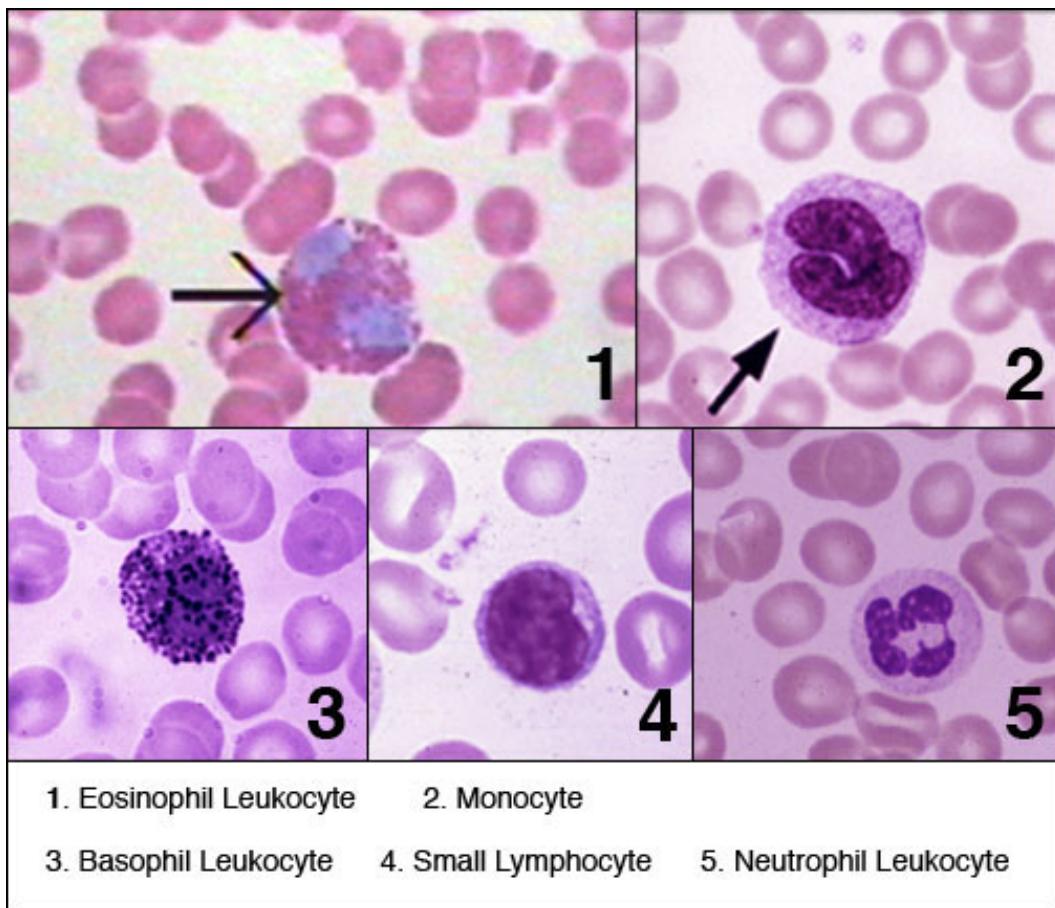


Peripheral Blood



Blood often is defined as a special connective tissue in which the intercellular substance is a fluid. Unlike other connective tissues, however, the intercellular substance of blood lacks a fibrous component, and most of the intercellular protein is produced by cells in other tissues (chiefly the liver) and not by the blood cells. Many of the formed elements of blood consist of anucleate elements (erythrocytes) and bits of cytoplasm (platelets). True cells - leukocytes - make up only a small part of the formed elements and are present only as transients that use the blood for transportation to other organs and tissues into which they migrate to carry out their functions. Only erythrocytes and platelets function while in the bloodstream. None of the formed elements normally replicate within the blood; as they are lost, new elements are added from special blood-forming tissues outside the circulation. Thus, blood can be considered the secretory product of several organs and tissues rather than a tissue per se. The total quantity of blood forms a rather constant proportion of the body mass. In humans the volume is about 5 liters, or approximately 7.5% of body weight.

Components of Blood

Blood consists of formed elements that include erythrocytes, platelets, leukocytes, and a fluid intercellular material called plasma. These elements can be separated by centrifugation, and when done in calibrated tubes, the result (hematocrit) gives an estimate of the volume of the formed elements. The heaviest components, erythrocytes, form the lower layer and make up about 45% of the blood volume. Platelets and leukocytes are present in the buffy coat, a

grayish white layer immediately above the erythrocytes, and form about 1% of the total blood volume. The uppermost layer consists of plasma, a proteinaceous solution, which contains three main types of protein: albumin, globulin, and fibrinogen. Albumin, the most abundant and smallest of the plasma proteins maintains blood colloidal osmotic (oncotic) pressure, is formed in the liver, as is fibrinogen, an essential component for blood clotting. The globulins (alpha, beta, and gamma) include several proteins of different sizes. Globulins participate in the transport of hormones, ions, metals and lipids. The gamma globulins are immunoglobulins (antibodies) synthesized by cells of the lymphatic organs and tissues. The plasma also contains hormones from various endocrine organs, metabolites, nutrients and several other substances. Plasma is obtained from blood after treatment with an anticoagulant and contains all the components of the fluid portion of blood. It makes up about 55% of the blood volume. In contrast, serum is obtained from clotted or defibrinated blood and does not contain fibrinogen; it does contain other components elaborated during the process of blood clotting.

