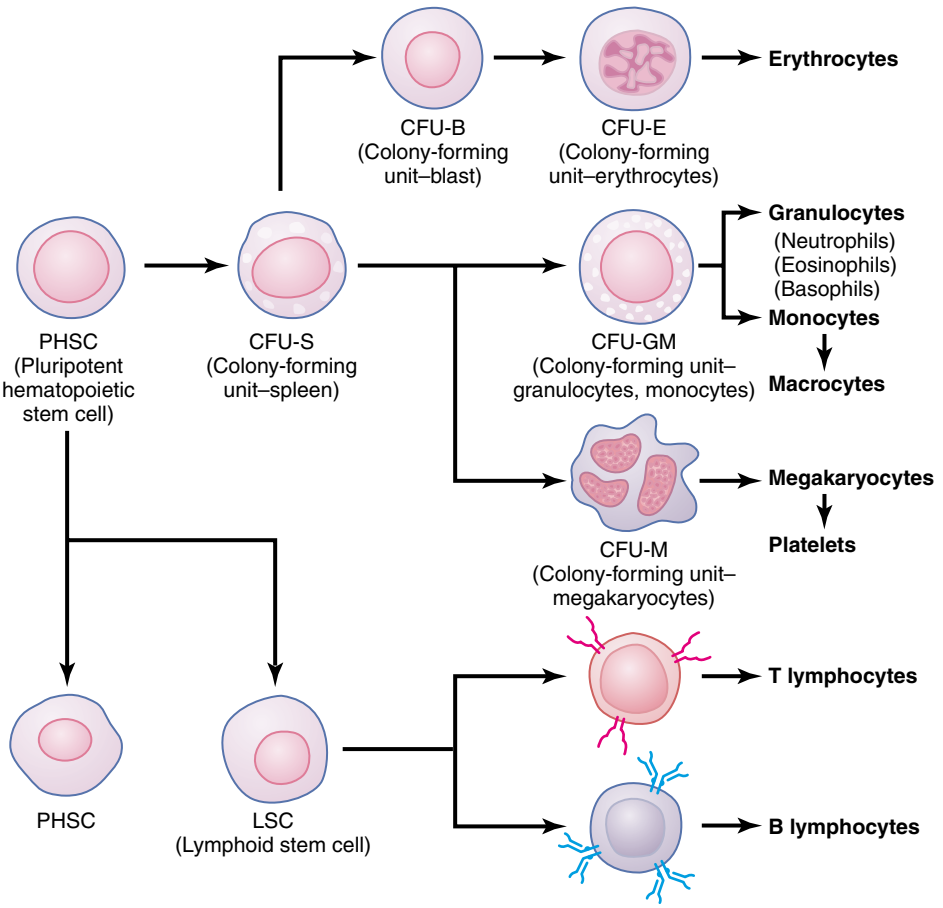


Figure 32-2 Formation of the multiple different blood cells from the original *pluripotent hematopoietic stem cell (PHSC)* in the bone marrow.

The different committed stem cells, when grown in culture, will produce colonies of specific types of blood cells. A committed stem cell that produces erythrocytes is called a *colony-forming unit-erythrocyte,* and the abbreviation CFU-E is used to designate this type of stem cell. Likewise, colony-forming units that form granulocytes and monocytes have the designation CFU-GM and so forth.

Growth and reproduction of the different stem cells are controlled by multiple proteins called *growth inducers.* Four major growth inducers have been described, each having different characteristics. One of these, *interleukin-3,* promotes growth and reproduction of virtually all the different types of committed stem cells, whereas the others induce growth of only specific types of cells.

The growth inducers promote growth but not differentiation of the cells. This is the function of another set of proteins called *differentiation inducers.* Each of these causes one type of committed stem cell to differentiate one or more steps toward a final adult blood cell.

Formation of the growth inducers and differentiation inducers is itself controlled by factors outside the bone marrow. For instance, in the case of erythrocytes (red blood cells), exposure of the blood to low oxygen for a long time causes growth induction, differentiation, and production of greatly increased numbers of erythrocytes, as discussed later in the chapter. In the case of some of the white blood cells, infectious diseases cause growth, differentiation, and eventual formation of specific types of white blood cells that are needed to combat each infection.

Stages of Differentiation of Red Blood Cells

The first cell that can be identified as belonging to the red blood cell series is the *proerythroblast,* shown at the starting point in Figure 32-3. Under appropriate stimulation, large numbers of these cells are formed from the CFU-E stem cells.

Once the proerythroblast has been formed, it divides multiple times, eventually forming many mature red blood cells. The first-generation cells are called *basophil erythroblasts* because they stain with basic dyes; the cell at this time has accumulated very little hemoglobin. In the succeeding generations, as shown in Figure 32-3, the cells become filled with hemoglobin to a concentration of about 34 percent, the nucleus condenses to a small size, and its final remnant is absorbed or extruded from the cell. At the same time, the endoplasmic reticulum is also reabsorbed. The cell at this stage is called a *reticulocyte* because it still contains a small amount of basophilic material, consisting of remnants of the Golgi apparatus, mitochondria, and a few other cytoplasmic organelles. During this reticulocyte stage, the cells pass from the bone marrow into the blood capillaries by *diapedesis* (squeezing through the pores of the capillary membrane).

The remaining basophilic material in the reticulocyte normally disappears within 1 to 2 days, and the cell is then a *mature erythrocyte.* Because of the short life of the reticulocytes, their concentration among all the red cells of the blood is normally slightly less than 1 percent.

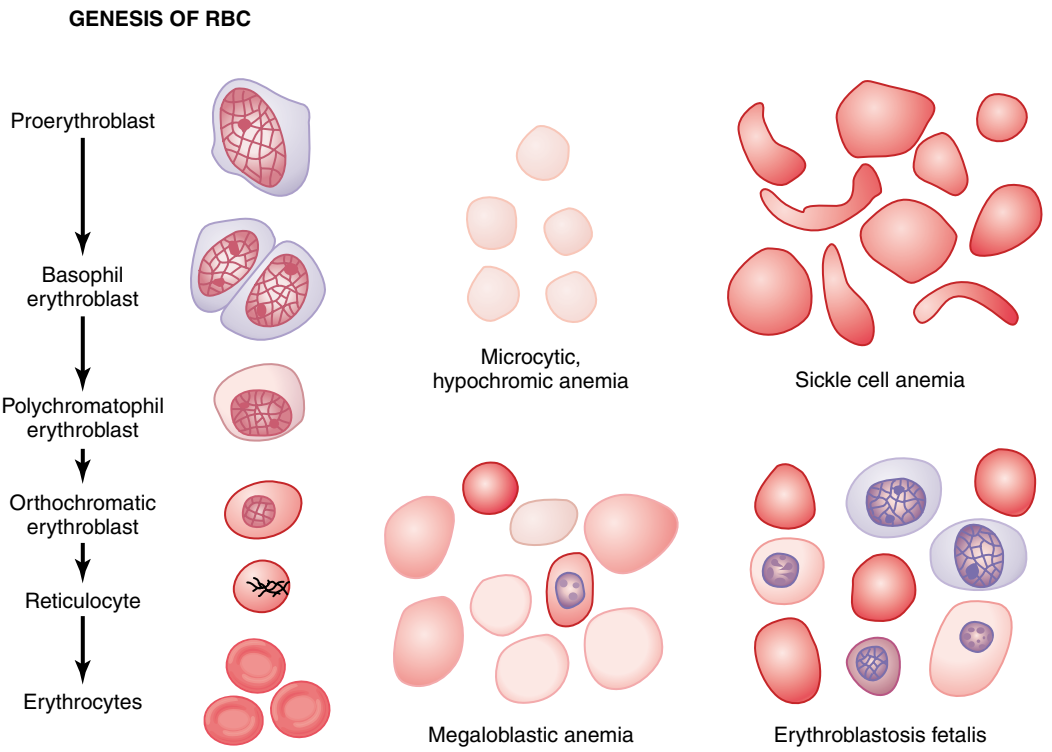


Figure 32-3 Genesis of normal red blood cells (RBCs) and characteristics of RBCs in different types of anemias.

Regulation of Red Blood Cell Production—Role of Erythropoietin

The total mass of red blood cells in the circulatory system is regulated within narrow limits, so (1) adequate red cells are always available to provide sufficient transport of oxygen from the lungs to the tissues, yet (2) the cells do not become so numerous that they impede blood flow. This control mechanism is diagrammed in Figure 32-4 and is as follows.

Tissue Oxygenation Is the Most Essential Regulator of Red Blood Cell Production. Any condition that causes the quantity of oxygen transported to the tissues to decrease ordinarily increases the rate of red blood cell production. Thus, when a person becomes extremely *anemic* as a result of hemorrhage or any other condition, the bone marrow begins to produce large quantities of red blood cells. Also, destruction of major portions of the bone marrow by any means, especially by x-ray therapy, causes hyperplasia of the remaining bone marrow, thereby attempting to supply the demand for red blood cells in the body.

At very *high altitudes,* where the quantity of oxygen in the air is greatly decreased, insufficient oxygen is transported to the tissues and red cell production is greatly increased. In this case, it is not the concentration of red blood cells in the blood that controls red cell production but the amount of oxygen transported to the tissues in relation to tissue demand for oxygen.

Various diseases of the circulation that cause decreased tissue blood flow, and particularly those that cause failure of oxygen absorption by the blood as it passes through the lungs, can also increase the rate of red cell production. This is especially apparent in prolonged *cardiac failure* and in many *lung diseases* because the tissue hypoxia resulting from these conditions increases red cell production, with a resultant increase in hematocrit and usually total blood volume as well.

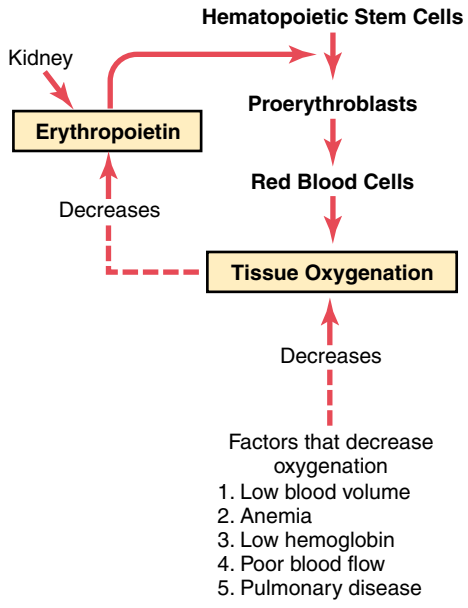


Figure 32-4 Function of the erythropoietin mechanism to increase production of red blood cells when tissue oxygenation decreases.

Erythropoietin Stimulates Red Cell Production, and Its Formation Increases in Response to Hypoxia. The principal stimulus for red blood cell production in low oxygen states is a circulating hormone called *erythropoietin,* a glycoprotein with a molecular weight of about 34,000. In the absence of erythropoietin, hypoxia has little or no effect to stimulate red blood cell production. But when the erythropoietin system is functional, hypoxia causes a marked increase in erythropoietin production and the erythropoietin in turn enhances red blood cell production until the hypoxia is relieved.

Role of the Kidneys in Formation of Erythropoietin. Normally, about 90 percent of all erythropoietin is formed in the kidneys; the remainder is formed mainly in the liver. It is not known exactly where in the kidneys the erythropoietin is formed. Some studies suggest that erythropoietin is secreted mainly by fibroblast-like interstitial cells surrounding the tubules in the cortex and outer medulla secrete, where much of the kidney's oxygen consumption occurs. It is likely that other cells, including the renal epithelial cells themselves, also secrete the erythropoietin in response to hypoxia.

Renal tissue hypoxia leads to increased tissue levels of *hypoxia-inducible factor-1* (HIF-1), which serves as a transcription factor for a large number of hypoxia-inducible genes, including the erythropoietin gene. HIF-1 binds to a *hypoxia response element* residing in the erythropoietin gene, inducing transcription of mRNA and, ultimately, increased erythropoietin synthesis.

At times, hypoxia in other parts of the body, but not in the kidneys, stimulates kidney erythropoietin secretion, which suggests that there might be some nonrenal sensor that sends an additional signal to the kidneys to produce this hormone. In particular, both norepinephrine and epinephrine and several of the prostaglandins stimulate erythropoietin production.

When both kidneys are removed from a person or when the kidneys are destroyed by renal disease, the person invariably becomes very anemic because the 10 percent of the normal erythropoietin formed in other tissues (mainly in the liver) is sufficient to cause only one third to one half the red blood cell formation needed by the body.

Effect of Erythropoietin in Erythrogenesis. When an animal or a person is placed in an atmosphere of low oxygen, erythropoietin begins to be formed within minutes to hours, and it reaches maximum production within 24 hours. Yet almost no new red blood cells appear in the circulating blood until about 5 days later. From this fact, as well as from other studies, it has been determined that the important effect of erythropoietin is to stimulate the production of proerythroblasts from hematopoietic stem cells in the bone marrow. In addition, once the proerythroblasts are formed, the erythropoietin causes these cells to pass more rapidly through the different erythroblastic stages than they normally do, further speeding up the production of new red blood cells. The rapid production of

cells continues as long as the person remains in a low oxygen state or until enough red blood cells have been produced to carry adequate amounts of oxygen to the tissues despite the low oxygen; at this time, the rate of erythropoietin production decreases to a level that will maintain the required number of red cells but not an excess.

In the absence of erythropoietin, few red blood cells are formed by the bone marrow. At the other extreme, when large quantities of erythropoietin are formed and if there is plenty of iron and other required nutrients available, the rate of red blood cell production can rise to perhaps 10 or more times normal. Therefore, the erythropoietin mechanism for controlling red blood cell production is a powerful one.

Maturation of Red Blood Cells—Requirement for Vitamin B_{12} (Cyanocobalamin) and Folic Acid

Because of the continuing need to replenish red blood cells, the erythropoietic cells of the bone marrow are among the most rapidly growing and reproducing cells in the entire body. Therefore, as would be expected, their maturation and rate of production are affected greatly by a person's nutritional status.

Especially important for final maturation of the red blood cells are two vitamins, *vitamin B_{12}* and *folic acid.* Both of these are essential for the synthesis of DNA because each, in a different way, is required for the formation of thymidine triphosphate, one of the essential building blocks of DNA. Therefore, lack of either vitamin B_{12} or folic acid causes abnormal and diminished DNA and, consequently, failure of nuclear maturation and cell division. Furthermore, the erythroblastic cells of the bone marrow, in addition to failing to proliferate rapidly, produce mainly larger than normal red cells called *macrocytes* and the cell itself has a flimsy membrane and is often irregular, large, and oval instead of the usual biconcave disc. These poorly formed cells, after entering the circulating blood, are capable of carrying oxygen normally, but their fragility causes them to have a short life, one-half to one-third normal. Therefore, it is said that deficiency of either vitamin B_{12} or folic acid causes *maturation failure* in the process of erythropoiesis.

Maturation Failure Caused by Poor Absorption of Vitamin B_{12} from the Gastrointestinal Tract—Pernicious Anemia. A common cause of red blood cell maturation failure is failure to absorb vitamin B_{12} from the gastrointestinal tract. This often occurs in the disease *pernicious anemia,* in which the basic abnormality is an *atrophic gastric mucosa* that fails to produce normal gastric secretions. The parietal cells of the gastric glands secrete a glycoprotein called *intrinsic factor,* which combines with vitamin B_{12} in food and makes the B_{12} available for absorption by the gut. It does this in the following way: (1) Intrinsic factor binds tightly with the vitamin B_{12}. In this bound state, the B_{12} is protected from digestion by the gastrointestinal secretions. (2) Still in the bound state, intrinsic factor binds to specific receptor sites on the brush border membranes of the mucosal cells in the ileum. (3) Then, vitamin B_{12} is transported into the blood during the next few hours by the process of pinocytosis, carrying intrinsic factor and the vitamin together through the membrane. Lack of intrinsic factor, therefore, decreases availability of vitamin B_{12} because of faulty absorption of the vitamin.

Once vitamin B_{12} has been absorbed from the gastrointestinal tract, it is first stored in large quantities in the liver and then released slowly as needed by the bone marrow. The minimum amount of vitamin B_{12} required each day to maintain normal red cell maturation is only 1 to 3 micrograms, and the normal storage in the liver and other body tissues is about 1000 times this amount. Therefore, 3 to 4 years of defective B_{12} absorption are usually required to cause maturation failure anemia.

Failure of Maturation Caused by Deficiency of Folic Acid (Pteroylglutamic Acid). Folic acid is a normal constituent of green vegetables, some fruits, and meats (especially liver). However, it is easily destroyed during cooking. Also, people with gastrointestinal absorption abnormalities, such as the frequently occurring small intestinal disease called *sprue,* often have serious difficulty absorbing both folic acid and vitamin B_{12}. Therefore, in many instances of maturation failure, the cause is deficiency of intestinal absorption of both folic acid and vitamin B_{12}.

Formation of Hemoglobin

Synthesis of hemoglobin begins in the proerythroblasts and continues even into the reticulocyte stage of the red blood cells. Therefore, when reticulocytes leave the bone marrow and pass into the blood stream, they continue to form minute quantities of hemoglobin for another day or so until they become mature erythrocytes.

Figure 32-5 shows the basic chemical steps in the formation of hemoglobin. First, succinyl-CoA, formed in the Krebs metabolic cycle (as explained in Chapter 67), binds with glycine to form a pyrrole molecule. In turn, four pyrroles combine to form protoporphyrin IX, which then combines with iron to form the *heme* molecule. Finally, each heme molecule combines with a long polypeptide chain, a *globin* synthesized by ribosomes, forming a subunit of hemoglobin called a *hemoglobin chain* (Figure 32-6). Each chain has a molecular weight of about 16,000; four of these in turn bind together loosely to form the whole hemoglobin molecule.

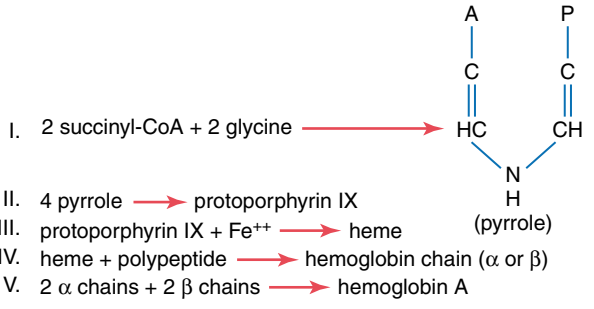


Figure 32-5 Formation of hemoglobin.

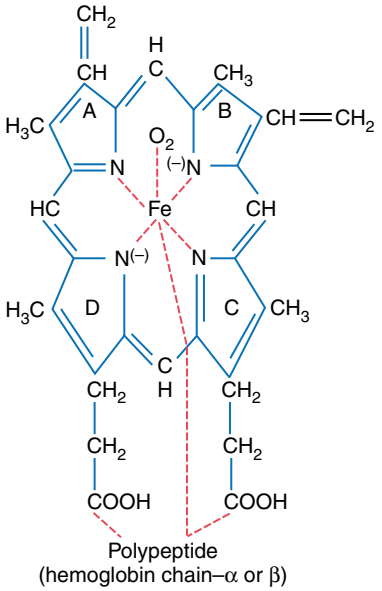


Figure 32-6 Basic structure of the hemoglobin molecule, showing one of the four heme chains that bind together to form the hemoglobin molecule.

There are several slight variations in the different subunit hemoglobin chains, depending on the amino acid composition of the polypeptide portion. The different types of chains are designated *alpha chains, beta chains, gamma chains,* and *delta chains.* The most common form of hemoglobin in the adult human being, *hemoglobin A,* is a combination of *two alpha chains* and *two beta chains.* Hemoglobin A has a molecular weight of 64,458.

Because each hemoglobin chain has a heme prosthetic group containing an atom of iron, and because there are four hemoglobin chains in each hemoglobin molecule, one finds four iron atoms in each hemoglobin molecule; each of these can bind loosely with one molecule of oxygen, making a total of four molecules of oxygen (or eight oxygen atoms) that can be transported by each hemoglobin molecule.

The types of hemoglobin chains in the hemoglobin molecule determine the binding affinity of the hemoglobin for oxygen. Abnormalities of the chains can alter the physical characteristics of the hemoglobin molecule as well. For instance, in *sickle cell anemia,* the amino acid *valine* is substituted for *glutamic acid* at one point in each of the two beta chains. When this type of hemoglobin is exposed to low oxygen, it forms elongated crystals inside the red blood cells that are sometimes 15 micrometers in length. These make it almost impossible for the cells to pass through many small capillaries, and the spiked ends of the crystals are likely to rupture the cell membranes, leading to sickle cell anemia.

Combination of Hemoglobin with Oxygen. The most important feature of the hemoglobin molecule is its ability to combine loosely and reversibly with oxygen. This ability is discussed in detail in Chapter 40 in relation to respiration because the primary function of hemoglobin in the body is to combine with oxygen in the lungs and then to release this oxygen readily in the peripheral tissue capillaries, where the gaseous tension of oxygen is much lower than in the lungs.

Oxygen *does not* combine with the two positive bonds of the iron in the hemoglobin molecule. Instead, it binds loosely with one of the so-called coordination bonds of the iron atom. This is an extremely loose bond, so the combination is easily reversible. Furthermore, the oxygen does not become ionic oxygen but is carried as molecular oxygen (composed of two oxygen atoms) to the tissues, where, because of the loose, readily reversible combination, it is released into the tissue fluids still in the form of molecular oxygen rather than ionic oxygen.

Iron Metabolism

Because iron is important for the formation not only of hemoglobin but also of other essential elements in the body (e.g., *myoglobin, cytochromes, cytochrome oxidase, peroxidase, catalase*), it is important to understand the means by which iron is utilized in the body. The total quantity of iron in the body averages 4 to 5 grams, about 65 percent of which is in the form of hemoglobin. About 4 percent is in the form of myoglobin, 1 percent is in the form of the various heme compounds that promote intracellular oxidation, 0.1 percent is combined with the protein transferrin in the blood plasma, and 15 to 30 percent is stored for later use, mainly in the reticuloendothelial system and liver parenchymal cells, principally in the form of ferritin.

Transport and Storage of Iron. Transport, storage, and metabolism of iron in the body are diagrammed in Figure 32-7 and can be explained as follows: When iron is absorbed from the small intestine, it immediately combines in the blood plasma with a beta globulin, *apotransferrin,* to form *transferrin,* which is then transported in the plasma. The iron is loosely bound in the transferrin and, consequently, can be released to any tissue cell at any point in the body. Excess iron in the blood is deposited especially in the liver hepatocytes and less in the reticuloendothelial cells of the bone marrow.

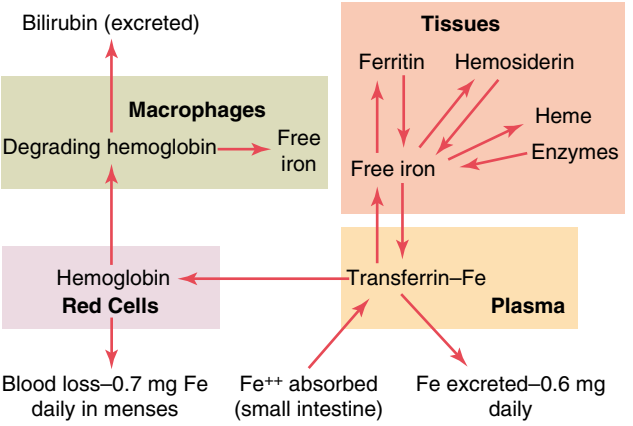


Figure 32-7 Iron transport and metabolism.

In the cell cytoplasm, iron combines mainly with a protein, *apoferritin,* to form *ferritin.* Apoferritin has a molecular weight of about 460,000, and varying quantities of iron can combine in clusters of iron radicals with this large molecule; therefore, ferritin may contain only a small amount of iron or a large amount. This iron stored as ferritin is called *storage iron.*

Smaller quantities of the iron in the storage pool are in an extremely insoluble form called *hemosiderin.* This is especially true when the total quantity of iron in the body is more than the apoferritin storage pool can accommodate. Hemosiderin collects in cells in the form of large clusters that can be observed microscopically as large particles. In contrast, ferritin particles are so small and dispersed that they usually can be seen in the cell cytoplasm only with the electron microscope.

When the quantity of iron in the plasma falls low, some of the iron in the ferritin storage pool is removed easily and transported in the form of transferrin in the plasma to the areas of the body where it is needed. A unique characteristic of the transferrin molecule is that it binds strongly with receptors in the cell membranes of erythroblasts in the bone marrow. Then, along with its bound iron, it is ingested into the erythroblasts by endocytosis. There the transferrin delivers the iron directly to the mitochondria, where heme is synthesized. In people who do not have adequate quantities of transferrin in their blood, failure to transport iron to the erythroblasts in this manner can cause severe *hypochromic anemia* (i.e., red cells that contain much less hemoglobin than normal).

When red blood cells have lived their life span of about 120 days and are destroyed, the hemoglobin released from the cells is ingested by monocyte-macrophage cells. There, iron is liberated and is stored mainly in the ferritin pool to be used as needed for the formation of new hemoglobin.

Daily Loss of Iron. A man excretes about 0.6 mg of iron each day, mainly into the feces. Additional quantities of iron are lost when bleeding occurs. For a woman, additional menstrual loss of blood brings long-term iron loss to an average of about 1.3 mg/day.

