153 | The White Blood Cell and Differential Count

MARTIN S. BLUMENREICH

Definition

White blood cells (WBC) are a heterogeneous group of nucleated cells that can be found in circulation for at least a period of their life. Their normal concentration in blood varies between 4000 and 10,000 per microliter. They play a most important role in phagocytosis and immunity and therefore in defense against infection.

Technique

Leukocytes can be evaluated through several techniques of varying complexity and sophistication. Both quantitative and qualitative properties can be assessed in the laboratory.

The simplest test is the WBC count and differential. White cells can be counted manually in specially designed chambers (Neubauer) or with automated counters. The latter are widely used, offering the advantage of higher accuracy and speed over manual techniques. To determine the differential, a drop of blood is thinly spread over a glass slide, air dried, and stained with a Romanofsky stain, most commonly the Wright or May-Grunewald-Giemsa technique. Two hundred cells are then counted and classified. Machines have been developed to perform automated differential counts, but they are still inferior to manual techniques as far as reliability and ability to discover morphologic abnormalities.

The absolute number of each type of WBC, often more informative than its proportion, can be calculated if the differential and the total number of leukocytes per volume unit are known.

Many of the conditions affecting the WBC can be diagnosed from studying the peripheral smear, but it may be necessary to evaluate the bone marrow for a better investigation. Bone marrow can be aspirated from the posterior iliac crest or the sternum. A core biopsy can be obtained percutaneously from the iliac crest. Biopsies allow assessment of the architecture of the marrow. Touch preparations can be made at the time the core bone marrow is obtained. Clumps of metastatic epithelial cancer cells can be recognized easily with this technique.

When evaluating a bone marrow specimen, note must be made of the overall cellularity and of the presence and proportion of normal bone marrow elements of abnormal hematopoietic cells or extrinsic cells. Myeloid precursors are two- to fourfold more numerous than erythroid precursors.

Occasionally, the morphologic examination is not sufficient to differentiate among cells of myeloid, monocyte, or lymphoid origin. *Histochemistry* can be helpful in this regard. In principle it identifies differences in the cellular content of different substances, mainly cytoplasmic enzymes. Among those most commonly used are:

- Leukocyte peroxidase, which is present in myeloid cells and ANLL (acute nonlymphoblastic leukemia) blasts. It plays a role in the killing of bacteria. It is not found in ALL (acute lymphoblastic leukemia) cells.
- Leukocyte alkaline phosphatase is found in the more mature cells of the myeloid series, bands and neutrophils. It is useful for the differential diagnosis between CML (chronic myelocytic leukemia) where it is low, from leukemoid reactions, where it is normal.
- Sudan Black B is a lipid stain positive in the neutrophilic granules of precursors and mature granulocytes. It is also found in ANLL but not ALL blasts.
- Periodic acid–Schiff (PAS) demonstrates the presence of polysaccharides. Neutrophilic granules stain with this technique. Lymphocytes may have PAS-positive granules. PAS is negative in ANLL blasts, but ALL blasts may show a variable positivity.
- Acid phosphatase. Macrophages and osteoclasts possess this enzyme. T cell ALL blasts and hairy cells are also positive. Acid phosphatase is tartrate resistant in hairy cell leukemia.
- Leukocyte esterases are found in monocytes and neutrophils in varying concentrations. Alpha naphtyl esterase is strongly positive in monocytes and weakly positive in neutrophils. The reverse is true for AS-D chloroacetate esterase. These enzymes are useful to differentiate the monocytic from granulocytic precursors in ANLL.
- Terminal deoxynucleotidyl transferase (TdT) is present in thymocytes and lymphocyte precursors. It is positive in most patients with ALL, except for the rare B-cell ALL. It is absent in ANLL.

Histochemical stains can sometimes be difficult to interpret even in the most experienced hands, especially when leukemic blasts are undifferentiated enough to make a morphologic diagnosis difficult.

Surface markers refer to a group of membrane properties that are useful to differentiate B from T lymphocytes. Many of these techniques utilize antibodies to detect the presence of the marker.

- Ia is an antigen present in mouse B lymphocyte precursors. It is also found on myeloid cells. An Ia-like antigen is present in human cells.
- Common ALL antigen (CALLA) is described on ALL cells.
- Surface immunoglobulins (sIg) are synthesized and carried attached to the membrane of B lymphocytes. Most commonly they belong to the IgM class.
- Receptors for the Fc fragment of immunoglobulins are also found on the membrane of B and T lymphocytes and monocytes.