Erythrocytes

At rest, the average human uses about 250 ml of oxygen and produces almost 200 ml of carbon dioxide per minute. With activity, these quantities can increase 10- to 20-fold. Oxygen and carbon dioxide are carried by the erythrocytes, which transport the gases with great efficiency. Exchange of gases occurs in the blood capillaries of the lung, through which the erythrocytes pass in less than 1 second, yet gaseous exchange is complete in approximately the first third of this time.

Structure

In blood smears and tissue sections, erythrocytes appear as anucleate, uniformly acidophilic bodies devoid of any internal structure. The pigment hemoglobin makes up about 95% of the dry weight. Erythroplastid more aptly describes these elements, but custom and common use has given the terms erythrocyte and red cell the status of proper terminology. Electron microscopy confirms the absence of organelles in the interior of mature red cells, which are unable to synthesize protein or renew constituents of their cell membrane. The cholesterol of the plasmalemma, important for the flexibility of the cell, is controlled by the plasma concentration of cholesterol, not by cell metabolism.

Carbohydrate chains extending from glycoproteins and glycolipids of the erythrocyte plasmalemma contain a number of antigenic determinants that are the basis for the ABO blood-group system. The cell membrane limiting erythrocytes of some individuals may have either antigen A (type A blood), antigen B (type B blood), or both antigens A and B (type AB blood). Erythrocytes of other individuals may lack both antigens, and such individuals are classified as having type O blood. Most individuals have antibodies in their blood plasma against erythrocyte antigens, with the exception of their own, and if a different blood type is transfused an immune reaction will occur. Therefore, it is essential to determine what antigens are present on the erythrocytes of donor blood and what antibodies are present in the plasma of the recipient before giving a transfusion.

Shape and Size

The human red cell usually is described as a biconcave disc. Face on, the normal erythrocyte presents a smooth, rounded contour with a central depression. In smears, human erythrocytes

average $7.6\text{ }\mu\text{m}$ in diameter, are slightly smaller in tissue sections ($6.5\text{-}7.0\text{ }\mu\text{m}$), and are slightly larger (about $8\text{ }\mu\text{m}$) in fresh blood. The thickness of the red cell is about $2\text{ }\mu\text{m}$. Apparently this thickness allows hemoglobin to maintain a distance from the cell surface that is optimal for its function.

Erythrocytes are enclosed in a typical cell membrane, whose flexibility and elasticity allow the red cell to accommodate its passage through the small capillaries. *In vivo*, the red cells often assume a cup shape as they pass through small blood vessels. A subplasmalemmal network of protein (spectrin) helps to maintain the biconcave shape and still allows flexibility. Spectrin, linked by actin, appears to form a web immediately beneath the plasmalemma and may act as a cytoskeleton. The network is attached to the interior of the cell membrane by the protein ankyrin so that the cytoskeleton and cell membrane are linked to act as a unit. Hemoglobin also may play a role in maintaining cell shape, since marked changes in shape are associated with the abnormal hemoglobin of sickle cell anemia.

Number and Survival

Consistent sex differences in the number of red cells are seen, with lower values occurring in women. Values of $4,500,000$ to $6,000,000/\text{mm}^3$ in men and $3,800,000$ to $5,000,000/\text{mm}^3$ in women are considered normal. The survival time of red cells is about 120 days. Although red cells generally are removed as they age and wear out, a certain amount of random destruction also occurs; many red cells are destroyed in the bone marrow without ever being released. As erythrocytes age, they use up most of the enzymes associated with adenosine triphosphate (ATP) and are unable to maintain themselves, synthesize protein, or renew their cell membranes. By the end of their life span, red cells have become rigid due to loss of cholesterol from the cell membrane and to degradation of protein and its cross-linkage with calcium. These aged red cells are trapped and destroyed by phagocytes mainly in the spleen but also in the liver and bone marrow. About 1% of circulating erythrocytes are destroyed daily. Iron in hemoglobin is recovered by the liver, stored, and recycled to new red cells.