Absorption of Iron from the Intestinal Tract

Iron is absorbed from all parts of the small intestine, mostly by the following mechanism. The liver secretes moderate amounts of *apotransferrin* into the bile, which flows through the bile duct into the duodenum. Here, the apotransferrin binds with free iron and also with certain iron compounds, such as hemoglobin and myoglobin from meat, two of the most important sources of iron in the diet. This combination is called *transferrin.* It, in turn, is attracted to and binds with receptors in the membranes of the intestinal epithelial cells. Then, by pinocytosis, the transferrin molecule, carrying its iron store, is absorbed into the epithelial cells and later released into the blood capillaries beneath these cells in the form of *plasma transferrin.*

Iron absorption from the intestines is extremely slow, at a maximum rate of only a few milligrams per day. This means that even when tremendous quantities of iron are present in the food, only small proportions can be absorbed.

Regulation of Total Body Iron by Controlling Rate of Absorption. When the body has become saturated with iron so that essentially all apoferritin in the iron storage areas is already combined with iron, the rate of additional iron absorption from the intestinal tract becomes greatly decreased. Conversely, when the iron stores have become depleted, the rate of absorption can accelerate probably five or more times normal. Thus, total body iron is regulated mainly by altering the rate of absorption.

Life Span of Red Blood Cells is About 120 Days

When red blood cells are delivered from the bone marrow into the circulatory system, they normally circulate an average of 120 days before being destroyed. Even though mature red cells do not have a nucleus, mitochondria, or endoplasmic reticulum, they do have cytoplasmic enzymes that are capable of metabolizing glucose and forming small amounts of ATP. These enzymes also (1) maintain pliability of the cell membrane, (2) maintain membrane transport of ions, (3) keep the iron of the cells' hemoglobin in the ferrous form rather than ferric form, and (4) prevent oxidation of the proteins in the red cells. Even so, the metabolic systems of old red cells become progressively less active and the cells become more and more fragile, presumably because their life processes wear out.

Once the red cell membrane becomes fragile, the cell ruptures during passage through some tight spot of the circulation. Many of the red cells self-destruct in the spleen, where they squeeze through the red pulp of the spleen. There, the spaces between the structural trabeculae of the red pulp, through which most of the cells must pass, are only 3 micrometers wide, in comparison with the 8-micrometer diameter of the red cell. When the spleen is removed, the number of old abnormal red cells circulating in the blood increases considerably.

Destruction of Hemoglobin. When red blood cells burst and release their hemoglobin, the hemoglobin is phagocytized almost immediately by macrophages in many parts of the body, but especially by the Kupffer cells of the liver and macrophages of the spleen and bone marrow. During the next few hours to days, the macrophages release iron from the hemoglobin and pass it back into the blood, to be carried by transferrin either to the bone marrow for the production of new red blood cells or to the liver and other tissues for storage in the form of ferritin. The porphyrin portion of the hemoglobin molecule is converted by the macrophages, through a series of stages, into the bile pigment *bilirubin,* which is released into

the blood and later removed from the body by secretion through the liver into the bile; this is discussed in relation to liver function in Chapter 70.

Anemias

Anemia means deficiency of hemoglobin in the blood, which can be caused by either too few red blood cells or too little hemoglobin in the cells. Some types of anemia and their physiologic causes are the following.

Blood Loss Anemia. After rapid hemorrhage the body replaces the fluid portion of the plasma in 1 to 3 days, but this leaves a low concentration of red blood cells. If a second hemorrhage does not occur, the red blood cell concentration usually returns to normal within 3 to 6 weeks.

In chronic blood loss a person frequently cannot absorb enough iron from the intestines to form hemoglobin as rapidly as it is lost. Red cells that are much smaller than normal and have too little hemoglobin inside them are then produced, giving rise to *microcytic, hypochromic anemia,* which is shown in Figure 32-3.

Aplastic Anemia. *Bone marrow aplasia* means lack of functioning bone marrow. For instance, a person exposed to high-dose radiation or chemotherapy for cancer treatment can damage stem cells of the bone marrow, followed in a few weeks by anemia. Likewise, high doses of certain toxic chemicals, such as insecticides or benzene in gasoline, may cause the same effect. In autoimmune disorders, such as lupus erythematosus, the immune system begins attacking healthy cells such as bone marrow stem cells, which may lead to aplastic anemia. In about half of aplastic anemia cases the cause is unknown, a condition called *idiopathic aplastic anemia*.

People with severe aplastic anemia usually die unless treated with blood transfusions, which can temporarily increase the numbers of red blood cells, or by bone marrow transplantation.

Megaloblastic Anemia. Based on the earlier discussions of vitamin B_{12}, folic acid, and intrinsic factor from the stomach mucosa, one can readily understand that loss of any one of these can lead to slow reproduction of erythroblasts in the bone marrow. As a result, the red cells grow too large, with odd shapes, and are called *megaloblasts.* Thus, atrophy of the stomach mucosa, as occurs in *pernicious anemia,* or loss of the entire stomach after surgical total gastrectomy can lead to megaloblastic anemia. Also, patients who have intestinal sprue, in which folic acid, vitamin B_{12}, and other vitamin B compounds are poorly absorbed, often develop megaloblastic anemia. Because in these states the erythroblasts cannot proliferate rapidly enough to form normal numbers of red blood cells, those red cells that are formed are mostly oversized, have bizarre shapes, and have fragile membranes. These cells rupture easily, leaving the person in dire need of an adequate number of red cells.

Hemolytic Anemia. Different abnormalities of the red blood cells, many of which are hereditarily acquired, make the cells fragile, so they rupture easily as they go through the capillaries, especially through the spleen. Even though the number of red blood cells formed may be normal, or even much greater than normal in some hemolytic diseases, the life span of the fragile red cell is so short that the cells are destroyed faster than they can be formed and serious anemia results.

In *hereditary spherocytosis,* the red cells are very small and *spherical* rather than being biconcave discs. These cells cannot withstand compression forces because they do not have the normal loose, baglike cell membrane structure of the biconcave discs. On passing through the splenic pulp and some other tight vascular beds, they are easily ruptured by even slight compression.

In *sickle cell anemia,* which is present in 0.3 to 1.0 percent of West African and American blacks, the cells have an abnormal type of hemoglobin called *hemoglobin S,* containing faulty beta chains in the hemoglobin molecule, as explained earlier in the chapter. When this hemoglobin is exposed to low concentrations of oxygen, it precipitates into long crystals inside the red blood cell. These crystals elongate the cell and give it the appearance of a sickle rather than a biconcave disc. The precipitated hemoglobin also damages the cell membrane, so the cells become highly fragile, leading to serious anemia. Such patients frequently experience a vicious circle of events called a sickle cell disease "crisis," in which low oxygen tension in the tissues causes sickling, which leads to ruptured red cells, which causes a further decrease in oxygen tension and still more sickling and red cell destruction. Once the process starts, it progresses rapidly, eventuating in a serious decrease in red blood cells within a few hours and, in some cases, death.

In *erythroblastosis fetalis,* Rh-positive red blood cells in the fetus are attacked by antibodies from an Rh-negative mother. These antibodies make the Rh-positive cells fragile, leading to rapid rupture and causing the child to be born with serious anemia. This is discussed in Chapter 35 in relation to the Rh factor of blood. The extremely rapid formation of new red cells to make up for the destroyed cells in erythroblastosis fetalis causes a large number of early *blast* forms of red cells to be released from the bone marrow into the blood.

Effects of Anemia on Function of the Circulatory System

The viscosity of the blood, which was discussed in Chapter 14, depends largely on the blood concentration of red blood cells. In severe anemia, the blood viscosity may fall to as low as 1.5 times that of water rather than the normal value of about 3. This decreases the resistance to blood flow in the peripheral blood vessels, so far greater than

normal quantities of blood flow through the tissues and return to the heart, thereby greatly increasing cardiac output. Moreover, hypoxia resulting from diminished transport of oxygen by the blood causes the peripheral tissue blood vessels to dilate, allowing a further increase in the return of blood to the heart and increasing the cardiac output to a still higher level—sometimes three to four times normal. Thus, one of the major effects of anemia is greatly *increased cardiac output,* as well as *increased pumping workload on the heart.*

The increased cardiac output in anemia partially offsets the reduced oxygen-carrying effect of the anemia because even though each unit quantity of blood carries only small quantities of oxygen, the rate of blood flow may be increased enough that almost normal quantities of oxygen are actually delivered to the tissues. However, when a person with anemia begins to exercise, the heart is not capable of pumping much greater quantities of blood than it is already pumping. Consequently, during exercise, which greatly increases tissue demand for oxygen, extreme tissue hypoxia results and *acute cardiac failure* may ensue.

Polycythemia

Secondary Polycythemia. Whenever the tissues become hypoxic because of too little oxygen in the breathed air, such as at high altitudes, or because of failure of oxygen delivery to the tissues, such as in cardiac failure, the blood-forming organs automatically produce large quantities of extra red blood cells. This condition is called *secondary polycythemia,* and the red cell count commonly rises to 6 to 7 million/mm^3, about 30 percent above normal.

A common type of secondary polycythemia, called *physiologic polycythemia,* occurs in natives who live at altitudes of 14,000 to 17,000 feet, where the atmospheric oxygen is very low. The blood count is generally 6 to 7 million/mm^3; this allows these people to perform reasonably high levels of continuous work even in a rarefied atmosphere.

Polycythemia Vera (Erythremia). In addition to those people who have physiologic polycythemia, others have a pathological condition known as *polycythemia vera,* in which the red blood cell count may be 7 to 8 million/mm^3 and the hematocrit may be 60 to 70 percent instead of the normal 40 to 45 percent. Polycythemia vera is caused by a genetic aberration in the hemocytoblastic cells that produce the blood cells. The blast cells no longer stop producing red cells when too many cells are already present. This causes excess production of red blood cells in the same manner that a breast tumor causes excess production of a specific type of breast cell. It usually causes excess production of white blood cells and platelets as well.

In polycythemia vera, not only does the hematocrit increase, but the total blood volume also increases, on some occasions to almost twice normal. As a result, the entire vascular system becomes intensely engorged. Also, many blood capillaries become plugged by the viscous blood; the viscosity of the blood in polycythemia vera sometimes increases from the normal of 3 times the viscosity of water to 10 times that of water.

Effect of Polycythemia on Function of the Circulatory System

Because of the greatly increased viscosity of the blood in polycythemia, blood flow through the peripheral blood vessels is often very sluggish. In accordance with the factors that regulate return of blood to the heart, as discussed in Chapter 20, increasing blood viscosity *decreases* the rate of venous return to the heart. Conversely, the blood volume is greatly increased in polycythemia, which tends to *increase* venous return. Actually, the cardiac output in polycythemia is not far from normal because these two factors more or less neutralize each other.

The arterial pressure is also normal in most people with polycythemia, although in about one third of them, the arterial pressure is elevated. This means that the blood pressure–regulating mechanisms can usually offset the tendency for increased blood viscosity to increase peripheral resistance and, thereby, increase arterial pressure. Beyond certain limits, however, these regulations fail and hypertension develops.

The color of the skin depends to a great extent on the quantity of blood in the skin subpapillary venous plexus. In polycythemia vera, the quantity of blood in this plexus is greatly increased. Further, because the blood passes sluggishly through the skin capillaries before entering the venous plexus, a larger than normal quantity of hemoglobin is deoxygenated. The blue color of all this deoxygenated hemoglobin masks the red color of the oxygenated hemoglobin. Therefore, a person with polycythemia vera ordinarily has a ruddy complexion with a bluish (cyanotic) tint to the skin.

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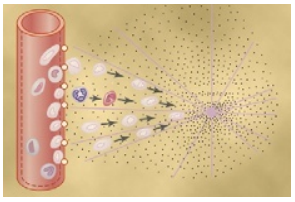
CHAPTER 33

Resistance of the Body to Infection: I. Leukocytes, Granulocytes, the Monocyte-Macrophage System, and Inflammation



Our bodies are exposed continually to bacteria, viruses, fungi, and parasites, all of which occur normally and to varying degrees in the skin, the mouth, the respiratory passageways, the intestinal tract, the lining membranes of the eyes, and even the urinary tract. Many of these infectious agents are capable of causing serious abnormal physiologic function or even death if they invade the deeper tissues. In addition, we are exposed intermittently to other highly infectious bacteria and viruses besides those that are normally present, and these can cause acute lethal diseases such as pneumonia, streptococcal infection, and typhoid fever.

Our bodies have a special system for combating the different infectious and toxic agents. This system is composed of blood leukocytes (white blood cells) and tissue cells derived from leukocytes. These cells work together in two ways to prevent disease: (1) by actually destroying invading bacteria or viruses by *phagocytosis* and (2) by forming *antibodies* and *sensitized lymphocytes,* which may destroy or inactivate the invader. This chapter is concerned with the first of these methods, and Chapter 34 with the second.

Leukocytes (White Blood Cells)

The leukocytes, also called *white blood cells,* are the *mobile units* of the body's protective system. They are formed partially in the bone marrow (*granulocytes* and *monocytes* and a few *lymphocytes*) and partially in the lymph tissue (*lymphocytes* and *plasma cells*). After formation, they are transported in the blood to different parts of the body where they are needed.

The real value of the white blood cells is that most of them are specifically transported to areas of serious infection and inflammation, thereby providing a rapid and potent defense against infectious agents. As we see later, the granulocytes and monocytes have a special ability to "seek out and destroy" a foreign invader.

General Characteristics of Leukocytes

Types of White Blood Cells. Six types of white blood cells are normally present in the blood. They are *polymorphonuclear neutrophils, polymorphonuclear eosinophils, polymorphonuclear basophils, monocytes, lymphocytes,* and, occasionally, *plasma cells.* In addition, there are large numbers of *platelets,* which are fragments of another type of cell similar to the white blood cells found in the bone marrow, the *megakaryocyte.* The first three types of cells, the polymorphonuclear cells, all have a granular appearance, as shown in cell numbers 7, 10, and 12 in Figure 33-1, and for this reason are called *granulocytes,* or, in clinical terminology, "polys," because of the multiple nuclei.

The granulocytes and monocytes protect the body against invading organisms mainly by ingesting them (i.e., by *phagocytosis*). The lymphocytes and plasma cells function mainly in connection with the immune system; this is discussed in Chapter 34. Finally, the function of platelets is specifically to activate the blood clotting mechanism, which is discussed in Chapter 36.

Concentrations of the Different White Blood Cells in the Blood. The adult human being has about 7000 white blood cells per *microliter* of blood (in comparison with 5 million red blood cells). Of the total white blood cells, the normal percentages of the different types are approximately the following:

Polymorphonuclear neutrophils	62.0%
Polymorphonuclear eosinophils	2.3%
Polymorphonuclear basophils	0.4%
Monocytes	5.3%
Lymphocytes	30.0%

The number of platelets, which are only cell fragments, in each microliter of blood is normally about 300,000.

Genesis of the White Blood Cells

Early differentiation of the pluripotential hematopoietic stem cell into the different types of committed stem cells is shown in Figure 32-2 in the previous chapter. Aside from those cells committed to form red blood cells, two major

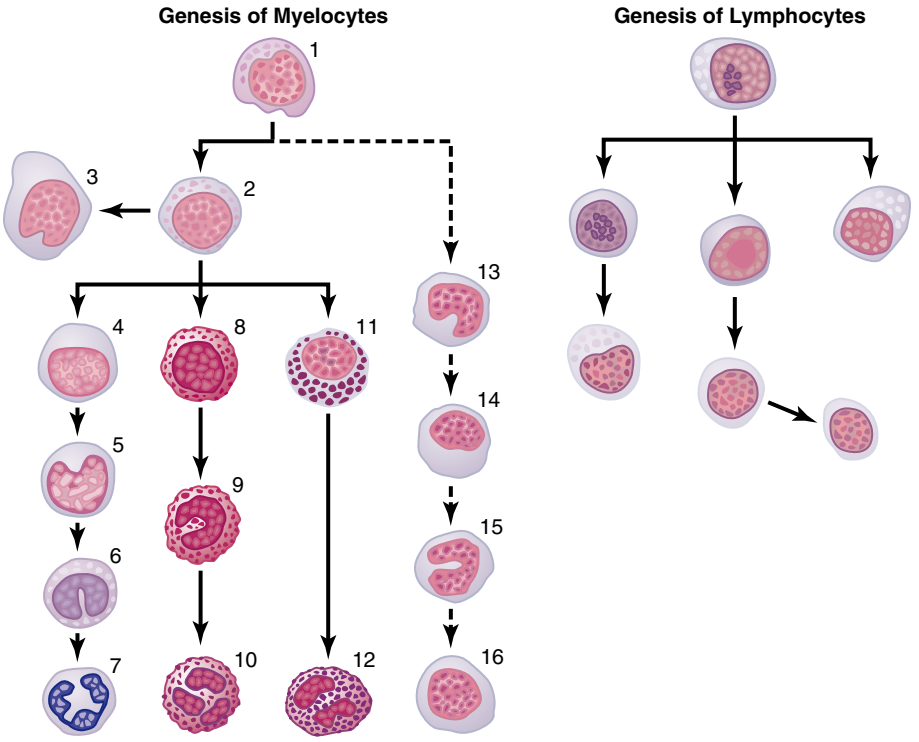


Figure 33-1 Genesis of white blood cells. The different cells of the myelocyte series are *1,* myeloblast; *2,* promyelocyte; *3,* megakaryocyte; *4,* neutrophil myelocyte; *5,* young neutrophil metamyelocyte; *6,* "band" neutrophil metamyelocyte; *7,* polymorphonuclear neutrophil; *8,* eosinophil myelocyte; *9,* eosinophil metamyelocyte; *10,* polymorphonuclear eosinophil; *11,* basophil myelocyte; *12,* polymorphonuclear basophil; *13-16,* stages of monocyte formation.

lineages of *white blood cells* are formed, the myelocytic and the lymphocytic lineages. The left side of Figure 33-1 shows the *myelocytic lineage,* beginning with the *myeloblast;* the right shows the *lymphocytic lineage,* beginning with the *lymphoblast.*

The granulocytes and monocytes are formed only in the bone marrow. Lymphocytes and plasma cells are produced mainly in the various lymphogenous tissues—especially the lymph glands, spleen, thymus, tonsils, and various pockets of lymphoid tissue elsewhere in the body, such as in the bone marrow and in so-called Peyer's patches underneath the epithelium in the gut wall.

The white blood cells formed in the bone marrow are stored within the marrow until they are needed in the circulatory system. Then, when the need arises, various factors cause them to be released (these factors are discussed later). Normally, about three times as many white blood cells are stored in the marrow as circulate in the entire blood. This represents about a 6-day supply of these cells.

The lymphocytes are mostly stored in the various lymphoid tissues, except for a small number that are temporarily being transported in the blood.

As shown in Figure 33-1, megakaryocytes (cell 3) are also formed in the bone marrow. These megakaryocytes fragment in the bone marrow; the small fragments, known as *platelets* (or *thrombocytes*), then pass into the blood. They are very important in the initiation of blood clotting.

Life Span of the White Blood Cells

The life of the granulocytes after being released from the bone marrow is normally 4 to 8 hours circulating in the blood and another 4 to 5 days in tissues where they are needed. In times of serious tissue infection, this total life span is often shortened to only a few hours because the granulocytes proceed even more rapidly to the infected area, perform their functions, and, in the process, are themselves destroyed.

The monocytes also have a short transit time, 10 to 20 hours in the blood, before wandering through the capillary membranes into the tissues. Once in the tissues, they swell to much larger sizes to become *tissue macrophages,* and, in this form, can live for months unless destroyed while performing phagocytic functions. These tissue macrophages are the basis of the *tissue macrophage system,* discussed in greater detail later, which provides continuing defense against infection.

Lymphocytes enter the circulatory system continually, along with drainage of lymph from the lymph nodes and other lymphoid tissue. After a few hours, they pass out of the blood back into the tissues by diapedesis. Then they reenter the lymph and return to the blood again and again; thus, there is continual circulation of lymphocytes through the body. The lymphocytes have life spans of weeks or months, depending on the body's need for these cells.

The platelets in the blood are replaced about once every 10 days; in other words, about 30,000 platelets are formed each day for each microliter of blood.

Neutrophils and Macrophages Defend Against Infections

It is mainly the neutrophils and tissue macrophages that attack and destroy invading bacteria, viruses, and other injurious agents. The neutrophils are mature cells that can attack and destroy bacteria even in the circulating blood. Conversely, the tissue macrophages begin life as blood monocytes, which are immature cells while still in the blood and have little ability to fight infectious agents at that time. However, once they enter the tissues, they begin to swell—sometimes increasing their diameters as much as fivefold—to as great as 60 to 80 micrometers, a size that can barely be seen with the naked eye. These cells are now called *macrophages,* and they are extremely capable of combating disease agents in the tissues.

White Blood Cells Enter the Tissue Spaces by Diapedesis. Neutrophils and monocytes can squeeze through the pores of the blood capillaries by *diapedesis.* That is, even though a pore is much smaller than a cell, a small portion of the cell slides through the pore at a time; the portion sliding through is momentarily constricted to the size of the pore, as shown in Figure 33-2 and 33-6.

White Blood Cells Move Through Tissue Spaces by Ameboid Motion. Both neutrophils and macrophages can move through the tissues by ameboid motion, described in Chapter 2. Some cells move at velocities as great as 40 μm/min, a distance as great as their own length each minute.

White Blood Cells Are Attracted to Inflamed Tissue Areas by Chemotaxis. Many different chemical substances in the tissues cause both neutrophils and macrophages to move toward the source of the chemical. This phenomenon, shown in Figure 33-2, is known as *chemotaxis.*

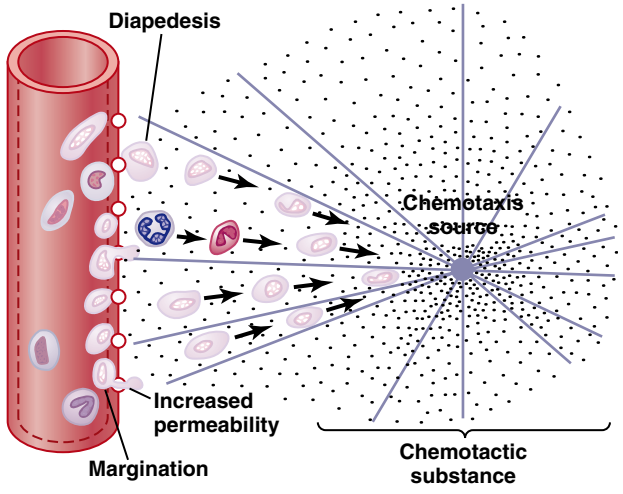


Figure 33-2 Movement of neutrophils by *diapedesis* through capillary pores and by *chemotaxis* toward an area of tissue damage.

When a tissue becomes inflamed, at least a dozen different products that can cause chemotaxis toward the inflamed area are formed. They include (1) some of the bacterial or viral toxins, (2) degenerative products of the inflamed tissues themselves, (3) several reaction products of the "complement complex" (discussed in Chapter 34) activated in inflamed tissues, and (4) several reaction products caused by plasma clotting in the inflamed area, as well as other substances.

As shown in Figure 33-2, chemotaxis depends on the concentration gradient of the chemotactic substance. The concentration is greatest near the source, which directs the unidirectional movement of the white cells. Chemotaxis is effective up to 100 micrometers away from an inflamed tissue. Therefore, because almost no tissue area is more than 50 micrometers away from a capillary, the chemotactic signal can easily move hordes of white cells from the capillaries into the inflamed area.

Phagocytosis

The most important function of the neutrophils and macrophages is *phagocytosis,* which means cellular ingestion of the offending agent. Phagocytes must be selective of the material that is phagocytized; otherwise, normal cells and structures of the body might be ingested. Whether phagocytosis will occur depends especially on three selective procedures.

First, most natural structures in the tissues have smooth surfaces, which resist phagocytosis. But if the surface is rough, the likelihood of phagocytosis is increased.

Second, most natural substances of the body have protective protein coats that repel the phagocytes. Conversely, most dead tissues and foreign particles have no protective coats, which makes them subject to phagocytosis.

Third, the immune system of the body (described in detail in Chapter 34) develops *antibodies* against infectious agents such as bacteria. The antibodies then adhere to the bacterial membranes and thereby make the bacteria especially susceptible to phagocytosis. To do this, the antibody molecule also combines with the C3 product of the *complement cascade,* which is an additional part of the immune system discussed in the next chapter. The C3 molecules, in turn, attach to receptors on the phagocyte membrane, thus initiating phagocytosis. This selection and phagocytosis process is called *opsonization.*

Phagocytosis by Neutrophils. The neutrophils entering the tissues are already mature cells that can immediately begin phagocytosis. On approaching a particle to be phagocytized, the neutrophil first attaches itself to the particle and then projects pseudopodia in all directions around the particle. The pseudopodia meet one another on the opposite side and fuse. This creates an enclosed chamber that contains the phagocytized particle. Then the chamber invaginates to the inside of the cytoplasmic cavity and breaks away from the outer cell membrane to

form a free-floating *phagocytic vesicle* (also called a *phagosome*) inside the cytoplasm. A single neutrophil can usually phagocytize 3 to 20 bacteria before the neutrophil itself becomes inactivated and dies.

Phagocytosis by Macrophages. Macrophages are the end-stage product of monocytes that enter the tissues from the blood. When activated by the immune system, as described in Chapter 34, they are much more powerful phagocytes than neutrophils, often capable of phagocytizing as many as 100 bacteria. They also have the ability to engulf much larger particles, even whole red blood cells or, occasionally, malarial parasites, whereas neutrophils are not capable of phagocytizing particles much larger than bacteria. Also, after digesting particles, macrophages can extrude the residual products and often survive and function for many more months.