- Complement receptors are present in lymphocytes and peripheral blood monocytes.
- Mouse rosettes are formed by B lymphocytes in the presence of mouse erythrocytes.

Several tests identify T cells. Sheep erythrocytes attach to the surface of T lymphocytes, forming characteristic rosettes. Monoclonal antibodies able to identify subsets of T cells are now available. For instance, CD4 (Ortho Company) identifies helper T cells, and CD8 identifies cytotoxic and suppressor T cells.

Chemotaxis and phagocytic function of neutrophils can be evaluated in the laboratory. The skin window technique is semiquantitative but has the advantage of simplicity. An abrasion is created in the epidermis and covered for a few hours with a glass coverslide that is then stained and evaluated for attached granulocytes. More complicated in vitro systems have been devised, most of which are based on the ability of neutrophils to traverse a certain obstacle to reach an attracting stimulus.

Phagocytosis and killing abilities can also be evaluated in vitro. These techniques are cumbersome and not universally available. The simplest way to assess the function of the cellular (T cell) immune system is delayed cutaneous hypersensitivity. Several antigens (PPD, mumps, histoplasmin, candida) can be injected intradermally, usually in the forearm. An area of induration appears after 48 hours if the reaction is positive. The ability of the patient to become sensitized to a new substance can also be tested, utilizing a compound to which the patient has had no previous exposure, like dinitrochlorobenzene (DNCB). In vitro techniques rely on stimulating the proliferation of T cells in culture with specific mitogens (concanavalin A, phytohemagglutinin) or antigens.

Humoral immunity can be investigated by measuring the end product of the B system, the immunoglobulins. In electrophoresis, serum proteins are separated in an electric field. Separation is obtained because the electric charge of the protein molecules varies. Protein can be better characterized and quantified by immunoelectrophoresis. Serum proteins are first separated in an electric field and then react with antibodies introduced in the support medium. Each arch of precipitation can then be identified and measured.

Basic Science

WBC are classified into granulocytes, lymphocytes, and monocytes. Granulocytes owe their name to the presence of distinct cytoplasmic granulation. Three varieties are recognized: neutrophils (or polymorphonuclear granulocytes), eosinophils, and basophils.

Myeloid cells originate from a common pluripotent stem cell, colony forming unit (CFU)-S or CFU-GEMM. A more primitive stem cell gives rise to lymphoid cells as well as to the myeloid precursor. Under the influence of poietins and microenvironmental factors, the stem cells evolve through a series of intermediate steps into the mature blood elements. From CFU-S derive burst forming unit (BFU)-E, which will give rise to CFU-E and through it to the erythroid series; CFU-MEGA, which will originate megakaryocytes; and CFU-GM, which will originate monocytes and granulocytes. Stem cells resemble lymphocytes morphologically. They can be recognized by growth characteristics in vitro in semisolid media containing different growth factors.

In the evolution of the neutrophilic granulocyte, the first cell identifiable morphologically is the myeloblast. As maturation progresses, the myeloblast becomes first a promyelocyte and later a myelocyte. These developmental stages constitute predominantly a proliferative compartment, in which the cell number increases geometrically. The next form, the metamyelocyte, is unable to undergo further mitosis but transforms into a band. This cell is either released into circulation (3 to 5% of WBC) where it completes its maturation or enters a storage compartment in the marrow where it becomes a neutrophil and is released later into the circulation.

About half of the intravascular polymorphonuclear cells are circulating, maintaining a dynamic equilibrium with the other half, which are marginated against the vascular endothelium. Only the circulating neutrophils are accounted for in the WBC count. The half-life of mature neutrophils in circulation is about 7 hours. They irreversibly traverse the vascular endothelium into the tissues, where they die after 1 or 2 days.

The main function of neutrophilic granulocytes is phagocytosis of bacteria. This is a complex multistage process that includes engulfment of the organism, incorporation into the cytoplasm, and fusion with a lysosome where enzymes are liberated that will destroy the bacterium while a burst of energy is generated.