Reticulocytes

Most erythrocytes stain an orange-red with the usual blood stains, but a few take on a bluish or slate gray tint. These polychromatophilic cells are erythrocytes that are not fully mature and contain a small amount of ribonucleoprotein that, when stained with brilliant cresyl blue or new methylene blue, precipitates as a network or web. These cells are called reticulocytes. Their numbers in peripheral blood provide a rough index of erythrocyte production. Normally, reticulocytes make up only 1 to 2% of the red cells in peripheral blood.

Rouleaux

Erythrocytes tend to adhere to each other by their broad surfaces to form stacks called rouleaux. Rouleaux depend on changes in the blood plasma rather than in the cell. Any condition that increases the net positive charge in the plasma produces changes in the surface charge of erythrocytes, allowing them to adhere to each other more readily. Rouleaux are temporary phenomena, may occur intravascularly, and appear to do no harm to the red cells. Increased rouleaux are reflected in an increase in the rate at which red cells settle out or sediment.

Abnormalities

Departures from normal size, shape, or staining properties of erythrocytes can be important indicators of disease, but to a much lesser degree, some of these abnormalities may be found in healthy individuals also. Anisocytosis describes abnormal variations in the size of red cells, which may be macrocytes (larger than normal) or microcytes (smaller than normal). In macrocytes the central pale area is less marked than in normal cells, but the concentration of hemoglobin is not increased. Irregularity in shape is called poikilocytosis; the cells may show blunt, pointed, or hook-shaped projections from their surfaces. Under various conditions, red cells *in vitro* may become shrunken and show numerous projections on their surfaces; these cells are said to be crenated and are called echinocytes. Crenated cells can be produced by subjecting normal red cells to fatty acids, anionic compounds, elevated pH, or hypertonic media. The changes are reversible. One of the most severe changes in shape occurs during sickling of red cells in sickle cell anemia, in which erythrocytes appear as crescents, holly leaves, or even tubes. Hypochromia denotes cells that stain poorly due to a decrease in hemoglobin; it frequently accompanies microcytosis. In extreme forms, staining may consist simply of a narrow peripheral band. Cells that are thinner than normal (leptocytes) often appear as target cells in which the staining is disposed as a central disc and an external band separated by an unstained zone. Howell-Jolly bodies are nuclear fragments left over from the nucleated precursors of the red cell. They appear as one or two rounded or rod-shaped basophilic granules. Siderocytes contain clusters of small, darkly stained granules that react positively for iron. They are rare in normal peripheral blood but are quite common in bone marrow. Normally the iron-containing granules are removed by the spleen without harming the cell. Allied to polychromatophilia is basophilic stippling, which appears as blue-black dots that may be coarse or fine and consists of precipitated ribonucleoprotein. It is found occasionally in leukemia and severe anemias but is of diagnostic importance in chronic lead poisoning. In some pathologic conditions, globular rather than bi-concave red cells are produced. These are called spherocytes and appear as small, deeply stained cells with a sharp, distinct outline.

Hemoglobin

Erythrocytes contain both hemoglobin and the enzyme, carbonic anhydrase. Oxygen is not soluble and transport is carried out by hemoglobin, an iron-containing respiratory pigment that gives the red color to human blood. In contrast, 90% of the carbon dioxide is transported as bicarbonate in the plasma. Dissolved carbon dioxide (CO_2) enters the erythrocyte and reacts with water to form carbonic acid (H_2CO_3). Carbonic anhydrase within the erythrocyte immediately converts the formed carbonic acid to hydrogen ion (H^+) and bicarbonate (HCO_3^-). Once formed the bicarbonate leaves the erythrocyte in exchange for chloride ion (Cl^-). On reaching the lung bicarbonate passes back into the erythrocyte, combines with hydrogen ion carried and buffered by the hemoglobin to form carbonic acid which is catalyzed by carbonic anhydrase back to carbon dioxide and water. The former then diffuses from the erythrocyte into the alveoli of the lung to be exhaled.

Structure

Hemoglobin is one of a group of catalytic compounds that has an iron porphyrin group, heme, attached to a protein, globulin. The heme group consists of four pyrrole rings combined with iron through their N groups. Structurally, hemoglobin is a symmetrical molecule formed by two

equal mirror-image halves. Each half molecule contains two different peptide chains, each bearing a heme group around which the chain is coiled. The heme groups form the functional component, and the peptide chains make up the globin part of the molecule. The amino acid composition and sequences in the globin confer species specificity to the whole molecule and also determine the type of hemoglobin present. Humans synthesize four structurally different globin chains: alpha, beta, gamma, and delta. About 95% of normal adult hemoglobin is hemoglobin A (HbA), which contains two alpha and two beta chains: about 2% is HbA2, which consists of two alpha and two delta chains. The remainder, fetal hemoglobin (HbF), is composed of two alpha and two gamma chains. Fetal hemoglobin predominates in fetal life but is replaced by the adult type postnatally. Fetal hemoglobin has a higher oxygen affinity than adult hemoglobin and therefore has the capacity to pull oxygen from the erythrocytes of maternal blood and into the fetal circulation during the prenatal period. Even minor modifications of the peptide chain can result in gross changes in the entire hemoglobin molecule, as shown by the sickle hemoglobin (HbS) associated with sickle cell anemia. In this condition the only fault in the molecule is in the sixth position of the beta chain, where the glutamic acid of normal hemoglobin has been replaced by valine.

