PART I

NON-NEOPLASTIC HEMATOLOGY

CHAPTER ONE

NON-NEOPLASTIC DISORDERS OF WHITE BLOOD CELLS

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OVERVIEW OF WBC PRODUCTION AND FUNCTION

Frequently the first test that suggests an imbalance or disturbance in hematopoiesis is the complete blood count (CBC). The CBC is a simple blood test that is ordered frequently. It may pick up incidental abnormalities or may yield a diagnosis of suspected abnormalities. The CBC is a count of multiple blood components and qualities, and can include a differential of the white blood cells (WBCs). The CBC can suggest infection, inflammatory processes, and malignant processes. Typically a peripheral blood smear and rarely a buffy coat (concentrated white blood cells) are analyzed to help determine a diagnosis (Efrati, 1960). A differential WBC assigns leukocytes to their specific categories as a percentage or absolute count. Manual differential counts tend to be accurate but imprecise, whereas automated counts are fairly precise but sometimes inaccurate (Bain, 2002). It may occasionally be necessary to evaluate both the bone marrow and blood smear to evaluate the quality and quantity of the blood lineages. WBC disorders are classified into quantitative and qualitative conditions, reflecting changes in their number and function, respectively (Stiene-Martin, 1998). This chapter discusses both nonneoplastic quantitative and qualitative disorders of WBCs.

Leukocytes

Hematopoeisis

Hematopoiesis occurs in different parts of the body, depending on the age of the embryo, child, or adult. Initially blood cell formation of the embryo occurs within the yolk sac, in blood cell aggregates called blood islands. As development progresses, the hematopoiesis location changes, and the spleen and liver become the primary sites. As bone marrow develops, it usurps the task of blood cell formation and becomes the site for trilineage hematopoiesis. The bone marrow contains pluripotent stem cells, which can develop into any of the blood cells, including granulocytes, monocytes, and lymphocytes, in response to specific stimulating factors (Andrews, 1994). Several white blood cells (leukocytes) are depicted in Figure 1.1. Maturation, activation, and some proliferation of lymphoid cells occur in secondary lymphoid organs, including the spleen, liver, and lymph nodes. Extramedullary hematopoiesis can take place in the liver, thymus, and spleen and may lead to organomegaly. There is a common myeloid progenitor cell called the CFU-GEMM (Colony-Forming Unit-Granulocyte–Erythroid-Macrophage-Megakaryocyte) that leads to the development of granulocytic, monocytic, eosinophilic, basophilic, erythrocytic, and megakaryocytic precursors.

Non-Neoplastic Hematopathology and Infections, First Edition. Edited by Hernani D. Cualing, Parul Bhargava, and Ramon L. Sandin.

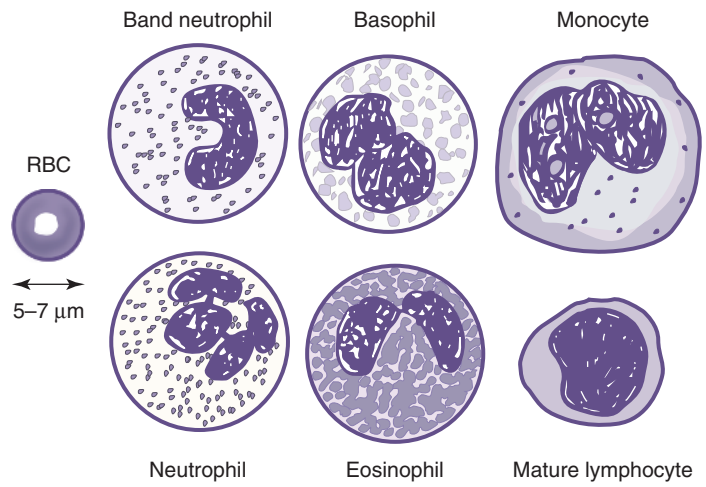

FIGURE 1.1 Schematic drawings of different white blood cells shown in relation to an erythrocyte. The band neutrophil (10–18 μm) is characterized by a deeply indented nucleus and secondary granules. The segmented neutrophil (10–15 μm) has 2 to 5 nuclear lobes (4 depicted) connected by thin filaments and contains several cytoplasmic secondary (specific) granules. The basophil (10–15 μm) contains a segmented nucleus and many coarse, dense granules of varying size (that may obscure the nucleus). The eosinophil (10–15 μm) has a bilobed nucleus and cytoplasm filled with coarse, uniform granules. The monocyte (12–20 μm) has an indented nucleus and abundant gray cytoplasm with sparse granules. The presence of nucleoli indicates that this is an immature monocyte (promonocyte). The mature lymphocyte (7–15 μm) has a high nuclear:cytoplasmic ratio (4:1), slightly notched nucleus with dense clumpy chromatin and no nucleolus, and only moderate agranular cytoplasm.