Once Phagocytized, Most Particles Are Digested by Intracellular Enzymes. Once a foreign particle has been phagocytized, lysosomes and other cytoplasmic granules in the neutrophil or macrophage immediately come in contact with the phagocytic vesicle, and their membranes fuse, thereby dumping many digestive enzymes and bactericidal agents into the vesicle. Thus, the phagocytic vesicle now becomes a *digestive vesicle,* and digestion of the phagocytized particle begins immediately.

Both neutrophils and macrophages contain an abundance of lysosomes filled with *proteolytic enzymes* especially geared for digesting bacteria and other foreign protein matter. The lysosomes of macrophages (but not of neutrophils) also contain large amounts of *lipases,* which digest the thick lipid membranes possessed by some bacteria such as the tuberculosis bacillus.

Both Neutrophils and Macrophages Can Kill Bacteria. In addition to the digestion of ingested bacteria in phagosomes, neutrophils and macrophages contain *bactericidal agents* that kill most bacteria even when the lysosomal enzymes fail to digest them. This is especially important because some bacteria have protective coats or other factors that prevent their destruction by digestive enzymes. Much of the killing effect results from several powerful *oxidizing agents* formed by enzymes in the membrane of the phagosome or by a special organelle called the *peroxisome.* These oxidizing agents include large quantities of *superoxide* (O_2^-), *hydrogen peroxide* (H_2O_2), and *hydroxyl ions* (OH^-), all of which are lethal to most bacteria, even in small quantities. Also, one of the lysosomal enzymes, myeloperoxidase, catalyzes the reaction between H_2O_2 and chloride ions to form hypochlorite, which is exceedingly bactericidal.

Some bacteria, notably the tuberculosis bacillus, have coats that are resistant to lysosomal digestion and also secrete substances that partially resist the killing effects of the neutrophils and macrophages. These bacteria are responsible for many of the chronic diseases, an example of which is tuberculosis.

Monocyte-Macrophage Cell System (Reticuloendothelial System)

In the preceding paragraphs, we described the macrophages mainly as mobile cells that are capable of wandering through the tissues. However, after entering the tissues and becoming macrophages, another large portion of monocytes becomes attached to the tissues and remains attached for months or even years until they are called on to perform specific local protective functions. They have the same capabilities as the mobile macrophages to phagocytize large quantities of bacteria, viruses, necrotic tissue, or other foreign particles in the tissue. And, when appropriately stimulated, they can break away from their attachments and once again become mobile macrophages that respond to chemotaxis and all the other stimuli related to the inflammatory process. Thus, the body has a widespread "monocyte-macrophage system" in virtually all tissue areas.

The total combination of monocytes, mobile macrophages, fixed tissue macrophages, and a few specialized endothelial cells in the bone marrow, spleen, and lymph nodes is called the *reticuloendothelial system.* However, all or almost all these cells originate from monocytic stem cells; therefore, the reticuloendothelial system is almost synonymous with the monocyte-macrophage system. Because the term *reticuloendothelial system* is much better known in medical literature than the term *monocyte-macrophage system,* it should be remembered as a generalized phagocytic system located in all tissues, especially in those tissue areas where large quantities of particles, toxins, and other unwanted substances must be destroyed.

Tissue Macrophages in the Skin and Subcutaneous Tissues (Histiocytes). Although the skin is mainly impregnable to infectious agents, this is no longer true when the skin is broken. When infection begins in a subcutaneous tissue and local inflammation ensues, local tissue macrophages can divide in situ and form still more macrophages. Then they perform the usual functions of attacking and destroying the infectious agents, as described earlier.

Macrophages in the Lymph Nodes. Essentially no particulate matter that enters the tissues, such as bacteria, can be absorbed directly through the capillary membranes into the blood. Instead, if the particles are not destroyed locally in the tissues, they enter the lymph and flow to the lymph nodes located intermittently along the course of the lymph flow. The foreign particles are then trapped in these nodes in a meshwork of sinuses lined by *tissue macrophages.*

Figure 33-3 illustrates the general organization of the lymph node, showing lymph entering through the lymph node capsule by way of *afferent lymphatics,* then flowing through the *nodal medullary sinuses,* and finally passing

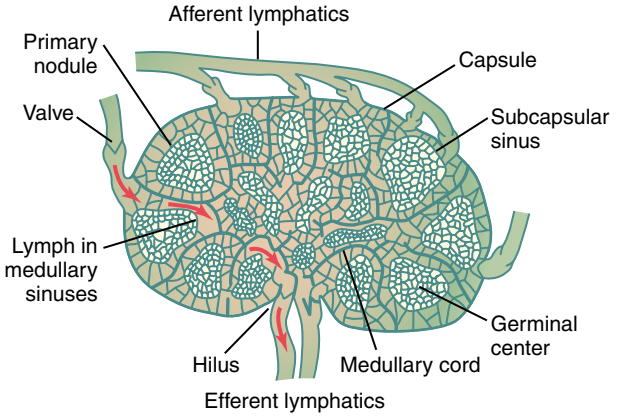


Figure 33-3 Functional diagram of a lymph node. (Redrawn from Ham AW: Histology, 6th ed. Philadelphia: JB Lippincott, 1969.) (Modified from Gartner LP, Hiatt JL: Color Textbook of Histology, 2nd ed. Philadelphia: WB Saunders, 2001.)

out the *hilus* into *efferent lymphatics* that eventually empty into the venous blood.

Large numbers of macrophages line the lymph sinuses, and if any particles enter the sinuses by way of the lymph, the macrophages phagocytize them and prevent general dissemination throughout the body.

Alveolar Macrophages in the Lungs. Another route by which invading organisms frequently enter the body is through the lungs. Large numbers of tissue macrophages are present as integral components of the alveolar walls. They can phagocytize particles that become entrapped in the alveoli. If the particles are digestible, the macrophages can also digest them and release the digestive products into the lymph. If the particle is not digestible, the macrophages often form a "giant cell" capsule around the particle until such time—if ever—that it can be slowly dissolved. Such capsules are frequently formed around tuberculosis bacilli, silica dust particles, and even carbon particles.

Macrophages (Kupffer Cells) in the Liver Sinusoids. Still another route by which bacteria invade the body is through the gastrointestinal tract. Large numbers of bacteria from ingested food constantly pass through the gastrointestinal mucosa into the portal blood. Before this blood enters the general circulation, it passes through the liver sinusoids, which are lined with tissue macrophages called *Kupffer cells,* shown in Figure 33-4. These cells form such an effective particulate filtration system that almost none of the bacteria from the gastrointestinal tract passes from the portal blood into the general systemic circulation. Indeed, motion pictures of phagocytosis by Kupffer cells have demonstrated phagocytosis of a single bacterium in less than $^{1}/_{100}$ of a second.

Macrophages of the Spleen and Bone Marrow. If an invading organism succeeds in entering the general circulation, there are other lines of defense by the tissue macrophage system, especially by macrophages of

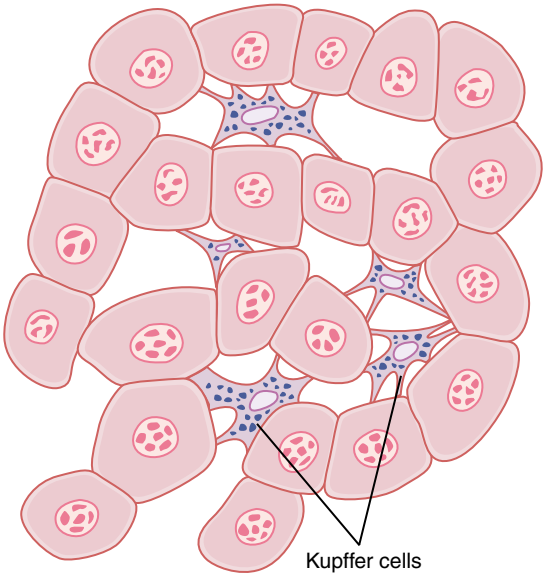


Figure 33-4 Kupffer cells lining the liver sinusoids, showing phagocytosis of India ink particles into the cytoplasm of the Kupffer cells. (Redrawn from Copenhaver WM et al: Bailey's Textbook of Histology, 10th ed. Baltimore: Williams & Wilkins, 1971.)

the spleen and bone marrow. In both these tissues, macrophages become entrapped by the reticular meshwork of the two organs and when foreign particles come in contact with these macrophages, they are phagocytized.

The spleen is similar to the lymph nodes, except that blood, instead of lymph, flows through the tissue spaces of the spleen. Figure 33-5 shows a small peripheral segment of spleen tissue. Note that a small artery penetrates from the splenic capsule into the *splenic pulp* and terminates in small capillaries. The capillaries are highly porous, allowing whole blood to pass out of the capillaries into *cords of red pulp.* The blood then gradually *squeezes* through the trabecular meshwork of these cords and eventually returns to the circulation through the endothelial walls of the *venous sinuses.* The trabeculae of the red pulp are lined with vast numbers of macrophages, and the venous

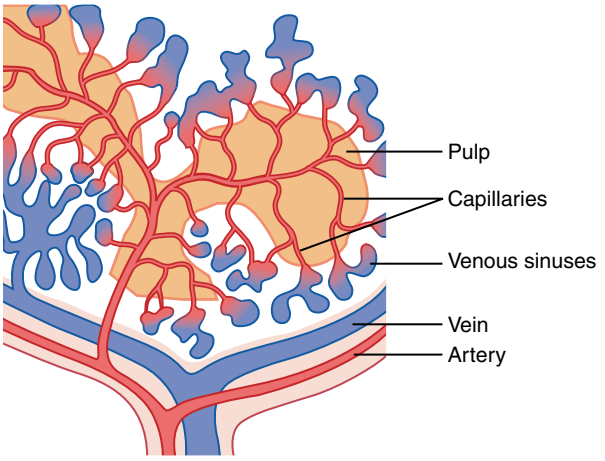


Figure 33-5 Functional structures of the spleen. (Modified from Bloom W, Fawcett DW: A Textbook of Histology, 10th ed. Philadelphia: WB Saunders, 1975.)

sinuses are also lined with macrophages. This peculiar passage of blood through the cords of the red pulp provides an exceptional means of phagocytizing unwanted debris in the blood, including especially old and abnormal red blood cells.

Inflammation: Role of Neutrophils and Macrophages

Inflammation

When tissue injury occurs, whether caused by bacteria, trauma, chemicals, heat, or any other phenomenon, multiple substances are released by the injured tissues and cause dramatic secondary changes in the surrounding uninjured tissues. This entire complex of tissue changes is called *inflammation.*

Inflammation is characterized by (1) vasodilation of the local blood vessels, with consequent excess local blood flow; (2) increased permeability of the capillaries, allowing leakage of large quantities of fluid into the interstitial spaces; (3) often clotting of the fluid in the interstitial spaces because of increased amounts of fibrinogen and other proteins leaking from the capillaries; (4) migration of large numbers of granulocytes and monocytes into the tissue; and (5) swelling of the tissue cells. Some of the many tissue products that cause these reactions are *histamine, bradykinin, serotonin, prostaglandins,* several different *reaction products of the complement system* (described in Chapter 34), *reaction products of the blood clotting system,* and multiple substances called *lymphokines* that are released by sensitized T cells (part of the immune system; also discussed in Chapter 34). Several of these substances strongly activate the macrophage system, and within a few hours, the macrophages begin to devour the destroyed tissues. But at times, the macrophages also further injure the still-living tissue cells.

"Walling-Off" Effect of Inflammation. One of the first results of inflammation is to "wall off" the area of injury from the remaining tissues. The tissue spaces and the lymphatics in the inflamed area are blocked by fibrinogen clots so that after a while, fluid barely flows through the spaces. This walling-off process delays the spread of bacteria or toxic products.

The intensity of the inflammatory process is usually proportional to the degree of tissue injury. For instance, when *staphylococci* invade tissues, they release extremely lethal cellular toxins. As a result, inflammation develops rapidly—indeed, much more rapidly than the staphylococci themselves can multiply and spread. Therefore, local staphylococcal infection is characteristically walled off rapidly and prevented from spreading through the body. Streptococci, in contrast, do not cause such intense local tissue destruction. Therefore, the walling-off process develops slowly over many hours, while many streptococci reproduce and migrate. As a result, streptococci often have a far greater tendency to spread through the body and cause death than do staphylococci, even though staphylococci are far more destructive to the tissues.

Macrophage and Neutrophil Responses During Inflammation

Tissue Macrophage Is a First Line of Defense Against Infection. Within minutes after inflammation begins, the macrophages already present in the tissues, whether histiocytes in the subcutaneous tissues, alveolar macrophages in the lungs, microglia in the brain, or others, immediately begin their phagocytic actions. When activated by the products of infection and inflammation, the first effect is rapid enlargement of each of these cells. Next, many of the previously sessile macrophages break loose from their attachments and become mobile, forming the first line of defense against infection during the first hour or so. The numbers of these early mobilized macrophages often are not great, but they are lifesaving.

Neutrophil Invasion of the Inflamed Area Is a Second Line of Defense. Within the first hour or so after inflammation begins, large numbers of neutrophils begin to invade the inflamed area from the blood. This is caused by inflammatory cytokines (e.g., TNF, IL-1) and other biochemical products produced by the inflamed tissues that initiate the following reactions:

1. They cause increased expression of *adhesion molecules, such as selectins and intracellular adhesion molecule-1 (ICAM-1)* on the surface of endothelial cells in the capillaries and venules. These adhesion molecules, reacting with complementary *integrin* molecules on the neutrophils, cause the neutrophils to stick to the capillary and venule walls in the inflamed area. This effect is called *margination* and is shown in Figure 33-2 and in more detail in Figure 33-6.
2. They also cause the intercellular attachments between the endothelial cells of the capillaries and small venules to loosen, allowing openings large enough for neutrophils to crawl through by *diapedesis,* directly from the blood into the tissue spaces.
3. They then cause *chemotaxis* of the neutrophils toward the injured tissues, as explained earlier.

Thus, within several hours after tissue damage begins, the area becomes well supplied with neutrophils. Because the blood neutrophils are already mature cells, they are ready to immediately begin their scavenger functions for killing bacteria and removing foreign matter.

Acute Increase in Number of Neutrophils in the Blood—"Neutrophilia." Also within a few hours after the onset of acute, severe inflammation, the number of neutrophils in the blood sometimes increases fourfold to fivefold—from a normal of 4000 to 5000 to 15,000 to 25,000 neutrophils per microliter. This is called *neutrophilia,* which means an increase in the number of neutrophils in

the blood. Neutrophilia is caused by products of inflammation that enter the blood stream, are transported to the bone marrow, and there act on the stored neutrophils of the marrow to mobilize these into the circulating blood. This makes even more neutrophils available to the inflamed tissue area.

Second Macrophage Invasion into the Inflamed Tissue Is a Third Line of Defense. Along with the invasion of neutrophils, monocytes from the blood enter the inflamed tissue and enlarge to become macrophages. However, the number of monocytes in the circulating blood is low: Also, the storage pool of monocytes in the bone marrow is much less than that of neutrophils. Therefore, the buildup of macrophages in the inflamed tissue area is much slower than that of neutrophils, requiring several days to become effective. Furthermore, even after invading the inflamed tissue, monocytes are still immature cells, requiring 8 hours or more to swell to much larger sizes and develop tremendous quantities of lysosomes; only then do they acquire the full capacity of *tissue macrophages* for phagocytosis. Yet, after several days to several weeks, the macrophages finally come to dominate the phagocytic cells of the inflamed area because of greatly increased bone marrow production of new monocytes, as explained later.

As already pointed out, macrophages can phagocytize far more bacteria (about five times as many) and far larger particles, including even neutrophils themselves and large quantities of necrotic tissue, than can neutrophils. Also, the macrophages play an important role in initiating the development of antibodies, as we discuss in Chapter 34.

Increased Production of Granulocytes and Monocytes by the Bone Marrow Is a Fourth Line of Defense. The fourth line of defense is greatly increased production of both granulocytes and monocytes by the bone marrow. This results from stimulation of the granulocytic and monocytic progenitor cells of the marrow. However, it takes 3 to 4 days before newly formed granulocytes and monocytes reach the stage of leaving the bone marrow. If the stimulus from the inflamed tissue continues, the bone marrow can continue to produce these cells in tremendous quantities for months and even years, sometimes at a rate 20 to 50 times normal.

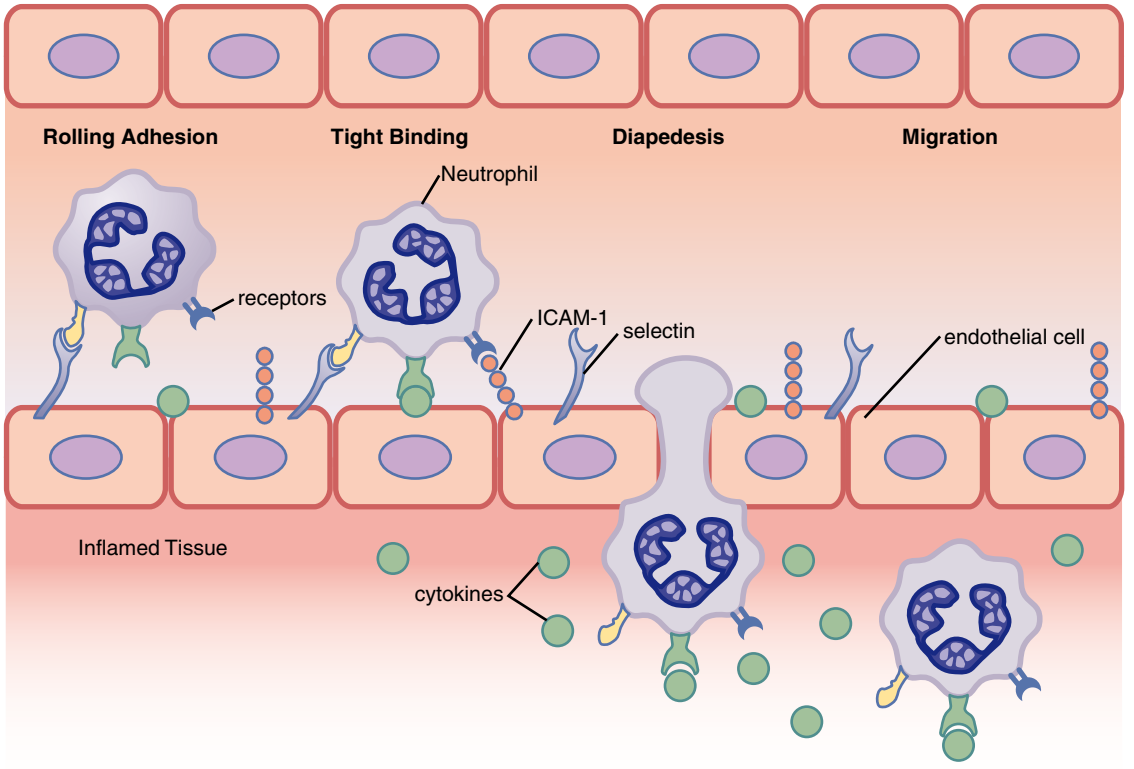


Figure 33-6 Migration of neutrophil from the blood into inflamed tissue. Cytokines and other biochemical products of the inflamed tissue cause increased expression of selectins and intracellular adhesion molecule-1 (ICAM-1) in the surface of endothelial cells. These adhesion molecules bind to complementary molecules/receptors on the neutrophil, causing it to adhere to the wall of the capillary or venule. The neutrophil then migrates through the vessel wall by diapedesis toward the site of tissue injury.

Feedback Control of the Macrophage and Neutrophil Responses

Although more than two dozen factors have been implicated in control of the macrophage response to inflammation, five of these are believed to play dominant roles. They are shown in Figure 33-7 and consist of (1) *tumor necrosis factor* (TNF), (2) *interleukin-1* (IL-1), (3) *granulocyte-monocyte colony-stimulating factor* (GM-CSF), (4) *granulocyte colony-stimulating factor* (G-CSF), and (5) *monocyte colony-stimulating factor* (M-CSF). These factors are formed by activated macrophage cells in the inflamed tissues and in smaller quantities by other inflamed tissue cells.

The cause of the increased production of granulocytes and monocytes by the bone marrow is mainly the three colony-stimulating factors, one of which, GM-CSF, stimulates both granulocyte and monocyte production; the other two, G-CSF and M-CSF, stimulate granulocyte and monocyte production, respectively. This combination of TNF, IL-1, and colony-stimulating factors provides a powerful feedback mechanism that begins with tissue inflammation and proceeds to formation of large numbers of defensive white blood cells that help remove the cause of the inflammation.

Formation of Pus

When neutrophils and macrophages engulf large numbers of bacteria and necrotic tissue, essentially all the neutrophils and many, if not most, of the macrophages eventually die. After several days, a cavity is often excavated in the inflamed tissues. It contains varying portions of necrotic tissue, dead neutrophils, dead macrophages, and tissue fluid. This mixture is commonly known as *pus.* After the infection has been suppressed, the dead cells and necrotic tissue in the pus gradually autolyze over a period of days, and the end products are eventually absorbed into the surrounding tissues and lymph until most of the evidence of tissue damage is gone.

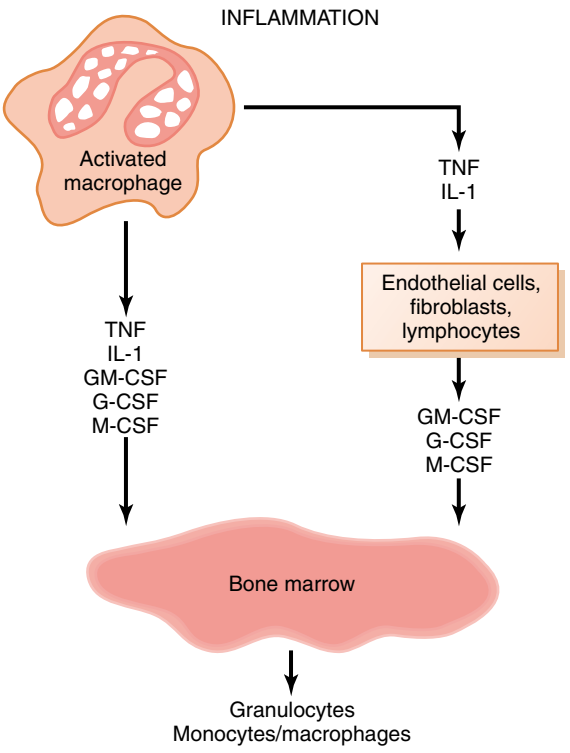


Figure 33-7 Control of bone marrow production of granulocytes and monocyte-macrophages in response to multiple growth factors released from activated macrophages in an inflamed tissue. G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-monocyte colony-stimulating factor; IL-1, interleukin-1; M-CSF, monocyte colony-stimulating factor; TNF, tumor necrosis factor.

Eosinophils

The eosinophils normally constitute about 2 percent of all the blood leukocytes. Eosinophils are weak phagocytes, and they exhibit chemotaxis, but in comparison with the neutrophils, it is doubtful that the eosinophils are significant in protecting against the usual types of infection.

Eosinophils, however, are often produced in large numbers in people with parasitic infections, and they migrate in large numbers into tissues diseased by parasites. Although most parasites are too large to be phagocytized by eosinophils or any other phagocytic cells, eosinophils attach themselves to the parasites by way of special surface molecules and release substances that kill many of the parasites. For instance, one of the most widespread infections is *schistosomiasis,* a parasitic infection found in as many as one third of the population of some developing countries in Asia, Africa, and South America; the parasite can invade any part of the body. Eosinophils attach themselves to the juvenile forms of the parasite and kill many of them. They do so in several ways: (1) by releasing hydrolytic enzymes from their granules, which are modified lysosomes; (2) probably by also releasing highly reactive forms of oxygen that are especially lethal to parasites; and (3) by releasing from the granules a highly larvacidal polypeptide called *major basic protein.*

In a few areas of the world, another parasitic disease that causes eosinophilia is *trichinosis.* This results from invasion of the body's muscles by the *Trichinella* parasite ("pork worm") after a person eats undercooked infested pork.

Eosinophils also have a special propensity to collect in tissues in which allergic reactions occur, such as in the peribronchial tissues of the lungs in people with asthma and in the skin after allergic skin reactions. This is caused at least partly by the fact that many mast cells and basophils participate in allergic reactions, as we discuss in the next paragraph. The mast cells and basophils release an *eosinophil chemotactic factor* that causes eosinophils to migrate toward the inflamed allergic tissue. The eosinophils are believed to detoxify some of the inflammation-inducing substances released by the mast cells and basophils and probably also to phagocytize and destroy allergen-antibody complexes, thus preventing excess spread of the local inflammatory process.

Basophils

The basophils in the circulating blood are similar to the large tissue *mast cells* located immediately outside many of the capillaries in the body. Both mast cells and basophils liberate *heparin* into the blood, a substance that can prevent blood coagulation.

The mast cells and basophils also release *histamine,* as well as smaller quantities of *bradykinin* and *serotonin.* Indeed, it is mainly the mast cells in inflamed tissues that release these substances during inflammation.

The mast cells and basophils play an important role in some types of allergic reactions because the type of antibody that causes allergic reactions, the immunoglobulin E (IgE) type, has a special propensity to become attached to mast cells and basophils. Then, when the specific antigen for the specific IgE antibody subsequently reacts with the antibody, the resulting attachment of antigen to antibody causes the mast cell or basophil to rupture and release large quantities of *histamine, bradykinin, serotonin, heparin, slow-reacting substance of anaphylaxis,* and a number of *lysosomal enzymes.* These cause local vascular and tissue reactions that cause many, if not most, of the allergic manifestations. These reactions are discussed in greater detail in Chapter 34.

Leukopenia

A clinical condition known as *leukopenia,* in which the bone marrow produces very few white blood cells, occasionally occurs. This leaves the body unprotected against many bacteria and other agents that might invade the tissues.