Eosinophils and basophils have a similar development. After release from the bone marrow, eosinophils promptly abandon the intravascular compartment (where they constitute up to 5% of WBC), entering the tissues. They are not able to reenter the blood. Heavy concentrations of eosinophils are found in the GI tract, lung, and skin. The precise function of these complex cells is not well known. They possibly play a role in defense against multicellular parasites and in limiting inflammation.

Basophils constitute about 1 to 2% of circulating leukocytes. Their physiologic role is also not known with precision. In their granules they carry heparin and histamine. IgE can be found bound to their surface.

Macrophages and lymphocytes are known collectively as mononuclear leukocytes. Both play important roles in cellular and humoral immunity. These cells are able to exit and reenter circulation, retaining their function. They may spend time in the tissues or in lymph nodes.

The cells of the monocyte–macrophage system have their origin in the bone marrow, deriving from the CFU-GM. They are not stored but are rapidly released into the circulation where they account for 5% of WBC. In tissues, they become macrophages.

Monocyte–macrophages phagocytose bacteria and particulate material, play a role in the inflammatory reaction, and are important in the immune apparatus where they process antigenic material and "communicate" with T lymphocytes through a cell–cell interaction process. Monocytes are able to secrete interleukin, a substance that potentiates B and T lymphocytes. They participate in fibrinolysis by secreting plasminogen activators.

Lymphocytes are immune cells fundamental in cellular and humoral immunity. In the blood they represent 20 to 45% of WBC. They belong to the B (*b*ursa or *b*one marrow) or T (*t*hymus) systems. Both cells are morphologically indistinguishable. The B system is responsible for synthesis of antibodies. When a B cell is properly stimulated, it proliferates first and transforms later into a plasma cell, the effector limb of the immune arch. Each B lymphocyte is able to synthesize only one species of antibody.

The T system constitutes the cellular immune system and regulates the whole immune apparatus. Several subsets of T cells can be identified with monoclonal antibodies specific against different membrane antigens. For instance, helper T cells favor the function of B cells, whereas suppressor T cells inhibit them. Some T cells are responsible for cell-mediated cytotoxicity; natural killer (NK) lymphocytes are responsible for nonspecific lysis of certain cells.

In the peripheral blood, approximately 15 to 25% of lymphocytes are B cells and 40 to 75% are T cells.

Clinical Significance

Leukocyte Disorders

WBC disorders can be classified as quantitative or qualitative. In quantitative alterations all cells appear normal but are present in abnormal quantities, either in excess or in defect of normal values. In qualitative defects, abnormal appearing cells or extrinsic cells are found in circulation.

Neutrophils

Quantitative abnormalities. Granulocytes can be increased in circulation by four different mechanisms: increased production, decreased egress from the circulation, demargination, and release from storage compartments.

Most instances of neutrophilia are secondary to a pathologic process outside the marrow. It can occur in infectious diseases, especially acute bacterial infections; neoplasia, either affecting the myeloid system (chronic myelogenous leukemia and other myeloproliferative disorders) or secondary to a solid tumor (paraneoplastic syndrome); inflammation secondary to tissue necrosis, metabolic and collagen diseases, hypersensitivity reactions; hemorrhage; hemolysis; and stress.

Neutropenia is due in the great majority of cases to decreased production of granulocytes. Antineoplastic agents and extensive radiation therapy produce neutropenia almost invariably. Drugs such as phenothiazines, phenylbutazone, and allopurinol can induce neutropenia through idiosyncratic reactions. Infections, most often viral but also bacterial or rickettsial, can lower the polymorphonuclear count. Other conditions causing a decrease in WBC production include cyclic neutropenia, congenital disorders, and idiopathic neutropenia.

If peripheral consumption exceeds production, neutropenia and concomitant marrow hypercellularity will result. It can be seen in hypersplenism, Felty's syndrome, and in the presence of antineutrophil antibodies.

The risk of infection increases when the absolute granulocyte count falls below 1000 per microliter. Gram-negative sepsis is common in this setting.

Qualitative abnormalities of neutrophils include functional defects in chemotaxis, phagocytosis, and bacterial killing. They can be due to extrinsic or intrinsic abnormalities of the granulocyte. Extrinsic abnormalities include treatment with antineoplastic agents and corticosteroids, deficiencies of complement and opsonizing antibody, hypophosphatemia, and sickle cell disease. Intrinsic abnormalities include defects of the killing mechanism of ingested bacteria (chronic granulomatous disease), and defects in lysosomal function (Chédiak-Higashi syndrome with giant lysosomes, premature graying of the hair, a bleeding diathesis, and a terminal

phase characterized by adenopathy, hepatosplenomegaly, and marrow failure).