Platelets

Platelets are the second-most numerous of the formed elements of blood. Platelets are not cells but merely fragments of cytoplasm derived from a large precursor cell (megakaryocyte) in the bone marrow.

Structure

Platelets are small, anucleate bodies' 2 to 5 μm in diameter. In stained smears, platelets show a granular part, the granulomere, and a pale, granule-free hyalomere. The granulomere (chromomere) often fills the central region of the platelet and may be so compact as to suggest a nucleus. Separation into two zones is not seen in circulating platelets or in those fixed immediately by drawing blood into fixative. In these conditions, the granulomere remains evenly distributed. Electron micrographs show that the granulomere consists of lysosomes, mitochondria, dense-cored granules that contain serotonin, adenosine diphosphate (ADP), ATP, calcium, alpha particles that contain platelet-specific proteins, fibrinogen and other clotting factors, actin, myosin, adenosinetriphosphatase (ATPase), and agents that increase vascular permeability. Mitochondria are small and few in number and have few cristae. Variable numbers of glycogen granules also are present. The hyalomere represents the cytoplasmic matrix and appears as a homogeneous, finely granular background. A crisscross arrangement of actin and myosin filaments is present just beneath the plasmalemma. A narrow peripheral zone remains free of granulomere elements and is the site of a system of microtubules that forms the marginal bundle. This structure has been described as a single, coiled microtubule that acts as a stiffening element to help maintain the discoidal shape. Each platelet is bounded by a typical cell membrane that limits the cytoplasm and becomes continuous with an elaborate system of channels.

Number

The number of platelets varies widely depending on the species and in individuals also. The variations reflect difficulties in obtaining accurate counts due to physiologic factors that affect

the numbers and to inherent properties of the platelets, such as their tendency to stick to foreign surfaces or to each other to form clumps. When in contact with a foreign surface, platelets spread to cover an area several hundred times that of their initial surface. Although platelet numbers in humans are given as 250,000 to 400,000/mm³ of blood, some estimates extend the range to 900,000/mm³. Transient fluctuations in platelets have been associated with variation in oxygen concentration, exposure to periods of cold, and food intake. Curiously, highly spiced foods have been reported to cause a significant reduction in circulating platelets. Emotional states such as fear and rage result in markedly elevated counts. A progressive decrease in the number of platelets occurs in women during the two weeks prior to menstruation, with a rapid return to normal values thereafter. A severe reduction in the number of platelets in circulating blood is referred to as thrombocytopenia.

Leukocytes

Leukocytes (white blood cells, WBCs) are true cells that have nuclei and cytoplasmic organelles and are capable of ameboid movement. They migrate from the blood into tissues, where they perform their functions.

Classification

Leukocytes can be classed as granular or agranular on the basis of cytoplasmic granulation or as polymorphonuclear or mononuclear according to the shape of the nucleus. Granular leukocytes contain multilobed nuclei and thus the terms granular leukocyte and polymorphonuclear leukocyte denote the same class of cell. Granular leukocytes (or granulocytes) can be subdivided into neutrophils (heterophils), eosinophils, or basophils according to the color of their cytoplasmic granules after staining with standard blood stains. Heterophil granules do not stain the same in all species, so the term is used inclusively for this type of granular leukocyte, regardless of staining reaction or species. The term neutrophil refers to the heterophil of humans. Agranular leukocytes consist of lymphocytes and monocytes, which have inconspicuous or no granules and a single, non-lobed nucleus. Thus, the terms mononuclear leukocytes and agranular leukocytes are used to denote the same class of cells. The distinction between granular and agranular leukocytes is not absolute: monocytes regularly show fine, dust like granules that are barely resolvable by light microscopy, and lymphocytes occasionally may show a few coarse granules. The distinction is an old one based on appearances with less refined dyes but remains useful because the granules of granular leukocytes are specific, distinctive, and prominent.

Neutrophil (Heterophil) Leukocytes

The neutrophil granulocyte varies from 12 to 15 μm in diameter and is characterized by the shape of the nucleus, which contains small lobes connected by fine filaments. The nucleus stains deeply, and the chromatin is aggregated into clumps that form a patchy network. Nucleoli are absent.