Normally, the human body lives in symbiosis with many bacteria because all the mucous membranes of the body are constantly exposed to large numbers of bacteria. The mouth almost always contains various spirochetal, pneumococcal, and streptococcal bacteria, and these same bacteria are present to a lesser extent in the entire respiratory tract. The distal gastrointestinal tract is especially loaded with colon bacilli. Furthermore, one can always find bacteria on the surfaces of the eyes, urethra, and vagina. Any decrease in the number of white blood cells immediately allows invasion of adjacent tissues by bacteria that are already present.

Within 2 days after the bone marrow stops producing white blood cells, ulcers may appear in the mouth and colon, or the person might develop some form of severe respiratory infection. Bacteria from the ulcers rapidly invade surrounding tissues and the blood. Without treatment, death often ensues in less than a week after acute total leukopenia begins.

Irradiation of the body by x-rays or gamma rays, or exposure to drugs and chemicals that contain benzene or anthracene nuclei, is likely to cause aplasia of the bone marrow. Indeed, some common drugs, such as chloramphenicol (an antibiotic), thiouracil (used to treat thyrotoxicosis), and even various barbiturate hypnotics, on very rare occasions cause leukopenia, thus setting off the entire infectious sequence of this malady.

After moderate irradiation injury to the bone marrow, some stem cells, myeloblasts, and hemocytoblasts may remain undestroyed in the marrow and are capable of regenerating the bone marrow, provided sufficient time is available. A patient properly treated with transfusions, plus antibiotics and other drugs to ward off infection, usually develops enough new bone marrow within weeks to months for blood cell concentrations to return to normal.

Leukemias

Uncontrolled production of white blood cells can be caused by cancerous mutation of a myelogenous or lymphogenous cell. This causes *leukemia,* which is usually characterized by greatly increased numbers of abnormal white blood cells in the circulating blood.

Types of Leukemia. Leukemias are divided into two general types: *lymphocytic leukemias* and *myelogenous leukemias.* The lymphocytic leukemias are caused by cancerous production of lymphoid cells, usually beginning in a lymph node or other lymphocytic tissue and spreading to other areas of the body. The second type of leukemia, myelogenous leukemia, begins by cancerous production of young myelogenous cells in the bone marrow and then spreads throughout the body so that white blood cells are produced in many extramedullary tissues—especially in the lymph nodes, spleen, and liver.

In myelogenous leukemia, the cancerous process occasionally produces partially differentiated cells, resulting in what might be called *neutrophilic leukemia, eosinophilic leukemia, basophilic leukemia,* or *monocytic leukemia.* More frequently, however, the leukemia cells are bizarre and undifferentiated and not identical to any of the normal white blood cells. Usually, the more undifferentiated the cell, the more *acute* is the leukemia, often leading to death within a few months if untreated. With some of the more differentiated cells, the process can be *chronic,* sometimes developing slowly over 10 to 20 years. Leukemic cells, especially the very undifferentiated cells, are usually nonfunctional for providing the normal protection against infection.

Effects of Leukemia on the Body

The first effect of leukemia is metastatic growth of leukemic cells in abnormal areas of the body. Leukemic cells from the bone marrow may reproduce so greatly that they invade the surrounding bone, causing pain and, eventually, a tendency for bones to fracture easily.

Almost all leukemias eventually spread to the spleen, lymph nodes, liver, and other vascular regions, regardless of whether the origin of the leukemia is in the bone marrow or the lymph nodes. Common effects in leukemia are

the development of infection, severe anemia, and a bleeding tendency caused by thrombocytopenia (lack of platelets). These effects result mainly from displacement of the normal bone marrow and lymphoid cells by the nonfunctional leukemic cells.

Finally, an important effect of leukemia on the body is excessive use of metabolic substrates by the growing cancerous cells. The leukemic tissues reproduce new cells so rapidly that tremendous demands are made on the body reserves for foodstuffs, specific amino acids, and vitamins. Consequently, the energy of the patient is greatly depleted, and excessive utilization of amino acids by the leukemic cells causes especially rapid deterioration of the normal protein tissues of the body. Thus, while the leukemic tissues grow, other tissues become debilitated. After metabolic starvation has continued long enough, this alone is sufficient to cause death.

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Resistance of the Body to Infection: II. Immunity and Allergy Innate Immunity

The human body has the ability to resist almost all types of organisms or toxins that tend to damage the tissues and organs. This capability is called *immunity.* Much of immunity is *acquired immunity* that does not develop until after the body is first attacked by a bacterium, virus, or toxin, often requiring weeks or months to develop the immunity. An additional portion of immunity results from general processes, rather than from processes directed at specific disease organisms. This is called *innate immunity.* It includes the following:

1. Phagocytosis of bacteria and other invaders by white blood cells and cells of the tissue macrophage system, as described in Chapter 33.
2. Destruction of swallowed organisms by the acid secretions of the stomach and the digestive enzymes.
3. Resistance of the skin to invasion by organisms.
4. Presence in the blood of certain chemical compounds that attach to foreign organisms or toxins and destroy them. Some of these compounds are (1) *lysozyme,* a mucolytic polysaccharide that attacks bacteria and causes them to dissolute; (2) *basic polypeptides,* which react with and inactivate certain types of gram-positive bacteria; (3) *the complement complex* that is described later, a system of about 20 proteins that can be activated in various ways to destroy bacteria; and (4) *natural killer lymphocytes* that can recognize and destroy foreign cells, tumor cells, and even some infected cells.

This innate immunity makes the human body resistant to such diseases as some paralytic viral infections of animals, hog cholera, cattle plague, and distemper—a viral disease that kills a large percentage of dogs that become afflicted with it. Conversely, many lower animals are resistant or even immune to many human diseases, such as poliomyelitis, mumps, human cholera, measles, and syphilis, which are very damaging or even lethal to human beings.

Acquired (Adaptive) Immunity

In addition to its generalized innate immunity, the human body has the ability to develop extremely powerful specific immunity against individual invading agents such as lethal bacteria, viruses, toxins, and even foreign tissues from other animals. This is called *acquired* or *adaptive immunity.* Acquired immunity is caused by a special immune system that forms antibodies and/or activated lymphocytes that attack and destroy the specific invading organism or toxin. It is with this acquired immunity mechanism and some of its associated reactions, especially the allergies, that this chapter is concerned.

Acquired immunity can often bestow extreme protection. For instance, certain toxins, such as the paralytic botulinum toxin or the tetanizing toxin of tetanus, can be protected against in doses as high as 100,000 times the amount that would be lethal without immunity. This is the reason the treatment process known as *immunization* is so important in protecting human beings against disease and against toxins, as explained in the course of this chapter.

Basic Types of Acquired Immunity—Humoral and Cell-Mediated

Two basic but closely allied types of acquired immunity occur in the body. In one of these the body develops circulating antibodies, which are globulin molecules in the blood plasma that are capable of attacking the invading agent. This type of immunity is called *humoral immunity* or *B-cell immunity* (because B lymphocytes produce the antibodies). The second type of acquired immunity is achieved through the formation of large numbers of activated *T lymphocytes* that are specifically crafted in the lymph nodes to destroy the foreign agent. This type of immunity is called *cell-mediated immunity* or *T-cell immunity* (because the activated lymphocytes are T lymphocytes). We shall see shortly that both the antibodies and the activated lymphocytes are formed in the lymphoid tissues of the body. Let us discuss the initiation of the immune process by *antigens.*

Both Types of Acquired Immunity Are Initiated by Antigens

Because acquired immunity does not develop until after invasion by a foreign organism or toxin, it is clear that the body must have some mechanism for recognizing this invasion. Each toxin or each type of organism almost always contains one or more specific chemical compounds in its makeup that are different from all other compounds. In general, these are proteins or large polysaccharides, and it is they that initiate the acquired immunity. These substances are called *antigens* (*anti*body *gen*erations).

For a substance to be antigenic, it usually must have a high molecular weight, 8000 or greater. Furthermore, the process of antigenicity usually depends on regularly recurring molecular groups, called *epitopes,* on the surface of the large molecule. This also explains why proteins and large polysaccharides are almost always antigenic, because both of these have this stereochemical characteristic.

Lymphocytes Are Responsible for Acquired Immunity

Acquired immunity is the product of the body's lymphocytes. In people who have a genetic lack of lymphocytes or whose lymphocytes have been destroyed by radiation or chemicals, no acquired immunity can develop. And within days after birth, such a person dies of fulminating bacterial infection unless treated by heroic measures. Therefore, it is clear that the lymphocytes are essential to survival of the human being.

The lymphocytes are located most extensively in the lymph nodes, but they are also found in special lymphoid tissues such as the spleen, submucosal areas of the gastrointestinal tract, thymus, and bone marrow. The lymphoid tissue is distributed advantageously in the body to intercept invading organisms or toxins before they can spread too widely.

In most instances, the invading agent first enters the tissue fluids and then is carried by lymph vessels to the lymph node or other lymphoid tissue. For instance, the lymphoid tissue of the gastrointestinal walls is exposed immediately to antigens invading from the gut. The lymphoid tissue of the throat and pharynx (the tonsils and adenoids) is well located to intercept antigens that enter by way of the upper respiratory tract. The lymphoid tissue in the lymph nodes is exposed to antigens that invade the peripheral tissues of the body. And, finally, the lymphoid tissue of the spleen, thymus, and bone marrow plays the specific role of intercepting antigenic agents that have succeeded in reaching the circulating blood.

Two Types of Lymphocytes Promote "Cell-Mediated" Immunity or "Humoral" Immunity—the T and B Lymphocytes. Although most lymphocytes in normal lymphoid tissue look alike when studied under a microscope, these cells are distinctly divided into two major populations. One of the populations, the T lymphocytes, is responsible for forming the activated lymphocytes that provide "cell-mediated" immunity, and the other population, the B lymphocytes, is responsible for forming antibodies that provide "humoral" immunity.

Both types of lymphocytes are derived originally in the embryo from *pluripotent hematopoietic stem cells* that form *common lymphoid progenitor cells* as one of their most important offspring as they differentiate. Almost all of the lymphocytes that are formed eventually end up in the lymphoid tissue, but before doing so, they are further differentiated or "preprocessed" in the following ways.

The lymphoid progenitor cells that are destined to eventually form activated T lymphocytes first migrate to and are preprocessed in the thymus gland, and thus they are called "T" lymphocytes to designate the role of the thymus. They are responsible for cell-mediated immunity.

The other population of lymphocytes—the B lymphocytes that are destined to form antibodies—are preprocessed in the liver during mid–fetal life and in the bone marrow in late fetal life and after birth. This population of cells was first discovered in birds, which have a special preprocessing organ called the *bursa of Fabricius.* For this reason, these lymphocytes are called "B" lymphocytes to designate the role of the bursa, and they are responsible for humoral immunity. Figure 34-1 shows the two lymphocyte systems for the formation, respectively, of (1) the activated T lymphocytes and (2) the antibodies.

Preprocessing of the T and B Lymphocytes

Although all lymphocytes in the body originate from *lymphocyte-committed stem cells* of the embryo, these stem cells themselves are incapable of forming directly either activated T lymphocytes or antibodies. Before they can do so, they must be further differentiated in appropriate processing areas as follows.

Thymus Gland Preprocesses the T Lymphocytes. The T lymphocytes, after origination in the bone marrow, first migrate to the thymus gland. Here they divide rapidly and at the same time develop extreme diversity for reacting against different specific antigens. That is, one thymic lymphocyte develops specific reactivity against one antigen. Then the next lymphocyte develops specificity against another antigen. This continues until there are thousands of different types of thymic lymphocytes with specific reactivities against many thousands of different antigens. These different types of preprocessed T lymphocytes now leave the thymus and spread by way of the blood throughout the body to lodge in lymphoid tissue everywhere.

The thymus also makes certain that any T lymphocytes leaving the thymus will not react against proteins or other antigens that are present in the body's own tissues; otherwise, the T lymphocytes would be lethal to the person's own body in only a few days. The thymus selects which T lymphocytes will be released by first mixing them with virtually all the specific "self-antigens" from the body's own tissues. If a T lymphocyte reacts, it is destroyed and phagocytized instead of being released. This happens to

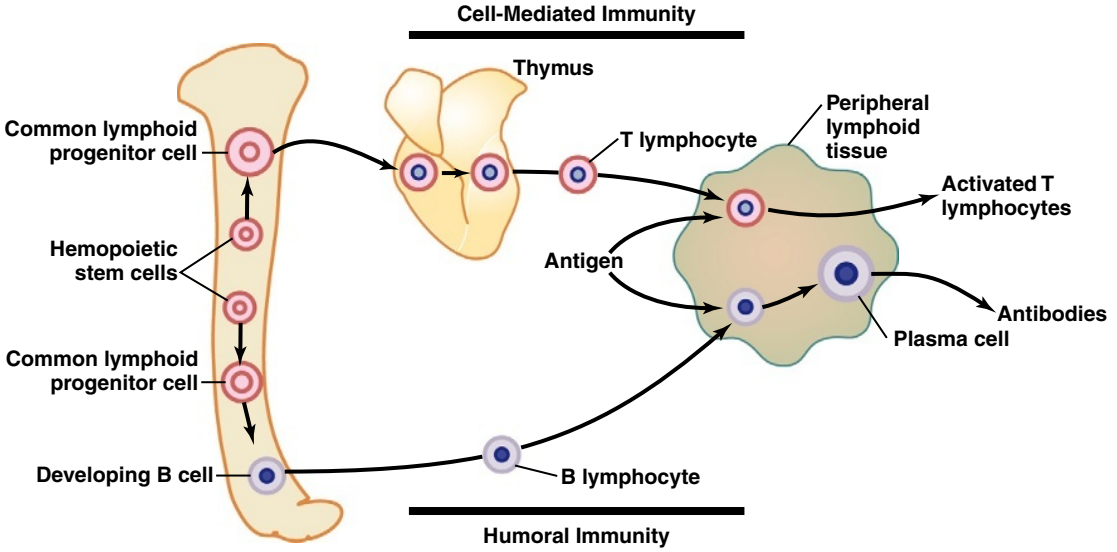

Figure 34-1 Formation of antibodies and sensitized lymphocytes by a lymph node in response to antigens. This figure also shows the origin of thymic (*T*) and bursal (*B*) lymphocytes that respectively are responsible for the cell-mediated and humoral immune processes.

up to 90 percent of the cells. Thus, the only cells that are finally released are those that are nonreactive against the body's own antigens—they react only against antigens from an outside source, such as from a bacterium, a toxin, or even transplanted tissue from another person.

Most of the preprocessing of T lymphocytes in the thymus occurs shortly before birth of a baby and for a few months after birth. Beyond this period, removal of the thymus gland diminishes (but does not eliminate) the T-lymphocytic immune system. However, removal of the thymus several months before birth can prevent development of all cell-mediated immunity. Because this cellular type of immunity is mainly responsible for rejection of transplanted organs, such as hearts and kidneys, one can transplant organs with much less likelihood of rejection if the thymus is removed from an animal a reasonable time before its birth.

Liver and Bone Marrow Preprocess the B Lymphocytes. Much less is known about the details for preprocessing B lymphocytes than for preprocessing T lymphocytes. In the human being, B lymphocytes are known to be preprocessed in the liver during mid–fetal life and in the bone marrow during late fetal life and after birth.

B lymphocytes are different from T lymphocytes in two ways: First, instead of the whole cell developing reactivity against the antigen, as occurs for the T lymphocytes, the B lymphocytes actively secrete *antibodies* that are the reactive agents. These agents are large protein molecules that are capable of combining with and destroying the antigenic substance, which is explained elsewhere in this chapter and in Chapter 33. Second, the B lymphocytes have even greater diversity than the T lymphocytes, thus forming many millions of types of B-lymphocyte antibodies with different specific reactivities. After preprocessing, the B lymphocytes, like the T lymphocytes, migrate to lymphoid tissue throughout the body, where they lodge near but slightly removed from the T-lymphocyte areas.

T Lymphocytes and B-Lymphocyte Antibodies React Highly Specifically Against Specific Antigens—Role of Lymphocyte Clones

When specific antigens come in contact with T and B lymphocytes in the lymphoid tissue, certain of the T lymphocytes become activated to form activated T cells, and certain of the B lymphocytes become activated to form antibodies. The activated T cells and antibodies in turn react highly specifically against the particular types of antigens that initiated their development. The mechanism of this specificity is the following.

Millions of Specific Types of Lymphocytes Are Stored in the Lymphoid Tissue. Millions of different types of preformed B lymphocytes and preformed T lymphocytes that are capable of forming highly specific types of antibodies or T cells have been stored in the lymph tissue, as explained earlier. Each of these preformed lymphocytes is capable of forming only one type of antibody or one type of T cell with a single type of specificity. And only the specific type of antigen with which it can react can activate it. Once the specific lymphocyte is activated by its antigen, it reproduces wildly, forming tremendous numbers of duplicate lymphocytes (Figure 34-2). If it is a B lymphocyte, its progeny will eventually secrete the specific type of antibody that then circulates throughout the body. If it is a T lymphocyte, its progeny are specific sensitized T cells that are released into the lymph and then carried to the blood and circulated through all the tissue fluids and back into the lymph, sometimes circulating around and around in this circuit for months or years.

All the different lymphocytes that are capable of forming one specific antibody or T cell are called a *clone of lymphocytes*. That is, the lymphocytes in each clone are

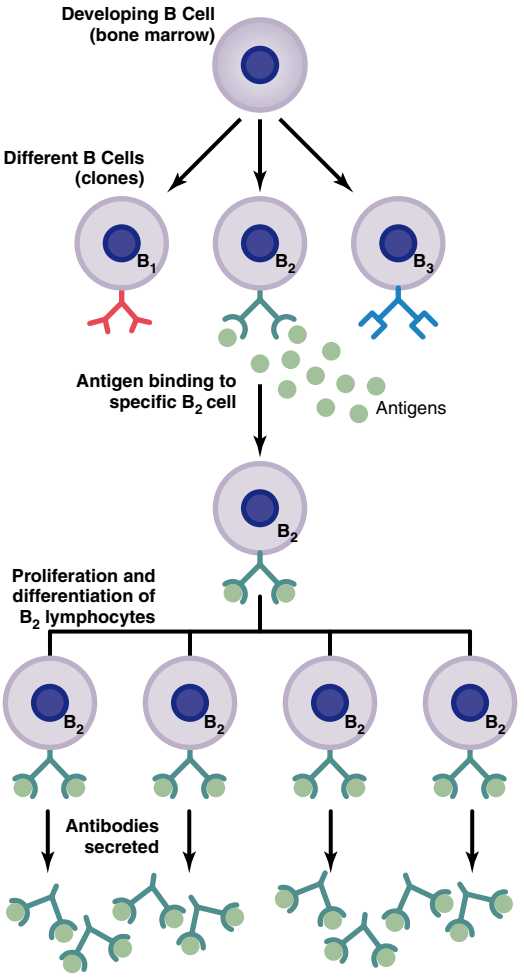

Figure 34-2 An antigen activates only the lymphocytes that have cell surface receptors that are complementary and recognize a specific antigen. Millions of different clones of lymphocytes exist (shown as *B1*, *B2,* and *B3*). When the lymphocyte clone (*B2* in this example) is activated by its antigen, it reproduces to form large numbers of duplicate lymphocytes, which then secrete antibodies.

alike and are derived originally from one or a few early lymphocytes of its specific type.

Origin of the Many Clones of Lymphocytes

Only several hundred to a few thousand genes code for the millions of different types of antibodies and T lymphocytes. At first, it was a mystery how it was possible for so few genes to code for the millions of different specificities of antibody molecules or T cells that can be produced by the lymphoid tissue, especially when one considers that a single gene is usually necessary for the formation of each different type of protein. This mystery has now been solved.

The whole gene for forming each type of T cell or B cell is never present in the original stem cells from which the functional immune cells are formed. Instead, there are only "gene segments"—actually, hundreds of such segments—but not whole genes. During preprocessing of the respective T- and B-cell lymphocytes, these gene segments become mixed with one another in random combinations, in this way finally forming whole genes.

Because there are several hundred types of gene segments, as well as millions of different combinations in which the segments can be arranged in single cells, one can understand the millions of different cell gene types that can occur. For each functional T or B lymphocyte that is finally formed, the gene structure codes for only a single antigen specificity. These mature cells then become the highly specific T and B cells that spread to and populate the lymphoid tissue.

Mechanism for Activating a Clone of Lymphocytes

Each clone of lymphocytes is responsive to only a single type of antigen (or to several similar antigens that have almost exactly the same stereochemical characteristics). The reason for this is the following: In the case of the B lymphocytes, each of these has on the surface of its cell membrane about 100,000 antibody molecules that will react highly specifically with only one specific type of antigen. Therefore, when the appropriate antigen comes along, it immediately attaches to the antibody in the cell membrane; this leads to the activation process, which we describe in more detail subsequently. In the case of the T lymphocytes, molecules similar to antibodies, called *surface receptor proteins* (or *T-cell markers*), are on the surface of the T-cell membrane, and these are also highly specific for one specified activating antigen. An antigen therefore stimulates only those cells that have complementary receptors for the antigen and are already committed to respond to it.

Role of Macrophages in the Activation Process. Aside from the lymphocytes in lymphoid tissue, literally millions of macrophages are also present in the same tissue. These line the sinusoids of the lymph nodes, spleen, and other lymphoid tissue, and they lie in apposition to many of the lymph node lymphocytes. Most invading organisms are first phagocytized and partially digested by the macrophages, and the antigenic products are liberated into the macrophage cytosol. The macrophages then pass these antigens by cell-to-cell contact directly to the lymphocytes, thus leading to activation of the specified lymphocytic clones. The macrophages, in addition, secrete a special activating substance, *interleukin-1*, that promotes still further growth and reproduction of the specific lymphocytes.

Role of the T Cells in Activation of the B Lymphocytes. Most antigens activate both T lymphocytes and B lymphocytes at the same time. Some of the T cells that are formed, called *helper cells,* secrete specific substances (collectively called *lymphokines*) that activate the specific B lymphocytes. Indeed, without the aid of these helper T cells, the quantity of antibodies formed by the B lymphocytes is usually slight. We discuss this cooperative relationship between helper T cells and B cells after we describe the mechanisms of the T-cell system of immunity.

Specific Attributes of the B-Lymphocyte System—Humoral Immunity and the Antibodies

Formation of Antibodies by Plasma Cells. Before exposure to a specific antigen, the clones of B lymphocytes remain dormant in the lymphoid tissue. On entry of a foreign antigen, macrophages in the lymphoid tissue phagocytize the antigen and then present it to adjacent B lymphocytes. In addition, the antigen is presented to T cells at the same time, and activated helper T cells are formed. These helper cells also contribute to extreme activation of the B lymphocytes, as we discuss more fully later.

Those B lymphocytes specific for the antigen immediately enlarge and take on the appearance of *lymphoblasts*. Some of the lymphoblasts further differentiate to form plasmablasts, which are precursors of plasma cells. In the *plasmablasts,* the cytoplasm expands and the rough endoplasmic reticulum vastly proliferates. The plasmablasts then begin to divide at a rate of about once every 10 hours for about nine divisions, giving in 4 days a total population of about 500 cells for each original plasmablast. The mature plasma cell then produces gamma globulin antibodies at an extremely rapid rate—about 2000 molecules per second for each plasma cell. In turn, the antibodies are secreted into the lymph and carried to the circulating blood. This process continues for several days or weeks until finally exhaustion and death of the plasma cells occur.

Formation of "Memory" Cells—Difference Between Primary Response and Secondary Response. A few of the lymphoblasts formed by activation of a clone of B lymphocytes do not go on to form plasma cells but instead form moderate numbers of new B lymphocytes similar to those of the original clone. In other words, the B-cell population of the specifically activated clone becomes greatly enhanced, and the new B lymphocytes are added to the original lymphocytes of the same clone. They also circulate throughout the body to populate all the lymphoid tissue; immunologically, however, they remain dormant until activated once again by a new quantity of the same antigen. These lymphocytes are called *memory cells.* Subsequent exposure to the same antigen will cause a much more rapid and much more potent antibody response this second time around, because there are many more memory cells than there were original B lymphocytes of the specific clone.

Figure 34-3 shows the differences between the primary response for forming antibodies that occurs on first exposure to a specific antigen and the secondary response that occurs after second exposure to the same antigen. Note the 1-week delay in the appearance of the primary response, its weak potency, and its short life. The secondary response, by contrast, begins rapidly after exposure to the antigen (often within hours), is far more potent, and forms antibodies for many months rather than for only a few weeks. The increased potency and duration of the secondary response explain why immunization is usually accomplished by injecting antigen in multiple doses with periods of several weeks or several months between injections.

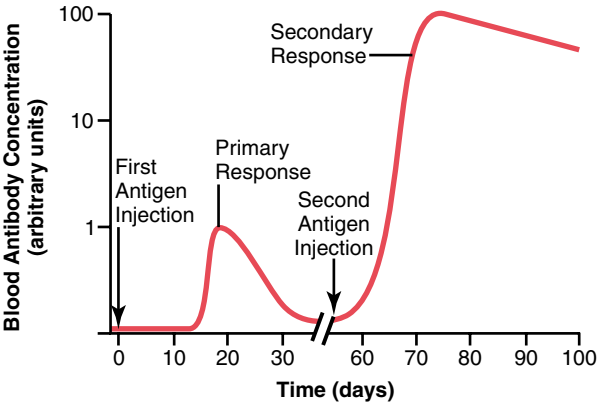

Figure 34-3 Time course of the antibody response in the circulating blood to a primary injection of antigen and to a secondary injection several weeks later.