Leukocytosis

Leukocytosis is defined as a total WBC count that is greater than two standard deviations above the mean, or a value >11,000/μL in adults. While leukocytosis is mainly due to a neutrophilia, it may reflect an increase in any of the other leukocytes. Patients with hyperleukocytosis, which is defined as a white cell count (WCC) >100,000/μL, may manifest with hyperviscosity (or so-called symptomatic hyperleukocytosis). The development of symptoms of hyperviscosity syndrome are often correlated with the underlying cause (e.g., hyperproteinemia, erythocytosis, hyperleukocytosis, and thrombocytosis) and is a medical emergency mainly seen with leukemia in blast crisis. The severity of hyperleukocytosis is related to the underlying disorder; hyperviscosity is typically evident in AML with a WCC of >100,100/μL, in ALL with a WCC of >250,000/μL, and in CLL with a WCC of 500, 000/μL (Adams, 2009; Rampling, 2003). Spurious leukocytosis can occur because of platelet clumping, increased nucleated erythrocytes, or in cryoglobulinemia (Savage, 1984; Patel, 1987).

Leukemoid Reaction

Leukemoid reaction is used to describe leukocytosis above 50, 000/μL. This is usually characterized by a significant increase in early neutrophil precursors including band forms (Figure 1.2). Infants with Down syndrome may have transient leukemoid reactions (Brodeur, 1980).

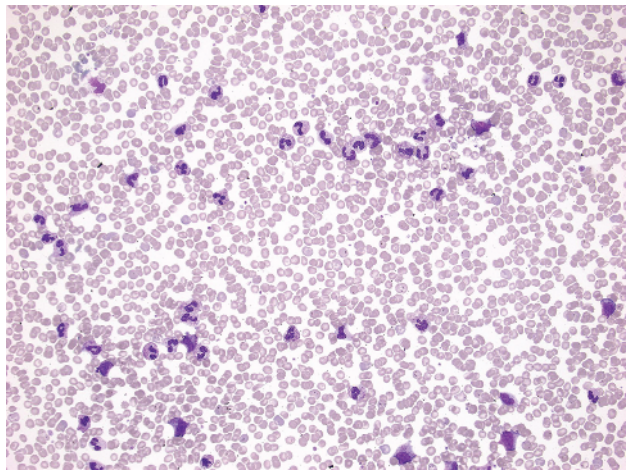

FIGURE 1.2 Leukemoid reaction observed in a peripheral blood smear. Notice the significant increase in early neutrophil precursors and band forms in addition to segmented neutrophils.

Granulocytes

Granulocytes have a single progenitor cell, the myeloblast, that can differentiate into neutrophils, eosinophils, and basophils (Lawrence, 1998). The differentiation process is based on the presence of certain stimulating factors. Neutrophils are the first

responders to infection or inflammation, and they respond to cytokines such as interleukin-8 (IL-8), interferon gamma (IFN-gamma), and C5a (Witko-Sarsat, 2000). These chemicals direct the neutrophils migration to areas of need.

Maturation Process

Neutrophils undergo a maturation process as they shift from myeloblasts, to promyelocytes, to myelocytes, to bands; eventually they finish the maturation process as neutrophils which is depicted in Figure 1.3. Only bands and mature neutrophils are normally present in the peripheral blood smear. Other immature cells are very rarely detected in small numbers in the blood of healthy individuals (Oertel, 1998). Band neutrophils constitute about 10 to 15% of the nucleated cells in the bone marrow and around 5 to 10% of the nucleated cells in the peripheral blood (Glassy, 1998). The nucleus of a band is indented to greater than 50% of the diameter of the nucleus (i.e., horseshoe shaped). Eosinophils have the same maturation process; however, the cells are distinctively eosinophilic, with coarse eosinophilic cytoplasmic granules. In this case the myeloblast becomes an eosinophilic myelocyte that matures into an eosinophilic metamyelocyte, then an eosinophilic band, and ultimately an eosinophil. Basophils have a shorter transition from a myeloblast to a basophilic myelocyte and eventually a basophil.