The number of lobes varies, but in humans, two to four are usual. A single, elongated, non-lobed nucleus is seen in a small number of granulocytes; called band forms, they represent cells recently released from the bone marrow. Excessive lobation occurs in some diseases or as an inherited anomaly in humans. In addition to nuclear lobation, nuclear appendages in the shape of hooks, hand racquets, clubs, or drumsticks may be present. Only the drumstick is significant: it represents female sex chromatin and usually is found on a terminal lobe in about

2 to 3% of the neutrophils from women. The cytoplasm of neutrophils contains three types of granules. Most numerous are the specific granules (secondary, type B granules), which are small and take on a pinkish hue. These granules contain lysozyme, an enzyme complex that acts against components of bacterial cell walls, alkaline phosphatase, collagenase, and lactoferrin, another antibacterial substance. Several other rather poorly characterized basic proteins (phagocytins) are present and also have antibacterial properties. Azurophil granules (primary, type A granules) are less numerous, somewhat larger, and stain a reddish purple. They contain myeloperoxidase as well as a battery of acid hydrolases and are considered to be modified lysosomes. Azurophil granules are large and homogeneous, whereas the smaller, less dense-specific granules may contain a crystalloid body. Myeloperoxidase complexes with peroxide to produce activated oxygen, a potent bactericide. Tertiary granules of neutrophils are secreted and promote cell adhesion. These factors also may be involved in the mechanics of phagocytosis.

Cytokines activate both white cells and the endothelium to produce adhesion molecules that allows the leukocytes to stick tightly to the endothelium. The activated white cells become motile, cross the endothelial cell barrier, and actively migrate by ameboid movement into adjacent tissues. It is estimated that about 40% of the neutrophils in the peripheral circulation are marginated (adhering to the endothelium of small blood vessels) under normal conditions. The leukocytes move in the direction of the highest concentration of chemotaxins. The crossing of the endothelial lining is referred to as diapedesis. The granules are scattered throughout the neutrophil cytoplasm, except in a narrow peripheral zone, that contains fine filaments and microtubules that appear to function in cell movement. A small Golgi complex and a pair of centrioles are located centrally in the cell. Rare profiles of endoplasmic reticulum, a few mitochondria, and ribosomes are present also. Neutrophils produce a protein that converts plasminogen to plasmin, the proteolytic enzyme responsible for fibrinolysis. Whereas the release of granule-associated factors is associated with death of the cell, release of plasminogen-activating substance is a secretory activity directly related to cell viability.

Neutrophils are the chief leukocytes in humans, forming 55 to 70% of the circulating white blood cells. Neutrophils circulate in a resting state. When they are activated they are short lived and die when their lysosomal enzymes are released which also causes liquefaction of adjacent tissues. The primary function of neutrophils is to engulf and/or destroy bacteria.

Eosinophil Leukocytes

Eosinophils form only a small proportion of the white cells and in humans normally makes up 1 to 3% of the circulating leukocytes. Their numbers in blood bear some relation to the activity of the adrenal glands; a decrease in eosinophils is seen in the alarm reaction. They increase in number during parasitic infestations. This relationship may account for the wide range of values reported. Distinct diurnal variations related to activity cycles also occur with highest numbers in the circulation during morning hours and minimal numbers reported in the afternoon. The eosinophil is about the same size as the neutrophil and also is characterized by nuclear lobation. Although often described as having a bilobed nucleus, eosinophils with three or four lobes are not uncommon. Conditions that produce hypersegmentation in neutrophils also affect the eosinophil granulocyte.

The distinctive feature of eosinophils is the closely packed, uniform, spherical granules that stain a brilliant red or orange-red. The granules are membrane-bound and show an internal

structure called a crystalloid, or internus. In humans, the crystalloid assumes various forms and may be multiple.

The crystalloid is embedded in a finely granular matrix. Eosinophil granules are lysosomes and contain the usual lysosomal enzymes. They show a higher content of myeloperoxidase than do the azurophil granules of neutrophils and lack lysozyme and phagocytin. The granules also contain lipid and major basic protein (MBP) that is responsible for the eosinophilia. Eosinophils respond chemotactically to bacterial products, complement components, and substances released by mast cells (histamine and eosinophilic chemotactic factor of anaphylaxis). Eosinophils have a special affinity for antigen-antibody complexes, which tend to bind complement and induce cell lysis. Phagocytosis of the complexes may suppress cell destruction in normal tissues. Factors such as histaminase released by eosinophils may dampen allergic responses by degrading histamine and histamine-like substances released from mast cells and basophils. Prostaglandins E1 and E2 released by eosinophils inhibit mast cell secretion. Eosinophils also have a major role in controlling parasitic infections (ascariasis, trichinosis, and schistosomiasis): eosinophils selectively interact with the larvae of some parasites and damage them by oxidative mechanisms. The major basic protein (MBP) in the crystalloid core of the eosinophil granules enhances the antibody-mediated destruction of parasites. Eosinophils have surface receptors for IgE antibody. The primary function of eosinophils is to dampen or terminate allergic reactions and parasitic infections.