Several abnormalities of cytoplasmic granulation can be found. Toxic granules appear in the cytoplasm of neutrophils during infectious processes and represent probably phagocytic vacuoles. Döhle bodies can be seen in similar circumstances as round, well-delineated structures. The May-Hegglin anomaly is characterized by large inclusion bodies in the cytoplasm of polymorphonuclear cells associated with thrombocytopenia and giant platelets.

The Pelger-Huët anomaly is manifested as a change in the morphology of the nucleus of the polymorphonuclear leukocyte, which has one or two smooth lobes with thick chromatin. The cell is functionally normal.

Monocytosis can follow chronic infectious disorders (tuberculosis, brucellosis), rheumatic diseases (lupus, rheumatoid arthritis), chronic inflammatory bowel disease, and some malignant processes (Hodgkin's and non-Hodgkin's lymphoma). Monocytes play an important role in other chronic granulomatous diseases: sarcoidosis, histiocytosis X, and storage diseases (Gaucher's disease, Niemann–Pick disease).

Eosinophilia occurs in association with hypersensitivity reactions, parasitic infestations, cancers (Hodgkin's disease, eosinophilic leukemia), connective tissue disorders (rheumatoid arthritis, polyarteritis nodosa), and the syndrome of pulmonary infiltrates with eosinophilia.

Basophilia can be found in chronic myelogenous leukemia and other myeloproliferative disorders, Hodgkin's disease, and some chronic inflammatory and infectious disorders.

Lymphocytopenia can be seen mainly in association with several congenital diseases of the immune system or following treatment with corticosteroids, antineoplastic agents, or radiation. *Lymphocytosis* can accompany some infections, both acute and chronic, usually viral, Addison's disease, and autoimmune diseases.

The Leukemias

Acute leukemia results from the malignant proliferation of cells of the myeloid (acute nonlymphoblastic leukemia or ANLL) or lymphoid (acute lymphoblastic leukemia or ALL) progeny. Untreated it is rapidly fatal. The clinical picture stems predominantly from bone marrow failure but also from infiltration of normal tissues by the leukemic cells. Anemia produces weakness, easy fatigability, dyspnea, palpitations, orthostasis, and pallor. Granulocytopenia results in infection, often with gram-negative organisms, but also with low-grade pathogens. Thrombocytopenia is manifested as purpura: epistaxis, petechiae, easy bruisability, and gum bleeding.

Infiltration of tissues results in lymphadenopathy and hepatosplenomegaly (more common and more marked in ALL than ANLL), chloromas, and, in monocytic leukemia, gum hypertrophy. Bone pain and tenderness are slightly more common in ALL than ANLL. In childhood ALL the long bones of the inferior limbs are predominantly affected. In the peripheral blood a normochromic normocytic anemia is found. Platelets are decreased, often to very low values. The total white cell count may be very elevated, normal, or low. In most cases blasts will be found. These are large, immature-looking cells with a high nucleus to cytoplasm ratio. Myeloblasts have a thin, lacy chromatin and well-defined nucleoli. The cytoplasm is pale and may exhibit Auer

rods, pathognomonic of ANLL. Monocytoid or promyelocytic features may be found in AML blasts. Lymphoblasts have a coarser chromatin with less sharply delineated nucleoli and a slightly basophilic cytoplasm with few granules. The bone marrow examination will show hypercellularity, decrease or absence of normal hemopoietic precursors, and infiltrations with blasts.

Sometimes it is difficult to differentiate ALL from ANLL on purely morphologic grounds. Histochemistry and cell surface markers can be helpful in these circumstances. Most patients with ALL have lymphoblasts that mark with neither T nor B cell markers: "null cells"; terminal deoxynucleotidyl transferase, CALLA, and Ia-like antigen are present. It appears that these cells are early B cell precursors. T cell ALL is diagnosed in approximately 20% of ALL patients. Large mediastinal lymphadenopathy is commonly found. B cell ALL is very rare.