Nature of the Antibodies

The antibodies are gamma globulins called *immunoglobulins* (abbreviated as *Ig*), and they have molecular weights between 160,000 and 970,000. They usually constitute about 20 percent of all the plasma proteins.

All the immunoglobulins are composed of combinations of *light* and *heavy polypeptide chains*. Most are a combination of two light and two heavy chains, as shown in Figure 34-4. However, some of the immunoglobulins have combinations of as many as 10 heavy and 10 light chains, which give rise to high-molecular-weight immunoglobulins. Yet, in all immunoglobulins, each heavy chain is paralleled by a light chain at one of its ends, thus forming a heavy-light pair, and there are always at least 2 and as many as 10 such pairs in each immunoglobulin molecule.

Figure 34-4 shows a designated end of each light and heavy chain, called the *variable portion*; the remainder of

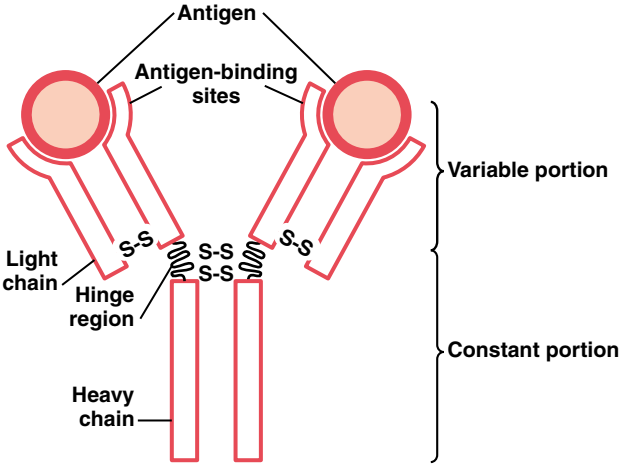

Figure 34-4 Structure of the typical IgG antibody, showing it to be composed of two heavy polypeptide chains and two light polypeptide chains. The antigen binds at two different sites on the variable portions of the chains.

each chain is called the *constant portion.* The variable portion is different for each specificity of antibody, and it is this portion that attaches specifically to a particular type of antigen. The constant portion of the antibody determines other properties of the antibody, establishing such factors as diffusivity of the antibody in the tissues, adherence of the antibody to specific structures within the tissues, attachment to the complement complex, the ease with which the antibodies pass through membranes, and other biological properties of the antibody. A combination of noncovalent and covalent bonds (disulfide) holds the light and heavy chains together.

Specificity of Antibodies. Each antibody is specific for a particular antigen; this is caused by its unique structural organization of amino acids in the variable portions of both the light and heavy chains. The amino acid organization has a different steric shape for each antigen specificity, so when an antigen comes in contact with it, multiple prosthetic groups of the antigen fit as a mirror image with those of the antibody, thus allowing rapid and tight bonding between the antibody and the antigen. When the antibody is highly specific, there are so many bonding sites that the antibody-antigen coupling is exceedingly strong, held together by (1) hydrophobic bonding, (2) hydrogen bonding, (3) ionic attractions, and (4) van der Waals forces. It also obeys the thermodynamic mass action law.

$$K_a = \frac{\text{Concentration of bound antibody-antigen}}{\text{Concentration of antibody} \times \text{Concentration of antigen}}$$

Ka is called the *affinity constant* and is a measure of how tightly the antibody binds with the antigen.

Note, especially, in Figure 34-4 that there are two variable sites on the illustrated antibody for attachment of antigens, making this type of antibody bivalent. A small proportion of the antibodies, which consist of combinations of up to 10 light and 10 heavy chains, have as many as 10 binding sites.

Classes of Antibodies. There are five general classes of antibodies, respectively named *IgM, IgG, IgA, IgD,* and *IgE.* Ig stands for immunoglobulin, and the other five respective letters designate the respective classes.

For the purpose of our present limited discussion, two of these classes of antibodies are of particular importance: IgG, which is a bivalent antibody and constitutes about 75 percent of the antibodies of the normal person, and IgE, which constitutes only a small percentage of the antibodies but is especially involved in allergy. The IgM class is also interesting because a large share of the antibodies formed during the primary response are of this type. These antibodies have 10 binding sites that make them exceedingly effective in protecting the body against invaders, even though there are not many IgM antibodies.

Mechanisms of Action of Antibodies

Antibodies act mainly in two ways to protect the body against invading agents: (1) by direct attack on the invader and (2) by activation of the "complement system" that then has multiple means of its own for destroying the invader.

Direct Action of Antibodies on Invading Agents. Figure 34-5 shows antibodies (designated by the red Y-shaped bars) reacting with antigens (designated by the shaded objects). Because of the bivalent nature of the antibodies and the multiple antigen sites on most invading agents, the antibodies can inactivate the invading agent in one of several ways, as follows:

1. *Agglutination*, in which multiple large particles with antigens on their surfaces, such as bacteria or red cells, are bound together into a clump
2. *Precipitation*, in which the molecular complex of soluble antigen (such as tetanus toxin) and antibody becomes so large that it is rendered insoluble and precipitates
3. *Neutralization*, in which the antibodies cover the toxic sites of the antigenic agent
4. *Lysis*, in which some potent antibodies are occasionally capable of directly attacking membranes of cellular agents and thereby cause rupture of the agent

These direct actions of antibodies attacking the antigenic invaders often are not strong enough to play a major role in protecting the body against the invader. Most of the protection comes through the amplifying effects of the complement system described next.

Complement System for Antibody Action

"Complement" is a collective term that describes a system of about 20 proteins, many of which are enzyme precursors. The principal actors in this system are 11 proteins designated C1 through C9, B, and D, shown in Figure 34-6. All these are present normally among the plasma proteins in the blood, as well as among the proteins that leak out of the capillaries into the tissue spaces. The enzyme precursors are normally inactive, but they can be activated mainly by the so-called *classic pathway*.

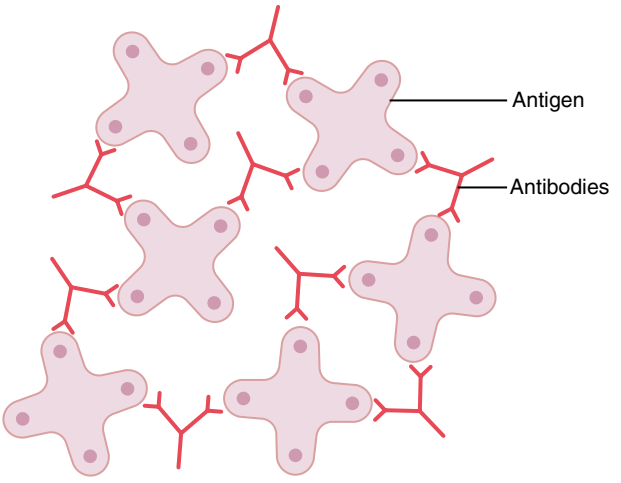

Figure 34-5 Binding of antigen molecules to one another by bivalent antibodies.

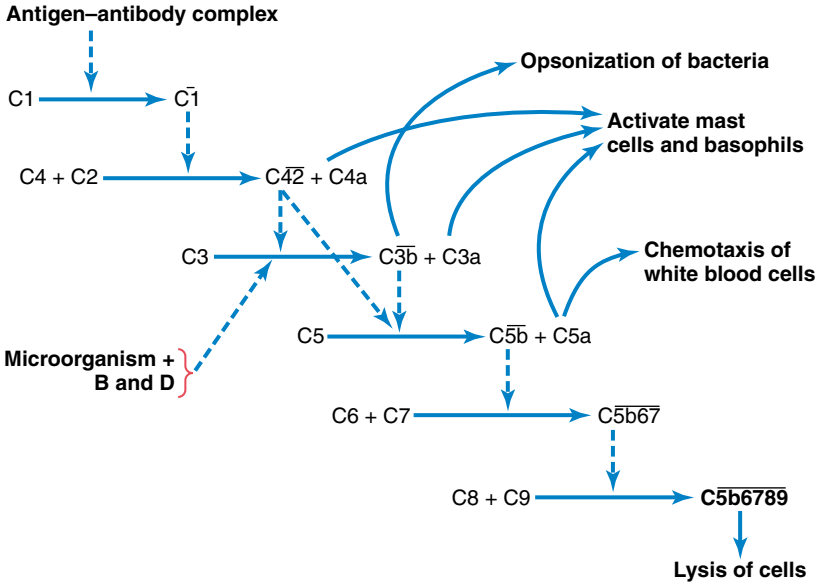

Figure 34-6 Cascade of reactions during activation of the classic pathway of complement. (Modified from Alexander JW, Good RA: Fundamentals of Clinical Immunology. Philadelphia: WB Saunders, 1977.)

Classic Pathway. The classic pathway is initiated by an antigen-antibody reaction. That is, when an antibody binds with an antigen, a specific reactive site on the "constant" portion of the antibody becomes uncovered, or "activated," and this in turn binds directly with the C1 molecule of the complement system, setting into motion a "cascade" of sequential reactions, shown in Figure 34-6, beginning with activation of the proenzyme C1 itself. The C1 enzymes that are formed then activate successively increasing quantities of enzymes in the later stages of the system so that from a small beginning, an extremely large "amplified" reaction occurs. Multiple end products are formed, as shown to the right in the figure, and several of these cause important effects that help to prevent damage to the body's tissues caused by the invading organism or toxin. Among the more important effects are the following:

1. *Opsonization and phagocytosis.* One of the products of the complement cascade, C3b, strongly activates phagocytosis by both neutrophils and macrophages, causing these cells to engulf the bacteria to which the antigen-antibody complexes are attached. This process is called *opsonization.* It often enhances the number of bacteria that can be destroyed by many hundredfold.
2. *Lysis.* One of the most important of all the products of the complement cascade is the lytic complex, which is a combination of multiple complement factors and designated C5b6789. This has a direct effect of rupturing the cell membranes of bacteria or other invading organisms.
3. *Agglutination.* The complement products also change the surfaces of the invading organisms, causing them to adhere to one another, thus promoting agglutination.
4. *Neutralization of viruses.* The complement enzymes and other complement products can attack the structures of some viruses and thereby render them nonvirulent.
5. *Chemotaxis.* Fragment C5a initiates chemotaxis of neutrophils and macrophages, thus causing large numbers of these phagocytes to migrate into the tissue area adjacent to the antigenic agent.
6. *Activation of mast cells and basophils.* Fragments C3a, C4a, and C5a activate mast cells and basophils, causing them to release histamine, heparin, and several other substances into the local fluids. These substances in turn cause increased local blood flow, increased leakage of fluid and plasma protein into the tissue, and other local tissue reactions that help inactivate or immobilize the antigenic agent. The same factors play a major role in inflammation (which was discussed in Chapter 33) and in allergy, as we discuss later.
7. *Inflammatory effects.* In addition to inflammatory effects caused by activation of the mast cells and basophils, several other complement products contribute to local inflammation. These products cause (1) the already increased blood flow to increase still further, (2) the capillary leakage of proteins to be increased, and (3) the interstitial fluid proteins to coagulate in the tissue spaces, thus preventing movement of the invading organism through the tissues.

Special Attributes of the T-Lymphocyte System—Activated T Cells and Cell-Mediated Immunity

Release of Activated T Cells from Lymphoid Tissue and Formation of Memory Cells. On exposure to the proper antigen, as presented by adjacent macrophages, the T lymphocytes of a specific lymphocyte clone proliferate and release large numbers of activated, specifically reacting T cells in ways that parallel antibody release by activated B cells. The principal difference is that instead of releasing antibodies, whole activated T cells are formed and released into the lymph. These then pass into the

circulation and are distributed throughout the body, passing through the capillary walls into the tissue spaces, back into the lymph and blood once again, and circulating again and again throughout the body, sometimes lasting for months or even years.

Also, *T-lymphocyte memory cells* are formed in the same way that B memory cells are formed in the antibody system. That is, when a clone of T lymphocytes is activated by an antigen, many of the newly formed lymphocytes are preserved in the lymphoid tissue to become additional T lymphocytes of that specific clone; in fact, these memory cells even spread throughout the lymphoid tissue of the entire body. Therefore, on subsequent exposure to the same antigen anywhere in the body, release of activated T cells occurs far more rapidly and much more powerfully than had occurred during first exposure.

Antigen-Presenting Cells, MHC Proteins, and Antigen Receptors on the T Lymphocytes. T-cell responses are extremely antigen specific, like the antibody responses of B cells, and are at least as important as antibodies in defending against infection. In fact, acquired immune responses usually require assistance from T cells to begin the process, and T cells play a major role in actually helping to eliminate invading pathogens.

Although B lymphocytes recognize intact antigens, T lymphocytes respond to antigens only when they are bound to specific molecules called *MHC proteins* on the surface of *antigen-presenting cells* in the lymphoid tissues (Figure 34-7). The three major types of antigen-presenting cells are *macrophages*, *B lymphocytes*, and *dendritic cells*. The dendritic cells, the most potent of the antigen-presenting cells, are located throughout the body, and their only known function is to present antigens to T cells. Interaction of cell adhesion proteins is critical in permitting the T cells to bind to antigen-presenting cells long enough to become activated.

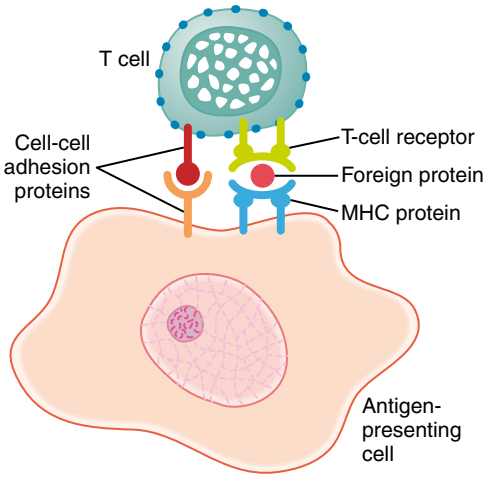

Figure 34-7 Activation of T cells requires interaction of T-cell receptors with an antigen (foreign protein) that is transported to the surface of the antigen-presenting cell by a major histocompatibility complex (MHC) protein. Cell-to-cell adhesion proteins enable the T cell to bind to the antigen-presenting cell long enough to become activated.

The MHC proteins are encoded by a large group of genes called the *major histocompatibility complex (MHC)*. The MHC proteins bind peptide fragments of antigen proteins that are degraded inside antigen-presenting cells and then transport them to the cell surface. There are two types of MHC proteins: (1) *MHC I proteins,* which present antigens to *cytotoxic T cells*, and (2) *MHC II proteins,* which present antigens to *T helper cells*. The specific functions of cytotoxic and helper T cells are discussed later.

The antigens on the surface of antigen-presenting cells bind with receptor molecules on the surfaces of T cells in the same way that they bind with plasma protein antibodies. These receptor molecules are composed of a variable unit similar to the variable portion of the humoral antibody, but its stem section is firmly bound to the cell membrane of the T lymphocyte. There are as many as 100,000 receptor sites on a single T cell.

Several Types of T Cells and Their Different Functions

It has become clear that there are multiple types of T cells. They are classified into three major groups: (1) *helper T cells*, (2) *cytotoxic T cells*, and (3) *suppressor T cells.* The functions of each of these are distinct.

Helper T Cells—Their Role in Overall Regulation of Immunity

The helper T cells are by far the most numerous of the T cells, usually constituting more than three quarters of all of them. As their name implies, they help in the functions of the immune system, and they do so in many ways. In fact, they serve as the major regulator of virtually all immune functions, as shown in Figure 34-8. They do this by forming a series of protein mediators, called *lymphokines*, that act on other cells of the immune system, as well as on bone marrow cells. Among the important lymphokines secreted by the helper T cells are the following:

Interleukin-2
Interleukin-3
Interleukin-4
Interleukin-5
Interleukin-6
Granulocyte-monocyte colony-stimulating factor
Interferon-γ

Specific Regulatory Functions of the Lymphokines. In the absence of the lymphokines from the helper T cells, the remainder of the immune system is almost paralyzed. In fact, it is the helper T cells that are inactivated or destroyed by the *acquired immunodeficiency syndrome (AIDS) virus*, which leaves the body almost totally unprotected against infectious disease, therefore leading

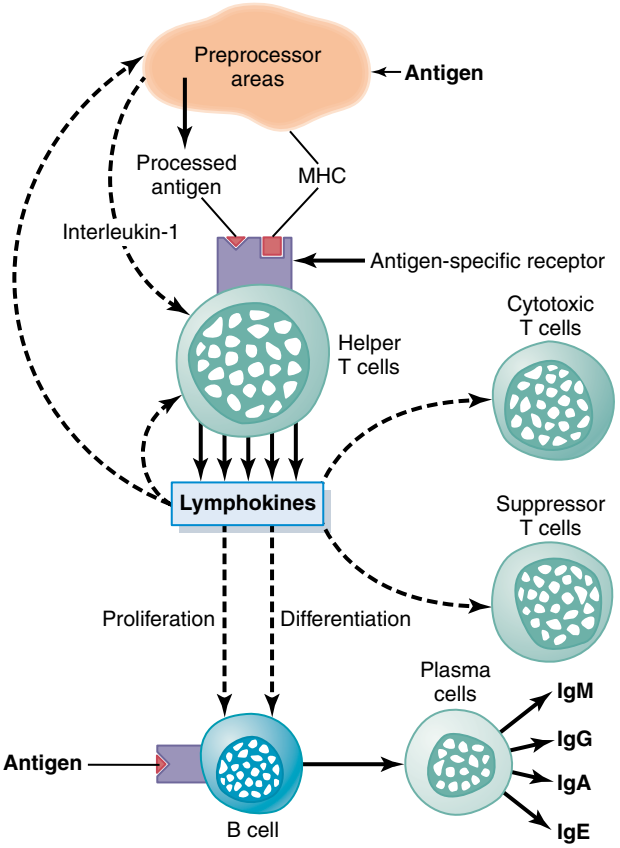

Figure 34-8 Regulation of the immune system, emphasizing a pivotal role of the helper T cells. MHC, major histocompatibility complex.

to the now well-known debilitating and lethal effects of AIDS. Some of the specific regulatory functions are the following.

Stimulation of Growth and Proliferation of Cytotoxic T Cells and Suppressor T Cells. In the absence of helper T cells, the clones for producing cytotoxic T cells and suppressor T cells are activated only slightly by most antigens. The lymphokine interleukin-2 has an especially strong stimulatory effect in causing growth and proliferation of both cytotoxic and suppressor T cells. In addition, several of the other lymphokines have less potent effects.

Stimulation of B-Cell Growth and Differentiation to Form Plasma Cells and Antibodies. The direct actions of antigen to cause B-cell growth, proliferation, formation of plasma cells, and secretion of antibodies are also slight without the "help" of the helper T cells. Almost all the interleukins participate in the B-cell response, but especially interleukins 4, 5, and 6. In fact, these three interleukins have such potent effects on the B cells that they have been called B-cell stimulating factors or B-cell growth factors.

Activation of the Macrophage System. The lymphokines also affect the macrophages. First, they slow or stop the migration of the macrophages after they have been chemotactically attracted into the inflamed tissue area, thus causing great accumulation of macrophages. Second, they activate the macrophages to cause far more efficient phagocytosis, allowing them to attack and destroy increasing numbers of invading bacteria or other tissue-destroying agents.

Feedback Stimulatory Effect on the Helper Cells Themselves. Some of the lymphokines, especially interleukin-2, have a direct positive feedback effect in stimulating activation of the helper T cells themselves. This acts as an amplifier by further enhancing the helper cell response, as well as the entire immune response to an invading antigen.

Cytotoxic T Cells Are "Killer" Cells

The cytotoxic T cell is a direct-attack cell that is capable of killing microorganisms and, at times, even some of the body's own cells. For this reason, these cells are called *killer cells.* The receptor proteins on the surfaces of the cytotoxic cells cause them to bind tightly to those organisms or cells that contain the appropriate binding-specific antigen. Then, they kill the attacked cell in the manner shown in Figure 34-9. After binding, the cytotoxic T cell secretes hole-forming proteins, called *perforins,* that literally punch round holes in the membrane of the attacked cell. Then fluid flows rapidly into the cell from the interstitial space. In addition, the cytotoxic T cell releases cytotoxic substances directly into the attacked cell. Almost immediately, the attacked cell becomes greatly swollen, and it usually dissolves shortly thereafter.

Especially important, these cytotoxic killer cells can pull away from the victim cells after they have punched holes and delivered cytotoxic substances and then move on to kill more cells. Indeed, some of these cells persist for months in the tissues.

Some of the cytotoxic T cells are especially lethal to tissue cells that have been invaded by viruses because many virus particles become entrapped in the membranes of the tissue cells and attract T cells in response to the viral antigenicity. The cytotoxic cells also play an important role in destroying cancer cells, heart transplant cells, or other types of cells that are foreign to the person's own body.

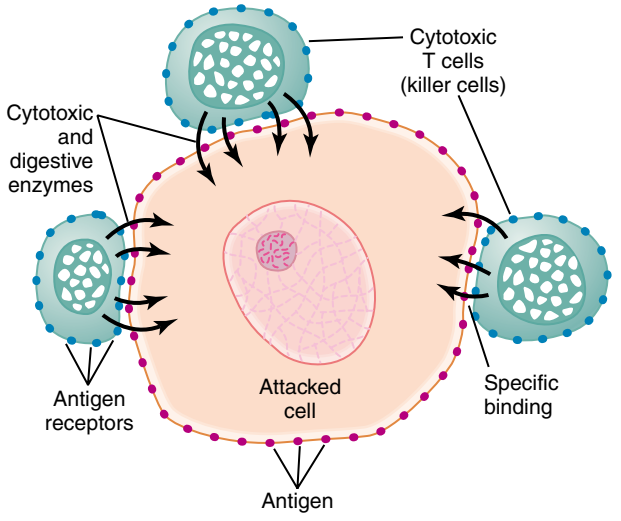

Figure 34-9 Direct destruction of an invading cell by sensitized lymphocytes (cytotoxic T cells).

Suppressor T Cells

Much less is known about the suppressor T cells than about the others, but they are capable of suppressing the functions of both cytotoxic and helper T cells. It is believed that these suppressor functions serve the purpose of preventing the cytotoxic cells from causing excessive immune reactions that might be damaging to the body's own tissues. For this reason, the suppressor cells are classified, along with the helper T cells, as *regulatory T cells*. It is probable that the suppressor T-cell system plays an important role in limiting the ability of the immune system to attack a person's own body tissues, called *immune tolerance*, as we discuss in the next section.

Tolerance of the Acquired Immunity System to One's Own Tissues—Role of Preprocessing in the Thymus and Bone Marrow

If a person should become immune to his or her own tissues, the process of acquired immunity would destroy the individual's own body. The immune mechanism normally "recognizes" a person's own tissues as being distinctive from bacteria or viruses, and the person's immunity system forms few antibodies or activated T cells against his or her own antigens.

Most Tolerance Results from Clone Selection During Preprocessing. It is believed that most tolerance develops during preprocessing of T lymphocytes in the thymus and of B lymphocytes in the bone marrow. The reason for this belief is that injecting a strong antigen into a fetus while the lymphocytes are being preprocessed in these two areas prevents development of clones of lymphocytes in the lymphoid tissue that are specific for the injected antigen. Experiments have shown that specific immature lymphocytes in the thymus, when exposed to a strong antigen, become lymphoblastic, proliferate considerably, and then combine with the stimulating antigen—an effect that is believed to cause the cells themselves to be destroyed by the thymic epithelial cells before they can migrate to and colonize the total body lymphoid tissue.

It is believed that during the preprocessing of lymphocytes in the thymus and bone marrow, all or most of those clones of lymphocytes that are specific to damage the body's own tissues are self-destroyed because of their continual exposure to the body's antigens.

Failure of the Tolerance Mechanism Causes Autoimmune Diseases. Sometimes people lose their immune tolerance of their own tissues. This occurs to a greater extent the older a person becomes. It usually occurs after destruction of some of the body's own tissues, which releases considerable quantities of "self-antigens" that circulate in the body and presumably cause acquired immunity in the form of either activated T cells or antibodies.

Several specific diseases that result from autoimmunity include (1) *rheumatic fever*, in which the body becomes immunized against tissues in the joints and heart, especially the heart valves, after exposure to a specific type of streptococcal toxin that has an epitope in its molecular structure similar to the structure of some of the body's own self-antigens; (2) one type of *glomerulonephritis*, in which the person becomes immunized against the basement membranes of glomeruli; (3) *myasthenia gravis*, in which immunity develops against the acetylcholine receptor proteins of the neuromuscular junction, causing paralysis; and (4) *lupus erythematosus*, in which the person becomes immunized against many different body tissues at the same time, a disease that causes extensive damage and often rapid death.

Immunization by Injection of Antigens

Immunization has been used for many years to produce acquired immunity against specific diseases. A person can be immunized by injecting dead organisms that are no longer capable of causing disease but that still have some of their chemical antigens. This type of immunization is used to protect against typhoid fever, whooping cough, diphtheria, and many other types of bacterial diseases.

Immunity can be achieved against toxins that have been treated with chemicals so that their toxic nature has been destroyed even though their antigens for causing immunity are still intact. This procedure is used in immunizing against tetanus, botulism, and other similar toxic diseases.

And, finally, a person can be immunized by being infected with live organisms that have been "attenuated." That is, these organisms either have been grown in special culture media or have been passed through a series of animals until they have mutated enough that they will not cause disease but do still carry specific antigens required for immunization. This procedure is used to protect against smallpox, yellow fever, poliomyelitis, measles, and many other viral diseases.