Left Shift

An increase (>20%) in the number of band cells (so-called bandemia) in relation to normal neutrophils is known as a left shift (Figure 1.4) (Nguyen, 2000). With a left shift the band count is usually >700/μL. When a left shift occurs, more immature granulocytes (blasts, promyelocytes, metamyelocytes, myelocytes) are typically released in the peripheral blood. Unless a patient is receiving G-CSF therapy, circulating blasts are not normally seen in reactive conditions. In reactive neutrophilia the left shift contains mainly bands. A left shift may be physiological (e.g., pregnancy) or in response to infection, inflammation, shock, hypoxia, or other marrow stimulation. Newborn infants may normally show leukocytosis and a leftward shift (Christensen, 1979). Although the diagnostic value of a left shift as an indicator for infection is limited (Seebach, 1997; Gombos, 1998), when the WBC is low, bands may be the only clue of an infection. Occasionally band cell counts are requested to detect infection (e.g., in the neonate) (Bain, 2002). The presence of a left shift and circulating nucleated red blood cells is referred to as a leukoerthyroblastic reaction (or leukoerythroblastosis). There is often also associated erythrocyte anisopoikilocytosis (e.g., teardrop cells or dacrocytes) with anemia and megakaryocyte fragments in a leukoerythrobalstic pattern, indicative of a space-occupying lesion within the marrow (i.e., myelophthisic process).

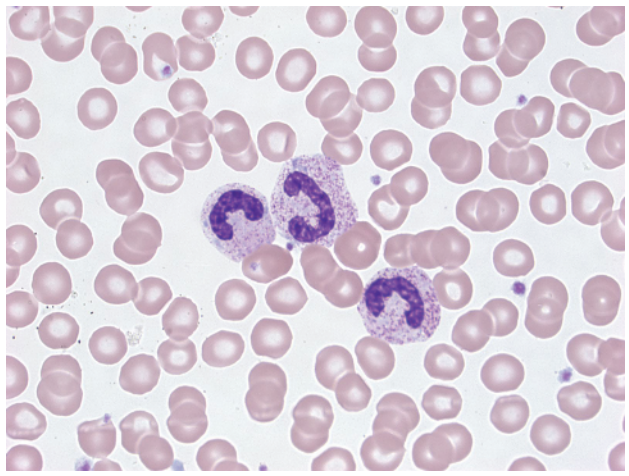

FIGURE 1.4 Blood smear from a patient with infection showing a bandemia and a left shift. These band neutrophils all have nuclei that are indented to greater than half the distance from the farthest nuclear margin. Their cytoplasm also contains many toxic granules.

Monocytes

Monocytes stem from monoblasts and undergo a maturation process as they progress from monoblasts to promonocytes. Monocytes reside in the peripheral blood. They may differentiate further and become macrophages (sometimes referred to as histiocytes) in the tissue.

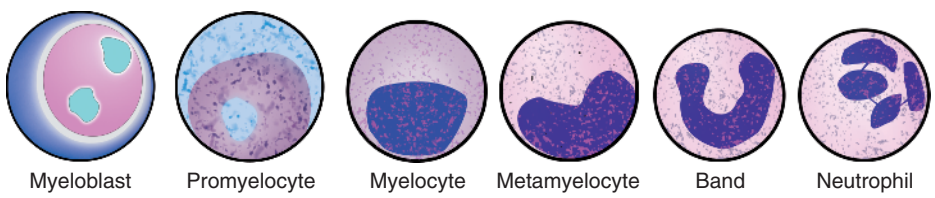

FIGURE 1.3 Neutrophil maturation process. Neutrophils undergo a maturation process as they shift from myeloblasts, to promyelocytes, to myelocytes, to bands, and eventually to neutrophils.

Lymphocytes

Lymphocytes differentiate from a precursor cell known as a common lymphoid progenitor cell. They can become a precursor T cell/natural killer (NK) cell or a precursor B cell. These cells then become committed to their designation as they develop into Pro-T cells, Pro-NK cells, and Pro-B cells. Further maturation is required as the cells proceed in their development and exhibit morphologically recognizable precursors as Pre-T cells and Pre-B cells. The cells undergo their maturation in distinct locations: B lymphocytes in the bone marrow and T lymphocytes in the thymus. Following this detailed maturation process (Figures 1.5 and 1.6), lymphocytes enter the blood circulation and reside in secondary lymphoid organs, including the spleen and lymph nodes.