Basophil Leukocytes

Basophils make up only about 0.5% of the total leukocytes. They are slightly smaller than neutrophil granulocytes and in blood smears measure 10 to 12 μm in diameter.

Nuclear lobes are less distinct than in the neutrophil or eosinophil, and nuclei with more than two or three lobes are rare. Filaments between lobes tend to be short and broad and rarely form the threadlike structures seen in neutrophils. The chromatin is relatively homogeneous and stains less deeply than in the other granulocyte types. Nucleoli are absent. The cytoplasm of basophils contains prominent, rather coarse granules that stain a deep violet with the standard blood stains and metachromatically with toluidine blue or thionin. Well-preserved granules are spherical and uniform but, being soluble in water or glycerin, frequently appear irregular in size and shape in fixed preparations. The granules are scattered unevenly throughout the cytoplasm, often overlie and obscure the nucleus and are neither as numerous nor as densely packed as the granules of eosinophil leukocytes. Electron microscopy shows an internal striated pattern to the granules. The granules contain myeloperoxidase, glycosaminoglycans, leukotriene 3, eosinophilic chemotactic factors, prostaglandins, and a platelet-activating factor. The basophilia of the granules is due to the presence of heparin. There is no evidence that the granules have lysosomal activity. Basophils have surface receptors for IgE produced in response to allergens. Basophil granules also have a high content of heparin (an anticoagulant), histamine, vasodilating agents, and slow-reacting substance (SRS). Histamine induces a prompt and transient vasodilation, whereas that induced by SRS is more sustained and occurs after a latent period. Both increase vascular permeability. Antigens that bind to specific sites on the basophil cell membrane cause degranulation with release of agents that cause smooth muscle spasm, mucous secretion, hives, itching, and rhinitis. Basophils play a role in immediate types of hypersensitivity reactions, anaphylactic reactions (hay fever), and some forms of asthma.

Lymphocytes

Lymphocytes are the predominant agranulocyte and in the adult human account for 20 to 35% of the circulating leukocytes.

Classification

Lymphocytes comprise a family of cells, each differing in function, life span, background, and size. They are found in blood, lymph, lymphatic tissues, and organs. The most common type is the small lymphocyte. In blood smears this cell has a diameter of 6 to 8 μm and appears to be little more than a nucleus surrounded by a thin rim of pale blue cytoplasm. At one aspect of the cell, the cytoplasm may be somewhat expanded. The round or slightly indented nucleus is heterochromatic and stains deeply. Except in living cells or in electron micrographs, nucleoli usually are not visible. Because of the round nucleus, lymphocytes are classified as mononuclear leukocytes. Electron micrographs reveal a small Golgi complex and a few mitochondria and lysosomes; a small amount of SER is present, but GER is very scarce. While lymphocytes are classified as agranular leukocytes, a few nonspecific azurophil granules may be present and represent lysosomes. Although morphologically indistinguishable by ordinary means, small lymphocytes are a mixture of functional types that can be divided into three major categories, B-lymphocytes, T-lymphocytes, and natural killer (NK) cells, by means of distinctive surface markers. B-lymphocytes (B-cells) play a central role in humoral immunity and represent cells that have been conditioned to transform into plasma cells and secrete antibody when stimulated by foreign antigens. The antibody reacts specifically with the antigen that induced its formation. The organ responsible for conditioning B-cells in mammals appears to be the bone marrow. In birds, B-cells are processed in the bursa of Fabricius, a lymphoreticular, appendix-like diverticulum of the cloaca. B-cells form about 5 to 10% of the circulating blood lymphocytes in humans. T-lymphocytes (T-cells) have been processed by the thymus, where they acquire distinctive T-cell markers and become immunologically competent. However, immunologic capacities are not fully developed until the cells are exported to peripheral lymphatic organs, especially the spleen. T-cells contribute to cell-mediated immunity and perform a variety of functions within the immune system. T-lymphocytes are generally organized into three functional subcategories. When appropriately stimulated, some T-cells interact with activated B-cells and, as T-helper cells, amplify the production of antibody; as T-suppressor cells, they dampen antibody formation; and as cytotoxic T cells, some have the capacity to kill target cells. Some T-cells produce lymphokines that have important roles in body defenses. Of the many lymphokines described, the best characterized are agents that damage or destroy foreign cells or interfere with their replication, such as lymphotoxin, interleukin, interferon, and tumor necrosis factor; agents that inhibit migration of macrophages (MIF) and thus concentrate macrophages at the site of an antigen; macrophage-activating factor, which stimulates macrophage activity; lymphocyte blastogenic factor (LBF), which induces blastic transformation, growth, and differentiation of small lymphocytes; factors that attract neutrophil, eosinophil, or basophil granulocytes to the sites of antigens (i.e., chemotactic agents); and factors that increase vascular permeability. Thus, lymphokines represent a large class of lymphocyte-derived substances that have a role in controlling immunologic and inflammatory responses or have cytotoxic activities. Some activated T-cells, the cytotoxic T-cell or killer (K) lymphocytes, take part in antibody-dependent, cell-to-cell interactions with foreign cells that result in lysis of the target cell. Only target cells that share antigens with the foreign cell that originally inciting the appearance of cytotoxic cells are killed.