Passive Immunity

Thus far, all the acquired immunity we have discussed has been *active immunity*. That is, the person's own body develops either antibodies or activated T cells in response to invasion of the body by a foreign antigen. However, temporary immunity can be achieved in a person without injecting any antigen. This is done by infusing antibodies, activated T cells, or both obtained from the blood of someone else or from some other animal that has been actively immunized against the antigen.

Antibodies last in the body of the recipient for 2 to 3 weeks, and during that time, the person is protected against the invading disease. Activated T cells last for a few weeks if transfused from another person but only for a few hours to a few days if transfused from an animal. Such transfusion of antibodies or T lymphocytes to confer immunity is called *passive immunity.*

Allergy and Hypersensitivity

An important undesirable side effect of immunity is the development, under some conditions, of allergy or other types of immune hypersensitivity. There are several types of allergy and other hypersensitivities, some of which occur only in people who have a specific allergic tendency.

Allergy Caused by Activated T Cells: Delayed-Reaction Allergy

Delayed-reaction allergy is caused by activated T cells and not by antibodies. In the case of poison ivy, the toxin of poison ivy in itself does not cause much harm to the tissues. However, on repeated exposure, it does cause the formation of activated helper and cytotoxic T cells. Then, after subsequent exposure to the poison ivy toxin, within a day or so, the activated T cells diffuse from the circulating blood in large numbers into the skin to respond to the poison ivy toxin. And, at the same time, these T cells elicit a cell-mediated type of immune reaction. Remembering that this type of immunity can cause release of many toxic substances from the activated T cells, as well as extensive invasion of the tissues by macrophages along with their subsequent effects, one can well understand that the eventual result of some delayed-reaction allergies can be serious tissue damage. The damage normally occurs in the tissue area where the instigating antigen is present, such as in the skin in the case of poison ivy, or in the lungs to cause lung edema or asthmatic attacks in the case of some airborne antigens.

Allergies in the "Allergic" Person Who Has Excess IgE Antibodies

Some people have an "allergic" tendency. Their allergies are called *atopic allergies* because they are caused by a nonordinary response of the immune system. The allergic tendency is genetically passed from parent to child and is characterized by the presence of large quantities of IgE antibodies in the blood. These antibodies are called *reagins* or *sensitizing antibodies* to distinguish them from the more common IgG antibodies. When an *allergen* (defined as an antigen that reacts specifically with a specific type of IgE reagin antibody) enters the body, an allergen-reagin reaction takes place and a subsequent allergic reaction occurs.

A special characteristic of the IgE antibodies (the reagins) is a strong propensity to attach to mast cells and basophils. Indeed, a single mast cell or basophil can bind as many as half a million molecules of IgE antibodies. Then, when an antigen (an allergen) that has multiple binding sites binds with several IgE antibodies that are already attached to a mast cell or basophil, this causes immediate change in the membrane of the mast cell or basophil, perhaps resulting from a physical effect of the antibody molecules to contort the cell membrane. At any rate, many of the mast cells and basophils rupture; others release special agents immediately or shortly thereafter, including *histamine, protease, slow-reacting substance of anaphylaxis* (which is a mixture of toxic leukotrienes), *eosinophil chemotactic substance, neutrophil chemotactic substance, heparin,* and *platelet activating factors*. These substances cause such effects as dilation of the local blood vessels; attraction of eosinophils and neutrophils to the reactive site; increased permeability of the capillaries with loss of fluid into the tissues; and contraction of local smooth muscle cells. Therefore, several different tissue responses can occur, depending on the type of tissue in which the allergen-reagin reaction occurs. Among the different types of allergic reactions caused in this manner are the following.

Anaphylaxis. When a specific allergen is injected directly into the circulation, the allergen can react with basophils of the blood and mast cells in the tissues located immediately outside the small blood vessels if the basophils and mast cells have been sensitized by attachment of IgE reagins. Therefore, a widespread allergic reaction occurs throughout the vascular system and closely associated tissues. This is called *anaphylaxis.* Histamine is released into the circulation and causes body-wide vasodilation, as well as increased permeability of the capillaries with resultant marked loss of plasma from the circulation. Occasionally, a person who experiences this reaction dies of circulatory shock within a few minutes unless treated with epinephrine to oppose the effects of the histamine.

Also released from the activated basophils and mast cells is a mixture of leukotrienes called *slow-reacting substance of anaphylaxis.* These leukotrienes can cause spasm of the smooth muscle of the bronchioles, eliciting an asthma-like attack, sometimes causing death by suffocation.

Urticaria. Urticaria results from antigen entering specific skin areas and causing localized anaphylactoid reactions. Histamine released locally causes (1) vasodilation that induces an immediate red flare and (2) increased local permeability of the capillaries that leads to local circumscribed areas of swelling of the skin within another few minutes. The swellings are commonly called *hives.* Administration of antihistamine drugs to a person before exposure will prevent the hives.

Hay Fever. In hay fever, the allergen-reagin reaction occurs in the nose. Histamine released in response to the reaction causes local intranasal vascular dilation, with resultant increased capillary pressure and increased capillary permeability. Both these effects cause rapid fluid leakage into the nasal cavities and into associated deeper tissues of the nose; and the nasal linings become swollen and secretory. Here again, use of antihistamine drugs can prevent this swelling reaction. But other products of the allergen-reagin reaction can still cause irritation of the nose, eliciting the typical sneezing syndrome.

Asthma. Asthma often occurs in the "allergic" type of person. In such a person, the allergen-reagin reaction occurs in the bronchioles of the lungs. Here, an important product released from the mast cells is believed to be the *slow-reacting substance of anaphylaxis*, which causes spasm of the bronchiolar smooth muscle. Consequently, the person has difficulty breathing until the reactive products of the allergic reaction have been removed. Administration of antihistamine medication has less effect on the course of asthma because histamine does not appear to be the major factor eliciting the asthmatic reaction.

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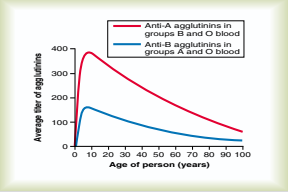
CHAPTER 35

Blood Types; Transfusion; Tissue and Organ Transplantation



Antigenicity Causes Immune Reactions of Blood

When blood transfusions from one person to another were first attempted, immediate or delayed agglutination and hemolysis of the red blood cells often occurred, resulting in typical transfusion reactions that frequently led to death. Soon it was discovered that the bloods of different people have different antigenic and immune properties so that antibodies in the plasma of one blood will react with antigens on the surfaces of the red cells of another blood type. If proper precautions are taken, one can determine ahead of time whether the antibodies and antigens present in the donor and recipient bloods will cause a transfusion reaction.

Multiplicity of Antigens in the Blood Cells. At least 30 commonly occurring antigens and hundreds of other rare antigens, each of which can at times cause antigen-antibody reactions, have been found on the surfaces of the cell membranes of human blood cells. Most of the antigens are weak and therefore are of importance principally for studying the inheritance of genes to establish parentage.

Two particular types of antigens are much more likely than the others to cause blood transfusion reactions. They are the *O-A-B* system of antigens and the *Rh* system.

O-A-B Blood Types

A and B Antigens—Agglutinogens

Two antigens—type A and type B—occur on the surfaces of the red blood cells in a large proportion of human beings. It is these antigens (also called *agglutinogens* because they often cause blood cell agglutination) that cause most blood transfusion reactions. Because of the way these agglutinogens are inherited, people may have neither of them on their cells, they may have one, or they may have both simultaneously.

Major O-A-B Blood Types. In transfusing blood from one person to another, the bloods of donors and recipients are normally classified into four major O-A-B blood types, as shown in Table 35-1, depending on the presence or absence of the two agglutinogens, the A and B agglutinogens. When neither A nor B agglutinogen is present, the blood is *type O*. When only type A agglutinogen is present, the blood is *type A*. When only type B agglutinogen is present, the blood is *type B*. When both A and B agglutinogens are present, the blood is *type AB*.

Genetic Determination of the Agglutinogens. Two genes, one on each of two paired chromosomes, determine the O-A-B blood type. These genes can be any one of three types but only one type on each of the two chromosomes: type O, type A, or type B. The type O gene is either functionless or almost functionless, so it causes no significant type O agglutinogen on the cells. Conversely, the type A and type B genes do cause strong agglutinogens on the cells.

The six possible combinations of genes, as shown in Table 35-1, are OO, OA, OB, AA, BB, and AB. These combinations of genes are known as the *genotypes,* and each person is one of the six genotypes.

One can also observe from Table 35-1 that a person with genotype OO produces no agglutinogens, and therefore the blood type is O. A person with genotype OA or AA produces type A agglutinogens and therefore has blood type A. Genotypes OB and BB give type B blood, and genotype AB gives type AB blood.

Relative Frequencies of the Different Blood Types. The prevalence of the different blood types among one group of persons studied was approximately:

O	47%
A	41%
B	9%
AB	3%

It is obvious from these percentages that the O and A genes occur frequently, whereas the B gene is infrequent.

Table 35-1 Blood Types with Their Genotypes and Their Constituent Agglutinogens and Agglutinins

Genotypes	Blood Types	Agglutinogens	Agglutinins
OO	O	–	Anti-A and Anti-B
OA or AA	A	A	Anti-B
OB or BB	B	B	Anti-A
AB	AB	A and B	–

Agglutinins

When type A agglutinogen *is not present* in a person's red blood cells, antibodies known as *anti-A agglutinins* develop in the plasma. Also, when type B agglutinogen *is not present* in the red blood cells, antibodies known as *anti-B agglutinins* develop in the plasma.

Thus, referring once again to Table 35-1, note that type O blood, although containing no agglutinogens, does contain both *anti-A* and *anti-B agglutinins;* type A blood contains type A agglutinogens and *anti-B agglutinins;* type B blood contains type B agglutinogens and anti-A agglutinins. Finally, type AB blood contains both A and B agglutinogens but no agglutinins.

Titer of the Agglutinins at Different Ages. Immediately after birth, the quantity of agglutinins in the plasma is almost zero. Two to 8 months after birth, an infant begins to produce agglutinins—anti-A agglutinins when type A agglutinogens are not present in the cells, and anti-B agglutinins when type B agglutinogens are not in the cells. Figure 35-1 shows the changing titers of the anti-A and anti-B agglutinins at different ages. A maximum titer is usually reached at 8 to 10 years of age, and this gradually declines throughout the remaining years of life.

Origin of Agglutinins in the Plasma. The agglutinins are gamma globulins, as are almost all antibodies, and they are produced by the same bone marrow and lymph gland cells that produce antibodies to any other antigens. Most of them are IgM and IgG immunoglobulin molecules.

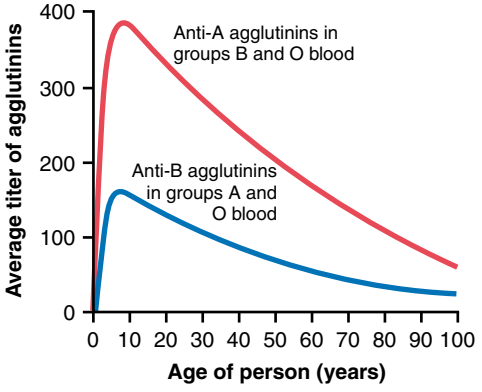


Figure 35-1 Average titers of anti-A and anti-B agglutinins in the plasmas of people with different blood types.

But why are these agglutinins produced in people who do not have the respective agglutinogens in their red blood cells? The answer to this is that small amounts of type A and B antigens enter the body in food, in bacteria, and in other ways, and these substances initiate the development of the anti-A and anti-B agglutinins.

For instance, infusion of group A antigen into a recipient having a non-A blood type causes a typical immune response with formation of greater quantities of anti-A agglutinins than ever. Also, the neonate has few, if any, agglutinins, showing that agglutinin formation occurs almost entirely after birth.

Agglutination Process in Transfusion Reactions

When bloods are mismatched so that anti-A or anti-B plasma agglutinins are mixed with red blood cells that contain A or B agglutinogens, respectively, the red cells agglutinate as a result of the agglutinins' attaching themselves to the red blood cells. Because the agglutinins have 2 binding sites (IgG type) or 10 binding sites (IgM type), a single agglutinin can attach to two or more red blood cells at the same time, thereby causing the cells to be bound together by the agglutinin. This causes the cells to clump, which is the process of "agglutination." Then these clumps plug small blood vessels throughout the circulatory system. During ensuing hours to days, either physical distortion of the cells or attack by phagocytic white blood cells destroys the membranes of the agglutinated cells, releasing hemoglobin into the plasma, which is called "*hemolysis*" of the red blood cells.

Acute Hemolysis Occurs in Some Transfusion Reactions. Sometimes, when recipient and donor bloods are mismatched, immediate hemolysis of red cells occurs in the circulating blood. In this case, the antibodies cause lysis of the red blood cells by activating the complement system, which releases proteolytic enzymes (the *lytic complex*) that rupture the cell membranes, as described in Chapter 34. *Immediate* intravascular hemolysis is far less common than agglutination followed by *delayed* hemolysis, because not only does there have to be a high titer of antibodies for lysis to occur, but also a different type of antibody seems to be required, mainly the IgM antibodies; these antibodies are called *hemolysins*.

Blood Typing

Before giving a transfusion to a person, it is necessary to determine the blood type of the recipient's blood and the blood type of the donor blood so that the bloods can be appropriately matched. This is called *blood typing* and *blood matching,* and these are performed in the following way: The red blood cells are first separated from the plasma and diluted with saline. One portion is then mixed with anti-A agglutinin and another portion with anti-B agglutinin. After several minutes, the mixtures are

Table 35-2 Blood Typing, Showing Agglutination of Cells of the Different Blood Types with Anti-A or Anti-B Agglutinins in the Sera

	Sera	
Red Blood Cell Types	***Anti-A***	***Anti-B***
O	–	–
A	+	–
B	–	+
AB	+	+

observed under a microscope. If the red blood cells have become clumped—that is, "agglutinated"—one knows that an antibody-antigen reaction has resulted.

Table 35-2 lists the presence (+) or absence (–) of agglutination of the four types of red blood cells. Type O red blood cells have no agglutinogens and therefore do not react with either the anti-A or the anti-B agglutinins. Type A blood has A agglutinogens and therefore agglutinates with anti-A agglutinins. Type B blood has B agglutinogens and agglutinates with anti-B agglutinins. Type AB blood has both A and B agglutinogens and agglutinates with both types of agglutinins.

Rh Blood Types

Along with the O-A-B blood type system, the Rh blood type system is also important when transfusing blood. The major difference between the O-A-B system and the Rh system is the following: In the O-A-B system, the plasma agglutinins responsible for causing transfusion reactions develop spontaneously, whereas in the Rh system, spontaneous agglutinins almost never occur. Instead, the person must first be massively exposed to an Rh antigen, such as by transfusion of blood containing the Rh antigen, before enough agglutinins to cause a significant transfusion reaction will develop.

Rh Antigens—"Rh-Positive" and "Rh-Negative" People. There are six common types of Rh antigens, each of which is called an *Rh factor*. These types are designated C, D, E, c, d, and e. A person who has a C antigen does not have the c antigen, but the person missing the C antigen always has the c antigen. The same is true for the D-d and E-e antigens. Also, because of the manner of inheritance of these factors, each person has one of each of the three pairs of antigens.

The type D antigen is widely prevalent in the population and considerably more antigenic than the other Rh antigens. Anyone who has this type of antigen is said to be *Rh positive,* whereas a person who does not have type D antigen is said to be *Rh negative.* However, it must be noted that even in Rh-negative people, some of the other Rh antigens can still cause transfusion reactions, although the reactions are usually much milder.

About 85 percent of all white people are Rh positive and 15 percent, Rh negative. In American blacks, the percentage of Rh-positives is about 95 percent, whereas in African blacks, it is virtually 100 percent.

Rh Immune Response

Formation of Anti-Rh Agglutinins. When red blood cells containing Rh factor are injected into a person whose blood does not contain the Rh factor—that is, into an Rh-negative person—anti-Rh agglutinins develop slowly, reaching maximum concentration of agglutinins about 2 to 4 months later. This immune response occurs to a much greater extent in some people than in others. With multiple exposures to the Rh factor, an Rh-negative person eventually becomes strongly "sensitized" to Rh factor.

Characteristics of Rh Transfusion Reactions. If an Rh-negative person has never before been exposed to Rh-positive blood, transfusion of Rh-positive blood into that person will likely cause no immediate reaction. However, anti-Rh antibodies can develop in sufficient quantities during the next 2 to 4 weeks to cause agglutination of those transfused cells that are still circulating in the blood. These cells are then hemolyzed by the tissue macrophage system. Thus, a *delayed* transfusion reaction occurs, although it is usually mild. On subsequent transfusion of Rh-positive blood into the same person, who is now already immunized against the Rh factor, the transfusion reaction is greatly enhanced and can be immediate and as severe as a transfusion reaction caused by mismatched type A or B blood.

Erythroblastosis Fetalis ("Hemolytic Disease of the Newborn")

Erythroblastosis fetalis is a disease of the fetus and newborn child characterized by agglutination and phagocytosis of the fetus's red blood cells. In most instances of erythroblastosis fetalis, the mother is Rh negative and the father Rh positive. The baby has inherited the Rh-positive antigen from the father, and the mother develops anti-Rh agglutinins from exposure to the fetus's Rh antigen. In turn, the mother's agglutinins diffuse through the placenta into the fetus and cause red blood cell agglutination.

Incidence of the Disease. An Rh-negative mother having her first Rh-positive child usually does not develop sufficient anti-Rh agglutinins to cause any harm. However, about 3 percent of second Rh-positive babies exhibit some signs of erythroblastosis fetalis; about 10 percent of third babies exhibit the disease; and the incidence rises progressively with subsequent pregnancies.

Effect of the Mother's Antibodies on the Fetus. After anti-Rh antibodies have formed in the mother, they diffuse slowly through the placental membrane into the fetus's blood. There they cause agglutination of the fetus's blood. The agglutinated red blood cells subsequently hemolyze, releasing hemoglobin into the blood. The fetus's macrophages then convert the hemoglobin into

bilirubin, which causes the baby's skin to become yellow (jaundiced). The antibodies can also attack and damage other cells of the body.

Clinical Picture of Erythroblastosis. The jaundiced, erythroblastotic newborn baby is usually anemic at birth, and the anti-Rh agglutinins from the mother usually circulate in the infant's blood for another 1 to 2 months after birth, destroying more and more red blood cells.

The hematopoietic tissues of the infant attempt to replace the hemolyzed red blood cells. The liver and spleen become greatly enlarged and produce red blood cells in the same manner that they normally do during the middle of gestation. Because of the rapid production of red cells, many early forms of red blood cells, including many *nucleated blastic forms,* are passed from the baby's bone marrow into the circulatory system, and it is because of the presence of these nucleated blastic red blood cells that the disease is called *erythroblastosis fetalis.*

Although the severe anemia of erythroblastosis fetalis is usually the cause of death, many children who barely survive the anemia exhibit permanent mental impairment or damage to motor areas of the brain because of precipitation of bilirubin in the neuronal cells, causing destruction of many, a condition called *kernicterus*.

Treatment of the Erythroblastotic Neonate. One treatment for erythroblastosis fetalis is to replace the neonate's blood with Rh-negative blood. About 400 milliliters of Rh-negative blood is infused over a period of 1.5 or more hours while the neonate's own Rh-positive blood is being removed. This procedure may be repeated several times during the first few weeks of life, mainly to keep the bilirubin level low and thereby prevent kernicterus. By the time these transfused Rh-negative cells are replaced with the infant's own Rh-positive cells, a process that requires 6 or more weeks, the anti-Rh agglutinins that had come from the mother will have been destroyed.

Prevention of Erythroblastosis Fetalis. The D antigen of the Rh blood group system is the primary culprit in causing immunization of an Rh-negative mother to an Rh-positive fetus. In the 1970s, a dramatic reduction in the incidence of erythroblastosis fetalis was achieved with the development of *Rh immunoglobulin globin, an anti-D antibody* that is administered to the expectant mother starting at 28 to 30 weeks of gestation. The anti-D antibody is also administered to Rh-negative women who deliver Rh-positive babies to prevent sensitization of the mothers to the D antigen. This greatly reduces the risk of developing large amounts of D antibodies during the second pregnancy.

The mechanism by which Rh immunoglobulin globin prevents sensitization of the D antigen is not completely understood, but one effect of the anti-D antibody is to inhibit antigen-induced B lymphocyte antibody production in the expectant mother. The administered anti-D antibody also attaches to D-antigen sites on Rh-positive fetal red blood cells that may cross the placenta and enter the circulation of the expectant mother, thereby interfering with the immune response to the D antigen.

Transfusion Reactions Resulting from Mismatched Blood Types

If donor blood of one blood type is transfused into a recipient who has another blood type, a transfusion reaction is likely to occur in which the red blood cells *of the donor blood* are agglutinated. It is rare that the transfused blood causes agglutination *of the recipient's cells,* for the following reason: The plasma portion of the donor blood immediately becomes diluted by all the plasma of the recipient, thereby decreasing the titer of the infused agglutinins to a level usually too low to cause agglutination. Conversely, the small amount of infused blood does not significantly dilute the agglutinins in the recipient's plasma. Therefore, the recipient's agglutinins can still agglutinate the mismatched donor cells.

As explained earlier, all transfusion reactions eventually cause either immediate hemolysis resulting from hemolysins or later hemolysis resulting from phagocytosis of agglutinated cells. The hemoglobin released from the red cells is then converted by the phagocytes into bilirubin and later excreted in the bile by the liver, as discussed in Chapter 70. The concentration of bilirubin in the body fluids often rises high enough to cause *jaundice*—that is, the person's internal tissues and skin become *colored with yellow bile pigment*. But if liver function is normal, the bile pigment will be excreted into the intestines by way of the liver bile, so jaundice usually does not appear in an adult person unless more than 400 milliliters of blood is hemolyzed in less than a day.

Acute Kidney Shutdown After Transfusion Reactions. One of the most lethal effects of transfusion reactions is *kidney failure,* which can begin within a few minutes to few hours and continue until the person dies of renal failure.

The kidney shutdown seems to result from three causes: First, the antigen-antibody reaction of the transfusion reaction releases toxic substances from the hemolyzing blood that cause powerful renal vasoconstriction. Second, loss of circulating red cells in the recipient, along with production of toxic substances from the hemolyzed cells and from the immune reaction, often causes circulatory shock. The arterial blood pressure falls very low, and renal blood flow and urine output decrease. Third, if the total amount of free hemoglobin released into the circulating blood is greater than the quantity that can bind with "*haptoglobin*" (a plasma protein that binds small amounts of hemoglobin), much of the excess leaks through the glomerular membranes into the kidney tubules. If this amount is still slight, it can be reabsorbed through the tubular epithelium into the blood and will cause no harm; if it is great, then only a small percentage is reabsorbed. Yet water continues to be reabsorbed, causing the tubular hemoglobin concentration to rise so high that the hemoglobin precipitates and blocks many of the kidney tubules. Thus, renal vasoconstriction, circulatory shock, and renal tubular blockage together cause acute renal shutdown.

If the shutdown is complete and fails to resolve, the patient dies within a week to 12 days, as explained in Chapter 31, unless treated with an artificial kidney.

Transplantation of Tissues and Organs

Most of the different antigens of red blood cells that cause transfusion reactions are also widely present in other cells of the body, and each bodily tissue has its own additional complement of antigens. Consequently, foreign cells transplanted anywhere into the body of a recipient can produce immune reactions. In other words, most recipients are just as able to resist invasion by foreign tissue cells as to resist invasion by foreign bacteria or red cells.

Autografts, Isografts, Allografts, and Xenografts. A transplant of a tissue or whole organ from one part of the same animal to another part is called an *autograft;* from one identical twin to another, an *isograft;* from one human being to another or from any animal to another animal of the same species, an *allograft;* and from a lower animal to a human being or from an animal of one species to one of another species, a *xenograft*.

Transplantation of Cellular Tissues. In the case of *autografts* and *isografts,* cells in the transplant contain virtually the same types of antigens as in the tissues of the recipient and will almost always continue to live normally and indefinitely if an adequate blood supply is provided.

At the other extreme, in the case of *xenografts,* immune reactions almost always occur, causing death of the cells in the graft within 1 day to 5 weeks after transplantation unless some specific therapy is used to prevent the immune reactions.

Some of the different cellular tissues and organs that have been transplanted as allografts, either experimentally or for therapeutic purposes, from one person to another are skin, kidney, heart, liver, glandular tissue, bone marrow, and lung. With proper "matching" of tissues between persons, many kidney allografts have been successful for at least 5 to 15 years, and allograft liver and heart transplants for 1 to 15 years.

Attempts to Overcome Immune Reactions in Transplanted Tissue

Because of the extreme potential importance of transplanting certain tissues and organs, serious attempts have been made to prevent antigen-antibody reactions associated with transplantation. The following specific procedures have met with some degrees of clinical or experimental success.

Tissue Typing—the Human Leukocyte Antigen (HLA) Complex of Antigens

The most important antigens for causing graft rejection are a complex called the *HLA antigens.* Six of these antigens are present on the tissue cell membranes of each person, but there are about 150 different HLA antigens to choose from. Therefore, this represents more than a trillion possible combinations. Consequently, it is virtually impossible for two persons, except in the case of identical twins, to have the same six HLA antigens. Development of significant immunity against any one of these antigens can cause graft rejection.

The HLA antigens occur on the white blood cells, as well as on the tissue cells. Therefore, tissue typing for these antigens is done on the membranes of lymphocytes that have been separated from the person's blood. The lymphocytes are mixed with appropriate antisera and complement; after incubation, the cells are tested for membrane damage, usually by testing the rate of trans-membrane uptake by the lymphocytic cells of a special dye.