Chronic myelogenous leukemia (CML) affects middle-aged patients (median age at diagnosis is 40 to 45 years). A juvenile form of the disease has been recognized. Patients present most often with signs of anemia or after finding a left upper quadrant abdominal mass. As CBC are performed nowadays almost routinely, CML is diagnosed incidentally and asymptomatically in many patients. On physical examination the dominant finding is splenomegaly, which can be enormous, penetrating into the pelvis and extending across the midline. Areas of infarction can result in tenderness on areas of the splenic surface. Palpable lymph nodes are seldom greater than 1 cm in diameter.

Examination of the peripheral smear confirms the diagnosis. The anemia is mild to moderate. Platelet count may be elevated, and counts above 10^6 per microliter are not uncommon; thrombocytopenia is rare. These platelets may be functionally impaired. The most striking abnormality is found in the white cell series. WBC counts are elevated, sometimes so that leukostasis may occur. Cells of all stages of granulopoiesis (including early progenitor cells) are found in the peripheral blood to the extent that it can resemble a bone marrow aspirate. Basophil and eosinophil counts are increased. The bone marrow appears hypercellular with a very heavy predominance of myeloid elements. Megakaryocytes are increased.

In 90% of patients a characteristic chromosomal abnormality, the Philadelphia chromosome (Ph_1), can be found. It results from the translocation of the long arm of chromosome 22 to chromosome 9. The cell primarily affected by the neoplastic process is an early stem cell. As a consequence, the Ph_1 chromosome can be found in neutrophils, erythroid precursors, megakaryocytes, and monocytes.

Treatment of CML improves symptoms but not survival. After a median of 3 to 4 years, the disease evolves into a blastic phase that resembles an aggressive acute leukemia on clinical and laboratory grounds. CML is a myeloproliferative disorder together with essential thrombocythemia, polycythemia vera, and agnogenic myeloid metaplasia.

Chronic lymphocytic leukemia (CLL) is a disorder seen usually in older persons (mean age = 60 years). It is not seen in children. In the majority of patients it results from the malignant proliferation of B lymphocytes, although occasionally a T cell CLL is found. Malignant cells resemble mature lymphocytes but have different morphologic features than their normal counterparts. Patients can be diagnosed while asymptomatic when a high lymphocyte count is found incidentally. A count higher than 15,000 lymphocytes per microliter is necessary to make the diagnosis.

After a variable asymptomatic period, lymphadenopathy will develop. It sometimes can be massive. Splenomegaly is common. As the disease progresses, anemia and thrombocytopenia will appear. Examination of the peripheral blood will invariably show an elevated WBC count at the expense of lymphocytosis, which can often be above 90%. Hypogammaglobulinemia develops in almost all patients, increasing the risk of infections, predominantly with encapsulated gram-positive organisms. Autoimmune hemolytic anemia and hypersplenism are common. The natural history of the disease is measured in years.

T cell CLL is a more aggressive disease. The skin is commonly involved and splenomegaly is seen early in the course of the disease.

Multiple myeloma results from the monoclonal proliferation of a plasma cell. The malignant population retains its ability to secrete immunoglobulins. As this production continues unchecked, it results in hyperglobulinemia that produces a single ("M") spike on protein electrophoresis. Most myelomas secrete IgG or IgA. IgE and IgM myelomas are extremely rare.

The clinical presentation includes bone pain and anemia. Symptoms of hypercalcemia, renal failure, and hyperviscosity may be present. On the peripheral smear a normochromic, normocytic anemia is encountered. Rouleaux formation is common. Plasma cells can be found in circulation, and when predominant, a diagnosis of plasma cell leukemia is made.

More than 10% plasma cells are found in the bone marrow. The protein electrophoresis reveals the M spike with greater than 3 g of the immunoglobulin. Immunoelectrophoresis identifies and quantifies the increased immunoglobulin. The hyperglobulinemia is exclusively related to the abnormal antibody; all other immunoglobulins are decreased. Consequently, susceptibility to infection is increased. In the urine, Bence Jones proteins are found. These are filtered light chains that precipitate at 50 to 60°C and redissolve when heated to 100°C. Myeloma cells secrete osteoclast activating factor, which produces hypercalcemia and lytic bone lesions, especially in flat bones. As there is little or no osteoblastic activity, bone scans and alkaline phosphatase often remain negative.

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