Cytolysis of the foreign cell requires intimate contact between the effector and its target, the latter then undergoing a series of membrane changes that result in rupture of the cell. The cytotoxic T-lymphocyte is unharmed by the interaction and remains free to react with other target cells. T-lymphocytes express cell surface markers called CDs (cluster designation molecules) that help lymphocytes perform their specific effector functions. T-helper cells express the CD4 molecule. They are necessary to induce B-lymphocytes to produce antibody and to activate the macrophage defense system. The T-helper cells recognize antigen when it is presented on cells that also express class II major histocompatibility (MHC) antigens. Cytotoxic T-cells express CD8 molecules and recognize and kill target cells. T-suppressor cells can express either CD4 or CD8 molecules and are capable of inhibiting the response to helper T-cells and modulate the immune response. An additional form of small lymphocyte, the null cell, also has been described. These cells lack B- or T-cell surface markers. Null cells also appear to be a diverse group among which may be circulating hemopoietic stem cells. Larger forms of lymphocytes, 10 to 14 μm in diameter, also are present in peripheral blood in small numbers. These cells have been called large granular lymphocytes. The cytoplasm of these cells is more abundant and more basophilic than that of the small lymphocyte. Electron micrographs show an increased number of ribosomes and mitochondria, a greater amount of endoplasmic reticulum, and an increase in the size of the Golgi apparatus. Scattered azurophil granules are present. These cells are directly cytotoxic and also lyse target cells by contact, but they appear to function in the absence of antibody and without known prior exposure to antigen. Thus, they have been named natural killer (NK) cells. Commonly considered to be null cells, there is growing evidence that they are a subset of T-cells and may be related to immune-dependent cytotoxic T-cells. Natural killer cells form the third main type of lymphocyte and have the ability to kill other cells. The primary role of this form of lymphocyte is the elimination of virus-infected cells and some tumor cells.

Stimulation by Antigens and Mitogens

Small lymphocytes are not simply terminal effector cells engaged in T- or B-cell activities; they also have the properties of "stem" cells and can propagate additional small lymphocytes when stimulated by antigens or nonspecific agents called mitogens. The best studied mitogens are plant extracts - phytohemagglutinin (PHA), concanavalin A (con A), pokeweed mitogen (PWM) - but other agents such as liposaccharide from *Escherichia coli*, antilymphocyte serum, and various pollens also have mitogenic activity. These agents have a common ability to bind to surface groups on small lymphocytes, thereby triggering blastogenic transformation. During transformation, small lymphocytes progressively enlarge; the nuclei increase in size and become euchromatic, nucleoli become visible, the Golgi complex enlarges, and the cell synthesizes deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Within 36 to 48 hours, the small lymphocyte has assumed the appearance of a lymphoblast that divides and gives rise to new T- or B-cells. The new cells can produce lymphokines (T-cells) or become plasma cells and synthesize antibody (B-cells). The reactions to mitogens show a degree of specificity: PHA and con A stimulate T-cells but not B-cells; *E. coli* lipopolysaccharide stimulates only B-cells; PWM activates both types of small lymphocyte. The mechanism of the reaction is not understood, but the reaction is specific to lymphocytes in that although these agents bind to the surface of other cells, they do not induce their transformation. The response of lymphocytes to mitogens resembles that after exposure to antigen or lymphoblastic lymphokine. Lymphocytes recirculate repeatedly between the blood, lymphoid organs, and lymph. Only small lymphocytes re-circulate, and most of these (80-85%) are long-lived cells of

T-cell origin, with a life span measured in years. A small proportion (12-15%) of the circulating cells are short-lived B-cells with a life expectancy of several months. Until the lymphocytes make contact with an antigen, the cells continue to recirculate. A small number of long-lived cells, of T- and B-origin, are present as memory cells. These are small lymphocytes that have been stimulated by antigen but have not responded with production of antibody or lymphokines. They remain for years as conditioned cells that can react immediately to the same antigen on subsequent exposure to it. Lymphocytes are highly motile cells that leave the bloodstream to enter lymphatic organs, migrate through connective tissues of the body, or penetrate into epithelia. They have little ability to adhere to or to spread on surfaces of any kind.