Some of the HLA antigens are not severely antigenic, for which reason a precise match of some antigens between donor and recipient is not always essential to allow allograft acceptance. Therefore, by obtaining the best possible match between donor and recipient, the grafting procedure has become far less hazardous. The best success has been with tissue-type matches between siblings and between parent and child. The match in identical twins is exact, so transplants between identical twins are almost never rejected because of immune reactions.

Prevention of Graft Rejection by Suppressing the Immune System

If the immune system were completely suppressed, graft rejection would not occur. In fact, in a person who has serious depression of the immune system, grafts can be successful without the use of significant therapy to prevent rejection. But in the normal person, even with the best possible tissue typing, allografts seldom resist rejection for more than a few days or weeks without use of specific therapy to suppress the immune system. Furthermore, because the T cells are mainly the portion of the immune system important for killing grafted cells, their suppression is much more important than suppression of plasma antibodies. Some of the therapeutic agents that have been used for this purpose include the following:

1. *Glucocorticoid hormones isolated from adrenal cortex glands (or drugs with glucocorticoid-like activity),* which suppress the growth of all lymphoid tissue and, therefore, decrease formation of antibodies and T cells.
2. *Various drugs that have a toxic effect on the lymphoid system* and, therefore, block formation of antibodies and T cells, especially the drug *azathioprine.*
3. *Cyclosporine,* which has a specific inhibitory effect on the formation of helper T cells and, therefore, is especially efficacious in blocking the T-cell rejection reaction. This has proved to be one of the most valuable of all the drugs because it does not depress some other portions of the immune system.

Use of these agents often leaves the person unprotected from infectious disease; therefore, sometimes bacterial

and viral infections become rampant. In addition, the incidence of cancer is several times as great in an immunosuppressed person, presumably because the immune system is important in destroying many early cancer cells before they can begin to proliferate.

Transplantation of living tissues in human beings has had important success mainly because of the development of drugs that suppress the responses of the immune system. With the introduction of improved immunosuppressive agents, successful organ transplantation has become much more common. The current approach to immunosuppressive therapy attempts to balance acceptable rates of rejection with moderation in the adverse effects of immunosuppressive drugs.

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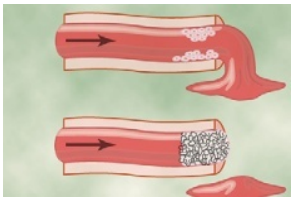
CHAPTER 36

Hemostasis and Blood Coagulation



Events in Hemostasis

The term *hemostasis* means prevention of blood loss. Whenever a vessel is severed or ruptured, hemostasis is achieved by several mechanisms: (1) vascular constriction, (2) formation of a platelet plug, (3) formation of a blood clot as a result of blood coagulation, and (4) eventual growth of fibrous tissue into the blood clot to close the hole in the vessel permanently.

Vascular Constriction

Immediately after a blood vessel has been cut or ruptured, the trauma to the vessel wall causes the smooth muscle in the wall to contract; this instantaneously reduces the flow of blood from the ruptured vessel. The contraction results from (1) local myogenic spasm, (2) local autacoid factors from the traumatized tissues and blood platelets, and (3) nervous reflexes. The nervous reflexes are initiated by pain nerve impulses or other sensory impulses that originate from the traumatized vessel or nearby tissues. However, even more vasoconstriction probably results from local *myogenic contraction* of the blood vessels initiated by direct damage to the vascular wall. And, for the smaller vessels, the platelets are responsible for much of the vasoconstriction by releasing a vasoconstrictor substance, *thromboxane* A_2.

The more severely a vessel is traumatized, the greater the degree of vascular spasm. The spasm can last for many minutes or even hours, during which time the processes of platelet plugging and blood coagulation can take place.

Formation of the Platelet Plug

If the cut in the blood vessel is very small—indeed, many very small vascular holes do develop throughout the body each day—the cut is often sealed by a *platelet plug,* rather than by a blood clot. To understand this, it is important that we first discuss the nature of platelets themselves.

Physical and Chemical Characteristics of Platelets

Platelets (also called *thrombocytes*) are minute discs 1 to 4 micrometers in diameter. They are formed in the bone marrow from *megakaryocytes,* which are extremely large cells of the hematopoietic series in the marrow; the megakaryocytes fragment into the minute platelets either in the bone marrow or soon after entering the blood, especially as they squeeze through capillaries. The normal concentration of platelets in the blood is between 150,000 and 300,000 per microliter.

Platelets have many functional characteristics of whole cells, even though they do not have nuclei and cannot reproduce. In their cytoplasm are such active factors as (1) *actin* and *myosin molecules,* which are contractile proteins similar to those found in muscle cells, and still another contractile protein, *thrombosthenin*, that can cause the platelets to contract; (2) residuals of both the *endoplasmic reticulum* and the *Golgi apparatus* that synthesize various enzymes and especially store large quantities of calcium ions; (3) mitochondria and enzyme systems that are capable of forming *adenosine triphosphate* (ATP) and *adenosine diphosphate* (ADP); (4) enzyme systems that synthesize *prostaglandins,* which are local hormones that cause many vascular and other local tissue reactions; (5) an important protein called *fibrin-stabilizing factor,* which we discuss later in relation to blood coagulation; and (6) a *growth factor* that causes vascular endothelial cells, vascular smooth muscle cells, and fibroblasts to multiply and grow, thus causing cellular growth that eventually helps repair damaged vascular walls.

The cell membrane of the platelets is also important. On its surface is a coat of *glycoproteins* that repulses adherence to normal endothelium and yet causes adherence to *injured* areas of the vessel wall, especially to injured endothelial cells and even more so to any exposed collagen from deep within the vessel wall. In addition, the platelet membrane contains large amounts of *phospholipids* that activate multiple stages in the blood-clotting process, as we discuss later.

Thus, the platelet is an active structure. It has a half-life in the blood of 8 to 12 days, so over several weeks its functional processes run out. Then it is eliminated from

the circulation mainly by the tissue macrophage system. More than one half of the platelets are removed by macrophages in the spleen, where the blood passes through a latticework of tight trabeculae.

Mechanism of the Platelet Plug

Platelet repair of vascular openings is based on several important functions of the platelet. When platelets come in contact with a damaged vascular surface, especially with collagen fibers in the vascular wall, the platelets immediately change their own characteristics drastically. They begin to swell; they assume irregular forms with numerous irradiating pseudopods protruding from their surfaces; their contractile proteins contract forcefully and cause the release of granules that contain multiple active factors; they become sticky so that they adhere to collagen in the tissues and to a protein called *von Willebrand factor* that leaks into the traumatized tissue from the plasma; they secrete large quantities of ADP; and their enzymes form *thromboxane* A_2. The ADP and thromboxane in turn act on nearby platelets to activate them as well, and the stickiness of these additional platelets causes them to adhere to the original activated platelets.

Therefore, at the site of any opening in a blood vessel wall, the damaged vascular wall activates successively increasing numbers of platelets that themselves attract more and more additional platelets, thus forming a *platelet plug.* This is at first a loose plug, but it is usually successful in blocking blood loss if the vascular opening is small. Then, during the subsequent process of blood coagulation, *fibrin threads* form. These attach tightly to the platelets, thus constructing an unyielding plug.

Importance of the Platelet Mechanism for Closing Vascular Holes. The platelet-plugging mechanism is extremely important for closing minute ruptures in very small blood vessels that occur many thousands of times daily. Indeed, multiple small holes through the endothelial cells themselves are often closed by platelets actually fusing with the endothelial cells to form additional endothelial cell membrane. A person who has few blood platelets develops each day literally thousands of small hemorrhagic areas under the skin and throughout the internal tissues, but this does not occur in the normal person.

Blood Coagulation in the Ruptured Vessel

The third mechanism for hemostasis is formation of the blood clot. The clot begins to develop in 15 to 20 seconds if the trauma to the vascular wall has been severe, and in 1 to 2 minutes if the trauma has been minor. Activator substances from the traumatized vascular wall, from platelets, and from blood proteins adhering to the traumatized vascular wall initiate the clotting process. The physical events of this process are shown in Figure 36-1, and Table 36-1 lists the most important of the clotting factors.

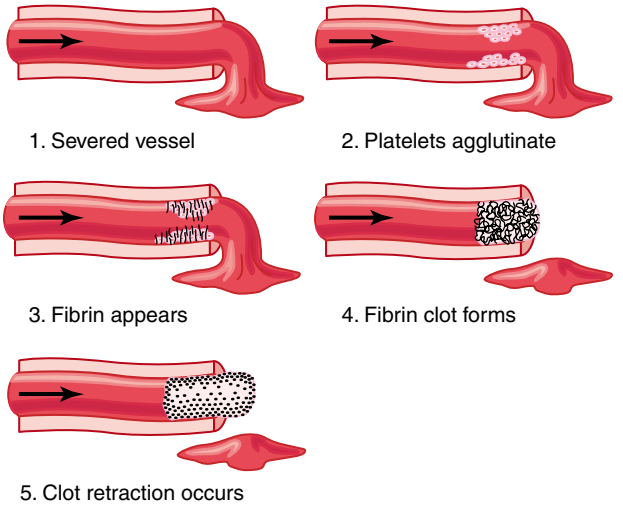


Figure 36-1 Clotting process in a traumatized blood vessel. (Modified from Seegers WH: Hemostatic Agents, 1948. Courtesy of Charles C Thomas, Publisher, Ltd., Springfield, Ill.)

Within 3 to 6 minutes after rupture of a vessel, if the vessel opening is not too large, the entire opening or broken end of the vessel is filled with clot. After 20 minutes to an hour, the clot retracts; this closes the vessel still further. Platelets also play an important role in this clot retraction, as is discussed later.

Table 36-1 Clotting Factors in Blood and Their Synonyms

Clotting Factor	Synonyms
Fibrinogen	Factor I
Prothrombin	Factor II
Tissue factor	Factor III; tissue thromboplastin
Calcium	Factor IV
Factor V	Proaccelerin; labile factor; Ac-globulin (Ac-G)
Factor VII	Serum prothrombin conversion accelerator (SPCA); proconvertin; stable factor
Factor VIII	Antihemophilic factor (AHF); antihemophilic globulin (AHG); antihemophilic factor A
Factor IX	Plasma thromboplastin component (PTC); Christmas factor; antihemophilic factor B
Factor X	Stuart factor; Stuart-Prower factor
Factor XI	Plasma thromboplastin antecedent (PTA); antihemophilic factor C
Factor XII	Hageman factor
Factor XIII	Fibrin-stabilizing factor
Prekallikrein	Fletcher factor
High-molecular-weight kininogen	Fitzgerald factor; HMWK (high-molecular-weight kininogen)
Platelets	

Fibrous Organization or Dissolution of the Blood Clot

Once a blood clot has formed, it can follow one of two courses: (1) It can become invaded by *fibroblasts,* which subsequently form connective tissue all through the clot, or (2) it can dissolve. The usual course for a clot that forms in a small hole of a vessel wall is invasion by fibroblasts, beginning within a few hours after the clot is formed (which is promoted at least partially by *growth factor* secreted by platelets). This continues to complete organization of the clot into fibrous tissue within about 1 to 2 weeks.

Conversely, when excess blood has leaked into the tissues and tissue clots have occurred where they are not needed, special substances within the clot itself usually become activated. These function as enzymes to dissolve the clot, as discussed later in the chapter.

Mechanism of Blood Coagulation

Basic Theory. More than 50 important substances that cause or affect blood coagulation have been found in the blood and in the tissues—some that promote coagulation, called *procoagulants,* and others that inhibit coagulation, called *anticoagulants.* Whether blood will coagulate depends on the balance between these two groups of substances. In the blood stream, the anticoagulants normally predominate, so the blood does not coagulate while it is circulating in the blood vessels. But when a vessel is ruptured, procoagulants from the area of tissue damage become "activated" and override the anticoagulants, and then a clot does develop.

General Mechanism. Clotting takes place in three essential steps: (1) In response to rupture of the vessel or damage to the blood itself, a complex cascade of chemical reactions occurs in the blood involving more than a dozen blood coagulation factors. The net result is formation of a complex of activated substances collectively called *prothrombin activator.* (2) The prothrombin activator catalyzes conversion of *prothrombin* into *thrombin.* (3) The thrombin acts as an enzyme to convert *fibrinogen* into *fibrin fibers* that enmesh platelets, blood cells, and plasma to form the clot.

Let us discuss first the mechanism by which the blood clot itself is formed, beginning with conversion of prothrombin to thrombin; then we will come back to the initiating stages in the clotting process by which prothrombin activator is formed.

Conversion of Prothrombin to Thrombin

First, prothrombin activator is formed as a result of rupture of a blood vessel or as a result of damage to special substances in the blood. Second, the prothrombin activator, in the presence of sufficient amounts of ionic Ca^{++}, causes conversion of prothrombin to thrombin (Figure 36-2). Third,

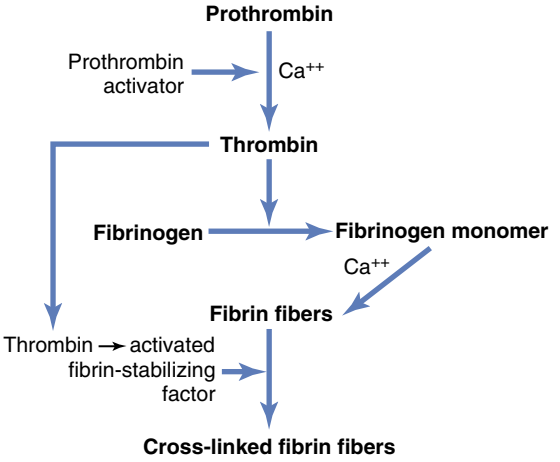


Figure 36-2 Schema for conversion of prothrombin to thrombin and polymerization of fibrinogen to form fibrin fibers.

the thrombin causes polymerization of fibrinogen molecules into fibrin fibers within another 10 to 15 seconds. Thus, the rate-limiting factor in causing blood coagulation is usually the formation of prothrombin activator and not the subsequent reactions beyond that point, because these terminal steps normally occur rapidly to form the clot.

Platelets also play an important role in the conversion of prothrombin to thrombin because much of the prothrombin first attaches to prothrombin receptors on the platelets already bound to the damaged tissue.

Prothrombin and Thrombin. Prothrombin is a plasma protein, an alpha2-globulin, having a molecular weight of 68,700. It is present in normal plasma in a concentration of about 15 mg/dl. It is an unstable protein that can split easily into smaller compounds, one of which is *thrombin,* which has a molecular weight of 33,700, almost exactly one half that of prothrombin.

Prothrombin is formed continually by the liver, and it is continually being used throughout the body for blood clotting. If the liver fails to produce prothrombin, in a day or so prothrombin concentration in the plasma falls too low to provide normal blood coagulation.

Vitamin K is required by the liver for normal activation of prothrombin, as well as a few other clotting factors. Therefore, either lack of vitamin K or the presence of liver disease that prevents normal prothrombin formation can decrease the prothrombin level so low that a bleeding tendency results.

Conversion of Fibrinogen to Fibrin—Formation of the Clot

Fibrinogen. Fibrinogen is a high-molecular-weight protein (MW = 340,000) that occurs in the plasma in quantities of 100 to 700 mg/dl. Fibrinogen is formed in the liver, and liver disease can decrease the concentration of circulating fibrinogen, as it does the concentration of prothrombin, pointed out earlier.

Because of its large molecular size, little fibrinogen normally leaks from the blood vessels into the interstitial

fluids, and because fibrinogen is one of the essential factors in the coagulation process, interstitial fluids ordinarily do not coagulate. Yet, when the permeability of the capillaries becomes pathologically increased, fibrinogen does then leak into the tissue fluids in sufficient quantities to allow clotting of these fluids in much the same way that plasma and whole blood can clot.

Action of Thrombin on Fibrinogen to Form Fibrin. Thrombin is a protein *enzyme* with weak proteolytic capabilities. It acts on fibrinogen to remove four low-molecular-weight peptides from each molecule of fibrinogen, forming one molecule of *fibrin monomer* that has the automatic capability to polymerize with other fibrin monomer molecules to form fibrin fibers. Therefore, many fibrin monomer molecules polymerize within seconds into *long fibrin fibers* that constitute the *reticulum* of the blood clot.

In the early stages of polymerization, the fibrin monomer molecules are held together by weak noncovalent hydrogen bonding, and the newly forming fibers are not cross-linked with one another; therefore, the resultant clot is weak and can be broken apart with ease. But another process occurs during the next few minutes that greatly strengthens the fibrin reticulum. This involves a substance called *fibrin-stabilizing factor* that is present in small amounts in normal plasma globulins but is also released from platelets entrapped in the clot. Before fibrin-stabilizing factor can have an effect on the fibrin fibers, it must itself be activated. The same thrombin that causes fibrin formation also activates the fibrin-stabilizing factor. Then this activated substance operates as an enzyme to cause *covalent bonds* between more and more of the fibrin monomer molecules, as well as multiple cross-linkages between adjacent fibrin fibers, thus adding tremendously to the three-dimensional strength of the fibrin meshwork.

Blood Clot. The clot is composed of a meshwork of fibrin fibers running in all directions and entrapping blood cells, platelets, and plasma. The fibrin fibers also adhere to damaged surfaces of blood vessels; therefore, the blood clot becomes adherent to any vascular opening and thereby prevents further blood loss.

Clot Retraction—Serum. Within a few minutes after a clot is formed, it begins to contract and usually expresses most of the fluid from the clot within 20 to 60 minutes. The fluid expressed is called *serum* because all its fibrinogen and most of the other clotting factors have been removed; in this way, serum differs from plasma. Serum cannot clot because it lacks these factors.

Platelets are necessary for clot retraction to occur. Therefore, failure of clot retraction is an indication that the number of platelets in the circulating blood might be low. Electron micrographs of platelets in blood clots show that they become attached to the fibrin fibers in such a way that they actually bond different fibers together. Furthermore, platelets entrapped in the clot continue to release procoagulant substances, one of the most important of which is *fibrin-stabilizing factor,* which causes more and more cross-linking bonds between adjacent fibrin fibers. In addition, the platelets themselves contribute directly to clot contraction by activating platelet thrombosthenin, actin, and myosin molecules, which are all contractile proteins in the platelets and cause strong contraction of the platelet spicules attached to the fibrin. This also helps compress the fibrin meshwork into a smaller mass. The contraction is activated and accelerated by thrombin, as well as by calcium ions released from calcium stores in the mitochondria, endoplasmic reticulum, and Golgi apparatus of the platelets.

As the clot retracts, the edges of the broken blood vessel are pulled together, thus contributing still further to hemostasis.

Positive Feedback of Clot Formation

Once a blood clot has started to develop, it normally extends within minutes into the surrounding blood. That is, the clot itself initiates a positive feedback to promote more clotting. One of the most important causes of this is the fact that the proteolytic action of thrombin allows it to act on many of the other blood-clotting factors in addition to fibrinogen. For instance, thrombin has a direct proteolytic effect on prothrombin itself, tending to convert this into still more thrombin, and it acts on some of the blood-clotting factors responsible for formation of prothrombin activator. (These effects, discussed in subsequent paragraphs, include acceleration of the actions of Factors VIII, IX, X, XI, and XII and aggregation of platelets.) Once a critical amount of thrombin is formed, a positive feedback develops that causes still more blood clotting and more and more thrombin to be formed; thus, the blood clot continues to grow until blood leakage ceases.

Initiation of Coagulation: Formation of Prothrombin Activator

Now that we have discussed the clotting process, we turn to the more complex mechanisms that initiate clotting in the first place. These mechanisms are set into play by (1) trauma to the vascular wall and adjacent tissues, (2) trauma to the blood, or (3) contact of the blood with damaged endothelial cells or with collagen and other tissue elements outside the blood vessel. In each instance, this leads to the formation of *prothrombin activator,* which then causes prothrombin conversion to thrombin and all the subsequent clotting steps.

Prothrombin activator is generally considered to be formed in two ways, although, in reality, the two ways interact constantly with each other: (1) by the *extrinsic pathway* that begins with trauma to the vascular wall and surrounding tissues and (2) by the *intrinsic pathway* that begins in the blood itself.

In both the extrinsic and the intrinsic pathways, a series of different plasma proteins called *blood-clotting*

factors plays a major role. Most of these proteins are *inactive* forms of proteolytic enzymes. When converted to the active forms, their enzymatic actions cause the successive, cascading reactions of the clotting process.

Most of the clotting factors, which are listed in Table 36-1, are designated by Roman numerals. To indicate the activated form of the factor, a small letter "a" is added after the Roman numeral, such as Factor VIIIa to indicate the activated state of Factor VIII.

Extrinsic Pathway for Initiating Clotting

The extrinsic pathway for initiating the formation of prothrombin activator begins with a traumatized vascular wall or traumatized extravascular tissues that come in contact with the blood. This leads to the following steps, as shown in Figure 36-3:

1. *Release of tissue factor.* Traumatized tissue releases a complex of several factors called *tissue factor* or *tissue thromboplastin.* This factor is composed especially of *phospholipids* from the membranes of the tissue plus a *lipoprotein complex* that functions mainly as a *proteolytic enzyme.*
2. *Activation of Factor X—role of Factor VII and tissue factor.* The lipoprotein complex of tissue factor further complexes with blood coagulation Factor VII and, in the presence of calcium ions, acts enzymatically on Factor X to form *activated Factor X* (Xa).
3. *Effect of Xa to form prothrombin activator—role of Factor V.* The activated Factor X combines immediately with tissue phospholipids that are part of tissue factors or with additional phospholipids released from platelets, as well as with Factor V to form the complex called *prothrombin activator.* Within a few seconds, in the presence of calcium ions (Ca^{++}), this splits prothrombin to form thrombin, and the clotting process proceeds as already explained. At first, the Factor V in the prothrombin activator complex is inactive, but once clotting begins and thrombin begins to form, the proteolytic action of thrombin activates Factor V. This then becomes an additional strong accelerator of prothrombin activation. Thus, in the final prothrombin activator complex, activated Factor X is the actual protease that causes splitting of prothrombin to form thrombin; activated Factor V greatly accelerates this protease activity, and platelet phospholipids act as a vehicle that further accelerates the process. Note especially the *positive feedback* effect of thrombin, acting through Factor V, to accelerate the entire process once it begins.

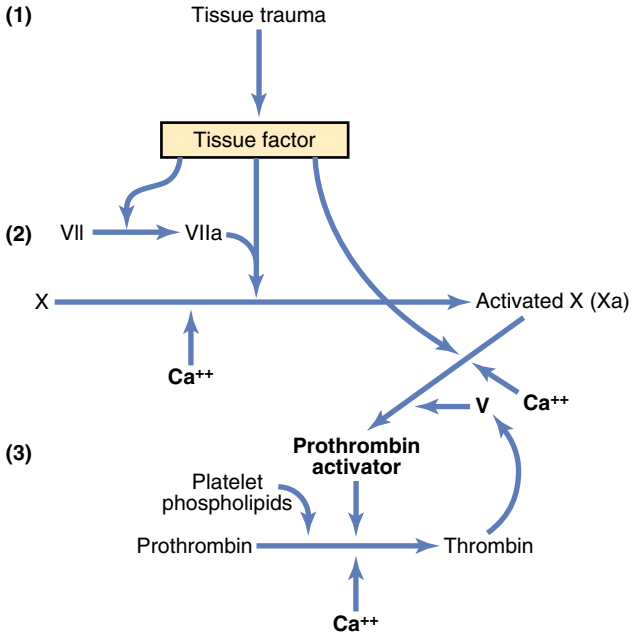


Figure 36-3 Extrinsic pathway for initiating blood clotting.

Intrinsic Pathway for Initiating Clotting

The second mechanism for initiating formation of prothrombin activator, and therefore for initiating clotting, *begins with trauma to the blood or exposure of the blood to collagen* from a traumatized blood vessel wall. Then the process continues through the series of cascading reactions shown in Figure 36-4.