Monocytes

Monocytes make up the second type of mononuclear leukocyte normally found in the blood. Their proportions are fairly consistent, and they regularly form 3 to 8% of the circulating leukocytes. Monocyte numbers are depressed by corticosteroid administration. They vary in size from 12 to 20 μm in diameter and contain a fairly large nucleus that may be rounded, kidney-shaped, or horseshoe-shaped. Segmentation of the nucleus and formation of filaments never occur, but coarse constrictions with blunt, broad lobes may be present. The chromatin is more loosely dispersed than in lymphocytes and therefore stains much less densely. Two or three nucleoli may be seen. The blue-gray cytoplasm is abundant and contains numerous fine azurophilic granules that impart an opacity to the cytoplasm, giving it a "ground glass" or "dusty" appearance. Ultrastructurally, the nucleus shows one or more nucleoli and the cytoplasm contains a small amount of GER and polyribosomes. Small, elongated mitochondria are present, and the Golgi apparatus is well formed. The azurophil granules represent primary lysosomes and in electron micrographs appear as dense, homogeneous structures. Monocytes are part of the mononuclear phagocyte system and represent the cells of this system in transit. They serve little function while in the blood but migrate into various organs and tissues throughout the body, where they differentiate into macrophages. Monocytes respond chemotactically to the presence of invading microorganisms and necrotic material. They express high levels of MHC class II molecules on their surface and are an important site for production of cytokine IL-1 which plays an important role in systemic responses to acute inflammation. In addition to serving as tissue scavengers, monocytes also have a role in processing antigen in the immune response and are able to fuse with one another to form various phagocytic giant cells. Some macrophages have antigen-presenting functions and these specialized macrophages form a family of antigen presenting cells. This type of macrophage phagocytoses endogenous antigens that are degraded into antigen peptide fragments. These fragments are then presented on the macrophage surface together with class II major histocompatibility complex (MCH). The presented antigen peptide fragment is recognized by CD4⁺ helper T lymphocytes which are stimulated and in turn promote B lymphocyte differentiation. Monocytes do not possess true storage granules for their enzymes as do granulocytes, and the cell behaves mostly as a secretory cell. They contain acid hydrolases, peroxidase, acid phosphatase, and aryl sulfatase.

Abnormalities

As with erythrocytes, the total number of circulating leukocytes tends to fall within a relatively narrow range in normal individuals. In humans, this range is 5,000 to 12,000/mm³. An increase

above normal is called a leukocytosis and may be due to disease or emotional or physical stress. A leukocytosis with counts of 25,000 to 35,000/mm³ has been reported after severe exercise. The increase represents the flushing of leukocytes sequestered in capillary beds and marginated at the edges of the bloodstream. A decrease in leukocytes is called leukopenia. Diurnal variations of variable degree are associated with activity cycles. Fluctuations in leukocyte numbers usually involve neutrophil granulocytes and lymphocytes. If neutrophil granulocytes are in excess, a neutrophilia is said to be present; if decreased, a neutropenia is present. An increase or decrease in lymphocytes is a lymphocytosis or lymphopenia, respectively. Increases in eosinophils, basophils, and monocytes are referred to as an eosinophilia, basophilia, and monocytosis, respectively. Decreases in these cells are difficult to establish from smears because of their normally low numbers, but they do exist. Various cytologic abnormalities may occur, especially among the granulocytes. Döhle's bodies are small, irregular blue clumps or patches, 0.5 to 2.0 μm in diameter that may appear in the cytoplasm of neutrophils during some infections, after burns, in cancer, or after treatment with some oncolytic drugs. They appear to consist of altered ribonucleoprotein. Toxic granulation is seen as coarse, black or purple granules scattered in the cytoplasm of neutrophils and represents altered azurophil granules. Toxic granulation often accompanies Döhle's bodies and, with cytoplasmic vacuolation, may occur during severe bacterial infections. Alder's anomaly is a rare abnormality characterized by large, coarse granules in the cytoplasm of neutrophils. An apparently inherited anomaly, it has no pathologic significance. Hypersegmentation of granulocyte nuclei occurs as an inherited anomaly of no significance and also accompanies anemia resulting from deficiency of vitamin B₁₂. In such anemia, the cells are abnormally large and have been called macropolycytes. At the other extreme, poorly segmented granulocytes are present in the Pelger-Huët anomaly, an inherited condition. All types of granulocyte are involved, and the mature cells rarely show more than two lobes; more frequently, the cells have round nuclei with no filaments. The anomaly is without significance. Atypical lymphocytes may show vacuolated cytoplasm, folded (monocytoid) nuclei, nuclear vacuolation, or prominent nucleoli. Most of the atypical cells are medium lymphocytes. Dark, basophilic cytoplasmic granules have been reported in lymphocytes in cases of gargoylism (Hurler's syndrome), and inclusions similar to Alder's granules occur in the lymphocytes of children as a familial disorder.

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