1. *Blood trauma causes (1) activation of Factor XII and (2) release of platelet phospholipids.* Trauma to the blood or exposure of the blood to vascular wall collagen alters two important clotting factors in the blood: Factor XII and the platelets. When Factor XII is disturbed, such as by coming into contact with collagen or with a wettable surface such as glass, it takes on a new molecular configuration that converts it into a proteolytic enzyme called "activated Factor XII." Simultaneously, the blood trauma also damages the platelets because of adherence to either collagen or a wettable surface (or by damage in other ways), and this releases platelet phospholipids that contain the lipoprotein called *platelet factor 3,* which also plays a role in subsequent clotting reactions.
2. *Activation of Factor XI.* The activated Factor XII acts enzymatically on Factor XI to activate this factor as well, which is the second step in the intrinsic pathway. This reaction also requires *HMW (high-molecular-weight) kininogen* and is accelerated by prekallikrein.
3. *Activation of Factor IX by activated Factor XI.* The activated Factor XI then acts enzymatically on Factor IX to activate this factor as well.
4. *Activation of Factor X—role of Factor VIII.* The activated Factor IX, acting in concert with activated Factor VIII and with the platelet phospholipids and factor 3 from the traumatized platelets, activates Factor X. It is clear that when either Factor VIII or platelets are in short supply, this step is deficient. Factor VIII is the factor that is missing in a person who has classic *hemophilia,* for which reason it is called *antihemophilic factor.* Platelets are the clotting factor that is lacking in the bleeding disease called *thrombocytopenia.*

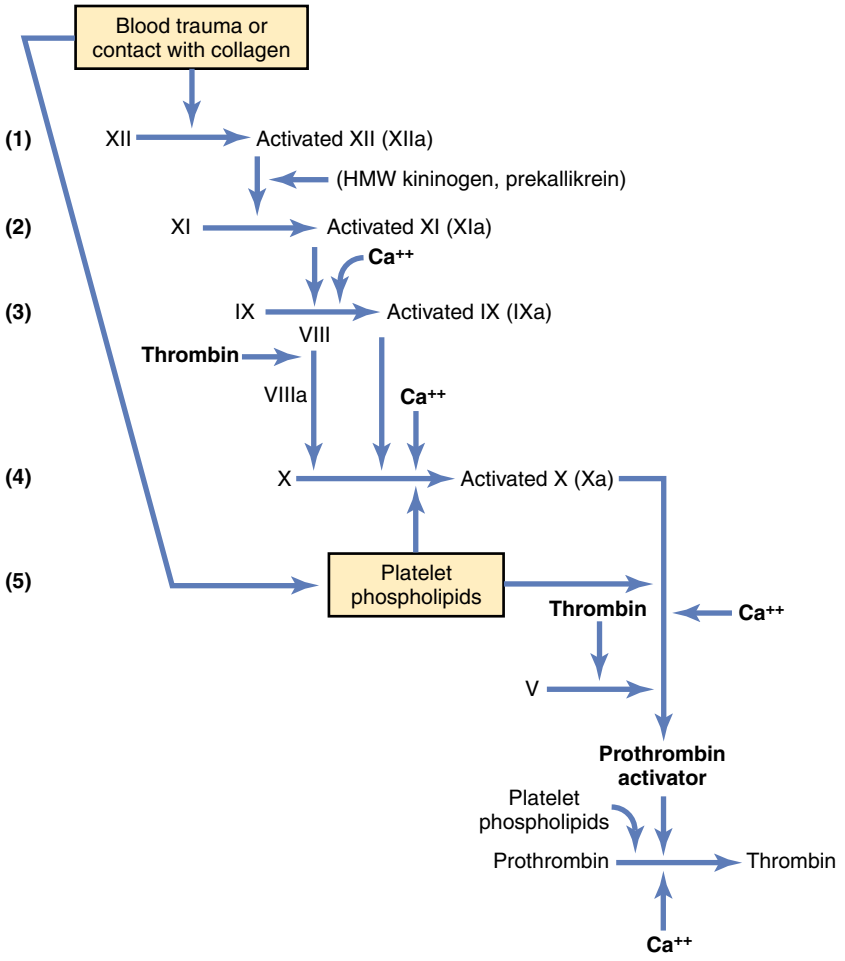


Figure 36-4 Intrinsic pathway for initiating blood clotting.

5. *Action of activated Factor X to form prothrombin activator—role of Factor V.* This step in the intrinsic pathway is the same as the last step in the extrinsic pathway. That is, activated Factor X combines with Factor V and platelet or tissue phospholipids to form the complex called *prothrombin activator.* The prothrombin activator in turn initiates within seconds the cleavage of prothrombin to form thrombin, thereby setting into motion the final clotting process, as described earlier.

Role of Calcium Ions in the Intrinsic and Extrinsic Pathways

Except for the first two steps in the intrinsic pathway, calcium ions are required for promotion or acceleration of all the blood-clotting reactions. Therefore, in the absence of calcium ions, blood clotting by either pathway does not occur.

In the living body, the calcium ion concentration seldom falls low enough to significantly affect the kinetics of blood clotting. But, when blood is removed from a person, it can be prevented from clotting by reducing the calcium ion concentration below the threshold level for clotting, either by deionizing the calcium by causing it to react with substances such as *citrate ion* or by precipitating the calcium with substances such as *oxalate ion.*

Interaction Between the Extrinsic and Intrinsic Pathways—Summary of Blood-Clotting Initiation

It is clear from the schemas of the intrinsic and extrinsic systems that after blood vessels rupture, clotting occurs by both pathways simultaneously. Tissue factor initiates the extrinsic pathway, whereas contact of Factor XII and platelets with collagen in the vascular wall initiates the intrinsic pathway.

An especially important difference between the extrinsic and intrinsic pathways is that *the extrinsic pathway* can be explosive; once initiated, its speed of completion to the final clot is limited only by the amount of tissue factor released from the traumatized tissues and by the quantities of Factors X, VII, and V in the blood. With severe tissue trauma, clotting can occur in as little as 15 seconds. The intrinsic pathway is much slower to proceed, usually requiring 1 to 6 minutes to cause clotting.

Prevention of Blood Clotting in the Normal Vascular System—Intravascular Anticoagulants

Endothelial Surface Factors. Probably the most important factors for preventing clotting in the normal vascular system are (1) the *smoothness* of the endothelial cell surface, which prevents contact activation of the intrinsic clotting system; (2) a layer of *glycocalyx* on the endothelium (glycocalyx is a mucopolysaccharide adsorbed

to the surfaces of the endothelial cells), which repels clotting factors and platelets, thereby preventing activation of clotting; and (3) a protein bound with the endothelial membrane, *thrombomodulin,* which binds thrombin. Not only does the binding of thrombin with thrombomodulin slow the clotting process by removing thrombin, but the thrombomodulin-thrombin complex also activates a plasma protein, *protein C,* that acts as an anticoagulant by *inactivating* activated Factors V and VIII.

When the endothelial wall is damaged, its smoothness and its glycocalyx-thrombomodulin layer are lost, which activates both Factor XII and the platelets, thus setting off the intrinsic pathway of clotting. If Factor XII and platelets come in contact with the subendothelial collagen, the activation is even more powerful.

Antithrombin Action of Fibrin and Antithrombin III. Among the most important *anticoagulants* in the blood are those that remove thrombin from the blood. The most powerful of these are (1) the *fibrin fibers* that are formed during the process of clotting and (2) an alpha-globulin called *antithrombin III* or *antithrombin-heparin cofactor.*

While a clot is forming, about 85 to 90 percent of the thrombin formed from the prothrombin becomes adsorbed to the fibrin fibers as they develop. This helps prevent the spread of thrombin into the remaining blood and, therefore, prevents excessive spread of the clot.

The thrombin that does not adsorb to the fibrin fibers soon combines with antithrombin III, which further blocks the effect of the thrombin on the fibrinogen and then also inactivates the thrombin itself during the next 12 to 20 minutes.

Heparin. Heparin is another powerful anticoagulant, but its concentration in the blood is normally low, so only under special physiologic conditions does it have significant anticoagulant effects. However, heparin is used widely as a pharmacological agent in medical practice in much higher concentrations to prevent intravascular clotting.

The heparin molecule is a highly negatively charged conjugated polysaccharide. By itself, it has little or no anticoagulant properties, but when it combines with antithrombin III, the effectiveness of antithrombin III for removing thrombin increases by a hundredfold to a thousandfold, and thus it acts as an anticoagulant. Therefore, in the presence of excess heparin, removal of free thrombin from the circulating blood by antithrombin III is almost instantaneous.

The complex of heparin and antithrombin III removes several other activated coagulation factors in addition to thrombin, further enhancing the effectiveness of anticoagulation. The others include activated Factors XII, XI, X, and IX.

Heparin is produced by many different cells of the body, but especially large quantities are formed by the basophilic *mast cells* located in the pericapillary connective tissue throughout the body. These cells continually secrete small quantities of heparin that diffuse into the circulatory system. The *basophil cells* of the blood, which are functionally almost identical to the mast cells, release small quantities of heparin into the plasma.

Mast cells are abundant in tissue surrounding the capillaries of the lungs and, to a lesser extent, capillaries of the liver. It is easy to understand why large quantities of heparin might be needed in these areas because the capillaries of the lungs and liver receive many embolic clots formed in slowly flowing venous blood; sufficient formation of heparin prevents further growth of the clots.

Lysis of Blood Clots—Plasmin

The plasma proteins contain a euglobulin called *plasminogen* (or *profibrinolysin*) that, when activated, becomes a substance called *plasmin* (or *fibrinolysin*). Plasmin is a proteolytic enzyme that resembles trypsin, the most important proteolytic digestive enzyme of pancreatic secretion. Plasmin digests fibrin fibers and some other protein coagulants such as fibrinogen, Factor V, Factor VIII, prothrombin, and Factor XII. Therefore, whenever plasmin is formed, it can cause lysis of a clot by destroying many of the clotting factors, thereby sometimes even causing hypocoagulability of the blood.

Activation of Plasminogen to Form Plasmin, Then Lysis of Clots. When a clot is formed, a large amount of plasminogen is trapped in the clot along with other plasma proteins. This will not become plasmin or cause lysis of the clot until it is activated. The injured tissues and vascular endothelium very slowly release a powerful activator called *tissue plasminogen activator* (t-PA) that a few days later, after the clot has stopped the bleeding, eventually converts plasminogen to plasmin, which in turn removes the remaining unnecessary blood clot. In fact, many small blood vessels in which blood flow has been blocked by clots are reopened by this mechanism. Thus, an especially important function of the plasmin system is to remove minute clots from millions of tiny peripheral vessels that eventually would become occluded were there no way to clear them.

Conditions That Cause Excessive Bleeding in Humans

Excessive bleeding can result from deficiency of any one of the many blood-clotting factors. Three particular types of bleeding tendencies that have been studied to the greatest extent are discussed here: bleeding caused by (1) vitamin K deficiency, (2) hemophilia, and (3) thrombocytopenia (platelet deficiency).

Decreased Prothrombin, Factor VII, Factor IX, and Factor X Caused by Vitamin K Deficiency

With few exceptions, almost all the blood-clotting factors are formed by the liver. Therefore, diseases of the liver such as *hepatitis*, *cirrhosis*, and *acute yellow atrophy* can

sometimes depress the clotting system so greatly that the patient develops a severe tendency to bleed.

Another cause of depressed formation of clotting factors by the liver is vitamin K deficiency. Vitamin K is an essential factor to a liver carboxylase that adds a carboxyl group to glutamic acid residues on five of the important clotting factors: *prothrombin*, *Factor VII*, *Factor IX*, *Factor X*, and *protein C*. In adding the carboxyl group to glutamic acid residues on the immature clotting factors, vitamin K is oxidized and becomes inactive. Another enzyme, *vitamin K epoxide reductase complex 1 (VKOR c1),* reduces vitamin K back to its active form.

In the absence of active vitamin K, subsequent insufficiency of these coagulation factors in the blood can lead to serious bleeding tendencies.

Vitamin K is continually synthesized in the intestinal tract by bacteria, so vitamin K deficiency seldom occurs in the normal person as a result of vitamin K absence from the diet (except in neonates before they establish their intestinal bacterial flora). However, in gastrointestinal disease, vitamin K deficiency often occurs as a result of poor absorption of fats from the gastrointestinal tract. The reason is that vitamin K is fat soluble and ordinarily absorbed into the blood along with the fats.

One of the most prevalent causes of vitamin K deficiency is failure of the liver to secrete bile into the gastrointestinal tract (which occurs either as a result of obstruction of the bile ducts or as a result of liver disease). Lack of bile prevents adequate fat digestion and absorption and, therefore, depresses vitamin K absorption as well. Thus, liver disease often causes decreased production of prothrombin and some other clotting factors both because of poor vitamin K absorption and because of the diseased liver cells. Because of this, vitamin K is injected into surgical patients with liver disease or with obstructed bile ducts before performing the surgical procedure. Ordinarily, if vitamin K is given to a deficient patient 4 to 8 hours before the operation and the liver parenchymal cells are at least one-half normal in function, sufficient clotting factors will be produced to prevent excessive bleeding during the operation.

Hemophilia

Hemophilia is a bleeding disease that occurs almost exclusively in males. In 85 percent of cases, it is caused by an *abnormality or deficiency of Factor VIII;* this type of hemophilia is called *hemophilia A* or *classic hemophilia.* About 1 of every 10,000 males in the United States has classic hemophilia. In the other 15 percent of hemophilia patients, the bleeding tendency is caused by deficiency of Factor IX. Both of these factors are transmitted genetically by way of the female chromosome. Therefore, almost never will a woman have hemophilia because at least one of her two X chromosomes will have the appropriate genes. If one of her X chromosomes is deficient, she will be a *hemophilia carrier,* transmitting the disease to half of her male offspring and transmitting the carrier state to half of her female offspring.

The bleeding trait in hemophilia can have various degrees of severity, depending on the character of the genetic deficiency. Bleeding usually does not occur except after trauma, but in some patients, the degree of trauma required to cause severe and prolonged bleeding may be so mild that it is hardly noticeable. For instance, bleeding can often last for days after extraction of a tooth.

Factor VIII has two active components, a large component with a molecular weight in the millions and a smaller component with a molecular weight of about 230,000. The smaller component is most important in the intrinsic pathway for clotting, and it is deficiency of this part of Factor VIII that causes classic hemophilia. Another bleeding disease with somewhat different characteristics, called *von Willebrand's disease,* results from loss of the large component.

When a person with classic hemophilia experiences severe prolonged bleeding, almost the only therapy that is truly effective is injection of purified Factor VIII. The cost of Factor VIII is high, because it is gathered from human blood and only in extremely small quantities. However, increasing production and use of recombinant Factor VIII will make this treatment available to more patients with classic hemophilia.

Thrombocytopenia

Thrombocytopenia means the presence of very low numbers of platelets in the circulating blood. People with thrombocytopenia have a tendency to bleed, as do hemophiliacs, except that the bleeding is usually from many small venules or capillaries, rather than from larger vessels, as in hemophilia. As a result, small punctate hemorrhages occur throughout all the body tissues. The skin of such a person displays many small, purplish blotches, giving the disease the name *thrombocytopenic purpura.* As stated earlier, platelets are especially important for repair of minute breaks in capillaries and other small vessels.

Ordinarily, bleeding will not occur until the number of platelets in the blood falls below 50,000/μl, rather than the normal 150,000 to 300,000. Levels as low as 10,000/μl are frequently lethal.

Even without making specific platelet counts in the blood, sometimes one can suspect the existence of thrombocytopenia if the person's blood fails to retract, because, as pointed out earlier, clot retraction is normally dependent on release of multiple coagulation factors from the large numbers of platelets entrapped in the fibrin mesh of the clot.

Most people with thrombocytopenia have the disease known as *idiopathic thrombocytopenia,* which means thrombocytopenia of unknown cause. In most of these people, it has been discovered that, for unknown reasons, specific antibodies have formed and react against the platelets themselves to destroy them. Relief from bleeding for 1 to 4 days can often be effected in a patient with thrombocytopenia by giving *fresh whole blood transfusions* that contain large numbers of platelets. Also, *splenectomy* is often helpful, sometimes effecting almost complete cure because the spleen normally removes large numbers of platelets from the blood.

Thromboembolic Conditions in the Human Being

Thrombi and Emboli. An abnormal clot that develops in a blood vessel is called a *thrombus.* Once a clot has developed, continued flow of blood past the clot is likely to break it away from its attachment and cause the clot to flow with the blood; such freely flowing clots are known as *emboli.* Also, emboli that originate in large arteries or in the left side of the heart can flow peripherally and plug arteries or arterioles in the brain, kidneys, or elsewhere. Emboli that originate in the venous system or in the right side of the heart generally flow into the lungs to cause pulmonary arterial embolism.

Cause of Thromboembolic Conditions. The causes of thromboembolic conditions in the human being are usually twofold: (1) Any *roughened endothelial surface of a vessel*—as may be caused by arteriosclerosis, infection, or trauma—is likely to initiate the clotting process. (2) Blood often clots *when it flows very slowly* through blood vessels, where small quantities of thrombin and other procoagulants are always being formed.

Use of t-PA in Treating Intravascular Clots. Genetically engineered t-PA (tissue plasminogen activator) is available. When delivered directly to a thrombosed area through a catheter, it is effective in activating plasminogen to plasmin, which in turn can dissolve some intravascular clots. For instance, if used within the first hour or so after thrombotic occlusion of a coronary artery, the heart is often spared serious damage.

Femoral Venous Thrombosis and Massive Pulmonary Embolism

Because clotting almost always occurs when blood flow is blocked for many hours in any vessel of the body, the immobility of patients confined to bed plus the practice of propping the knees with pillows often causes intravascular clotting because of blood stasis in one or more of the leg veins for hours at a time. Then the clot grows, mainly in the direction of the slowly moving venous blood, sometimes growing the entire length of the leg veins and occasionally even up into the common iliac vein and inferior vena cava. Then, about 1 time out of every 10, a large part of the clot disengages from its attachments to the vessel wall and flows freely with the venous blood through the right side of the heart and into the pulmonary arteries to cause massive blockage of the pulmonary arteries, called *massive pulmonary embolism.* If the clot is large enough to occlude both of the pulmonary arteries at the same time, immediate death ensues. If only one pulmonary artery is blocked, death may not occur, or the embolism may lead to death a few hours to several days later because of further growth of the clot within the pulmonary vessels. But, again, t-PA therapy can be a lifesaver.

Disseminated Intravascular Coagulation

Occasionally the clotting mechanism becomes activated in widespread areas of the circulation, giving rise to the condition called *disseminated intravascular coagulation.* This often results from the presence of large amounts of traumatized or dying tissue in the body that releases great quantities of tissue factor into the blood. Frequently, the clots are small but numerous, and they plug a large share of the small peripheral blood vessels. This occurs especially in patients with widespread septicemia, in which either circulating bacteria or bacterial toxins—especially *endotoxins*—activate the clotting mechanisms. Plugging of small peripheral vessels greatly diminishes delivery of oxygen and other nutrients to the tissues—a situation that leads to or exacerbates circulatory shock. It is partly for this reason that *septicemic shock* is lethal in 85 percent or more of patients.

A peculiar effect of disseminated intravascular coagulation is that the patient on occasion begins to bleed. The reason for this is that so many of the clotting factors are removed by the widespread clotting that too few procoagulants remain to allow normal hemostasis of the remaining blood.

Anticoagulants for Clinical Use

In some thromboembolic conditions, it is desirable to delay the coagulation process. Various anticoagulants have been developed for this purpose. The ones most useful clinically are *heparin* and the *coumarins.*

Heparin as an Intravenous Anticoagulant

Commercial heparin is extracted from several different animal tissues and prepared in almost pure form. Injection of relatively small quantities, about 0.5 to 1 mg/kg of body weight, causes the blood-clotting time to increase from a normal of about 6 minutes to 30 or more minutes. Furthermore, this change in clotting time occurs instantaneously, thereby immediately preventing or slowing further development of a thromboembolic condition.

The action of heparin lasts about 1.5 to 4 hours. The injected heparin is destroyed by an enzyme in the blood known as *heparinase*.

Coumarins as Anticoagulants

When a coumarin, such as *warfarin,* is given to a patient, the amounts of active prothrombin and Factors VII, IX, and X, all formed by the liver, begin to fall. Warfarin causes this effect by inhibiting the enzyme, *vitamin K epoxide reductase complex 1 (VKOR c1).* As discussed previously, this enzyme converts the inactive, oxidized form of vitamin K to its active, reduced form. By inhibiting VKOR c1, warfarin decreases the available active form of vitamin K in the tissues. When this occurs, the coagulation factors are no longer carboxylated and are biologically inactive. Over several days the body stores of the active

coagulation factors degrade and are replaced by inactive factors. Although the coagulation factors continue to be produced, they have greatly decreased coagulant activity.

After administration of an effective dose of warfarin, the coagulant activity of the blood decreases to about 50 percent of normal by the end of 12 hours and to about 20 percent of normal by the end of 24 hours. In other words, the coagulation process is not blocked immediately but must await the degradation of the active prothrombin and the other affected coagulation factors already present in the plasma. Normal coagulation usually returns 1 to 3 days after discontinuing coumarin therapy.

Prevention of Blood Coagulation Outside the Body

Although blood removed from the body and held in a glass test tube normally clots in about 6 minutes, blood collected in *siliconized containers* often does not clot for 1 hour or more. The reason for this delay is that preparing the surfaces of the containers with silicone prevents contact activation of platelets and Factor XII, the two principal factors that initiate the intrinsic clotting mechanism. Conversely, untreated glass containers allow contact activation of the platelets and Factor XII, with rapid development of clots.

Heparin can be used for preventing coagulation of blood outside the body, as well as in the body. Heparin is especially used in surgical procedures in which the blood must be passed through a heart-lung machine or artificial kidney machine and then back into the person.

Various substances that *decrease the concentration of calcium ions* in the blood can also be used for preventing blood coagulation *outside* the body. For instance, a soluble *oxalate* compound mixed in a very small quantity with a sample of blood causes precipitation of calcium oxalate from the plasma and thereby decreases the ionic calcium level so much that blood coagulation is blocked.

Any substance that deionizes the blood calcium will prevent coagulation. The negatively charged *citrate ion* is especially valuable for this purpose, mixed with blood usually in the form of *sodium*, *ammonium*, or *potassium citrate.* The citrate ion combines with calcium in the blood to cause an un-ionized calcium compound, and the lack of *ionic* calcium prevents coagulation. Citrate anticoagulants have an important advantage over the oxalate anticoagulants because oxalate is toxic to the body, whereas moderate quantities of citrate can be injected intravenously. After injection, the citrate ion is removed from the blood within a few minutes by the liver and is polymerized into glucose or metabolized directly for energy. Consequently, 500 milliliters of blood that has been rendered incoagulable by citrate can ordinarily be transfused into a recipient within a few minutes without dire consequences. But if the liver is damaged or if large quantities of citrated blood or plasma are given too rapidly (within fractions of a minute), the citrate ion may not be removed quickly enough, and the citrate can, under these conditions, greatly depress the level of calcium ion in the blood, which can result in tetany and convulsive death.

Blood Coagulation Tests

Bleeding Time

When a sharp-pointed knife is used to pierce the tip of the finger or lobe of the ear, bleeding ordinarily lasts for 1 to 6 minutes. The time depends largely on the depth of the wound and the degree of hyperemia in the finger or ear lobe at the time of the test. Lack of any one of several of the clotting factors can prolong the bleeding time, but it is especially prolonged by lack of platelets.

Clotting Time

Many methods have been devised for determining blood clotting times. The one most widely used is to collect blood in a chemically clean glass test tube and then to tip the tube back and forth about every 30 seconds until the blood has clotted. By this method, the normal clotting time is 6 to 10 minutes. Procedures using multiple test tubes have also been devised for determining clotting time more accurately.

Unfortunately, the clotting time varies widely, depending on the method used for measuring it, so it is no longer used in many clinics. Instead, measurements of the clotting factors themselves are made, using sophisticated chemical procedures.

Prothrombin Time and International Normalized Ratio

Prothrombin time gives an indication of the concentration of prothrombin in the blood. Figure 36-5 shows the relation of prothrombin concentration to prothrombin

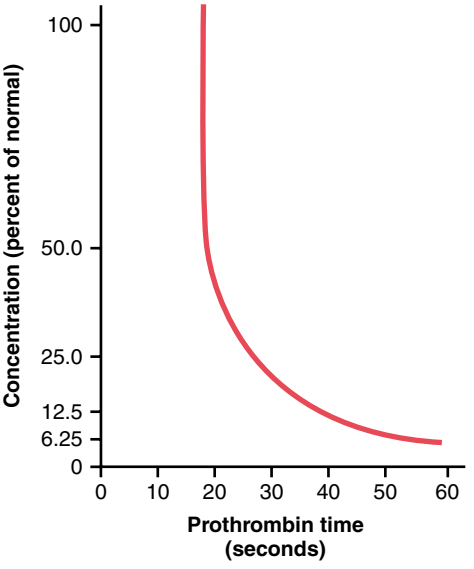


Figure 36-5 Relation of prothrombin concentration in the blood to "prothrombin time."

time. The method for determining prothrombin time is the following.

Blood removed from the patient is immediately oxalated so that none of the prothrombin can change into thrombin. Then, a large excess of calcium ion and tissue factor is quickly mixed with the oxalated blood. The excess calcium nullifies the effect of the oxalate, and the tissue factor activates the prothrombin-to-thrombin reaction by means of the extrinsic clotting pathway. The time required for coagulation to take place is known as the *prothrombin time.* The *shortness of the time* is determined mainly by prothrombin concentration. The normal prothrombin time is about 12 seconds. In each laboratory, a curve relating prothrombin concentration to prothrombin time, such as that shown in Figure 36-5, is drawn for the method used so that the prothrombin in the blood can be quantified.

The results obtained for prothrombin time may vary considerably even in the same individual if there are differences in activity of the tissue factor and the analytical system used to perform the test. Tissue factor is isolated from human tissues, such as placental tissue, and different batches may have different activity. The *international normalized ratio (INR)* was devised as a way to standardize measurements of prothrombin time. For each batch of tissue factor, the manufacturer assigns an international sensitivity index (ISI), which indicates the activity of the tissue factor with a standardized sample. The ISI usually varies between 1.0 and 2.0. The INR is the ratio of the person's prothrombin time to a normal control sample raised to the power of the ISI:

$$\text{INR} = \left(\frac{\text{PT}_{\text{test}}}{\text{PT}_{\text{normal}}} \right)^{\text{ISI}}$$

The normal range for INR in a healthy person is 0.9 to 1.3. A high INR level (e.g., 4 or 5) indicates a high risk of bleeding, whereas a low INR (e.g., 0.5) suggests that there is a chance of having a clot. Patients on warfarin therapy usually have an INR of 2.0 to 3.0.

Tests similar to that for prothrombin time and INR have been devised to determine the quantities of other blood clotting factors. In each of these tests, excesses of calcium ions and all the other factors *besides the one being tested* are added to oxalated blood all at once. Then the time required for coagulation is determined in the same manner as for prothrombin time. If the factor being tested is deficient, the coagulation time is prolonged. The time itself can then be used to quantitate the concentration of the factor.

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