QUANTITATIVE DISORDERS OF WBCS

Disorders of Neutrophils

Normal Neutrophil Physiology

In normal adults the bone marrow is the usual site of differentiation, proliferation, and terminal maturation of hematopoietic stem cells into neutrophil progenitors. Maturation of myeloblasts into segmented neutrophils usually occurs in five phases: blast, promyelocyte, myelocyte, metamyelocyte, and mature neutrophil. Division occurs only during the first three stages (i.e., neutrophil blast, promyelocyte, and myelocyte). After the myelocyte stage, the cells are no longer capable of mitosis and enter a large marrow storage pool. After 5 days the cells are released into the blood, where they circulate for a few hours before entering tissues (Nathan, 2006). The physiology of neutrophil function is covered in greater detail in the qualitative disorders of neutrophils section. A neutrophil is also referred to as a polymorphonuclear neutrophil (PMN). The qualitative and quantitative changes of neutrophils noted in response to infection include neutrophilia, left shift, toxic granulation, Döhle bodies, and vacuolization (see below).

Normal Neutrophil Morphology

The nucleus of the circulating neutrophil is segmented, usually with two to four interconnected lobes. The purpose of nuclear segmentation is unknown. In rare situations (mainly with hematological malignancy, but also following G-CSF therapy) neutrophils may have unusual nuclear shapes such as ring/donut or botryoid nuclei or

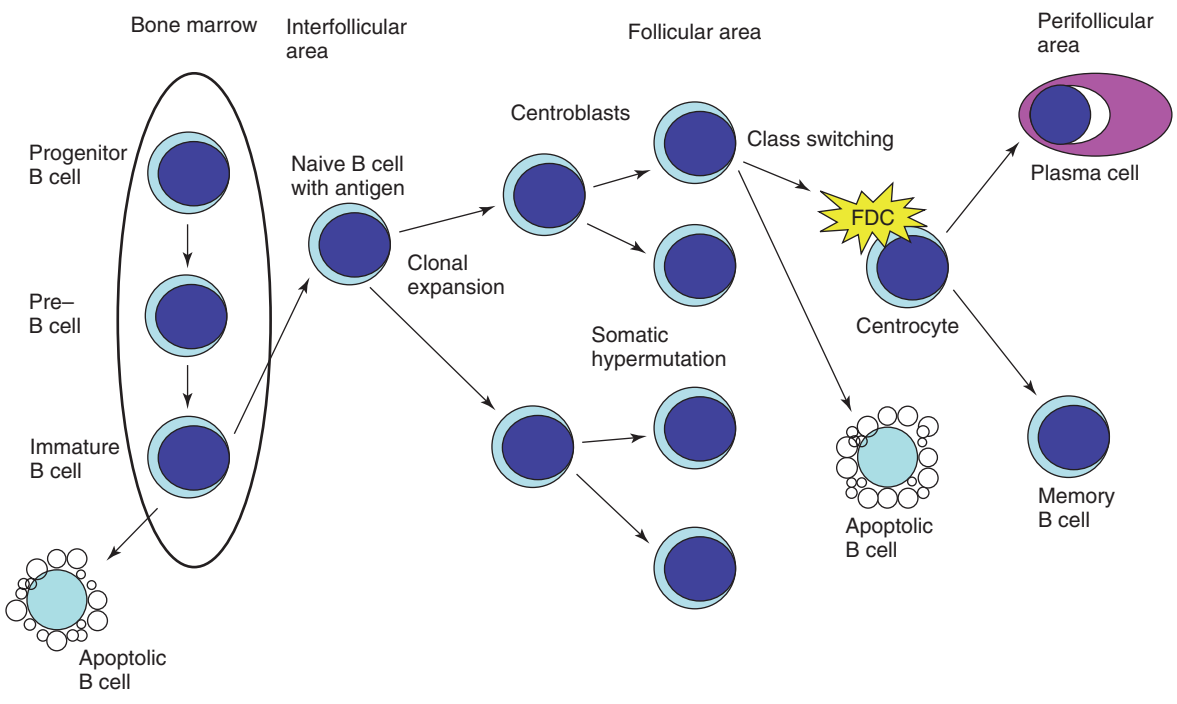

FIGURE 1.5 **Differentiation of B cells**. Precursor B cells may develop into naïve B cells, or may undergo apoptosis. Antigen stimulation of a naïve B cell starts a cascade of events including clonal expansion, somatic hypermutation and class switching of centroblasts. Centroblasts then develop into centrocytes within the follicle center and with the follicle dentritic cell (FDC) or undergo apoptosis. The perifollicular area contains either plasma cells or memory B cells.

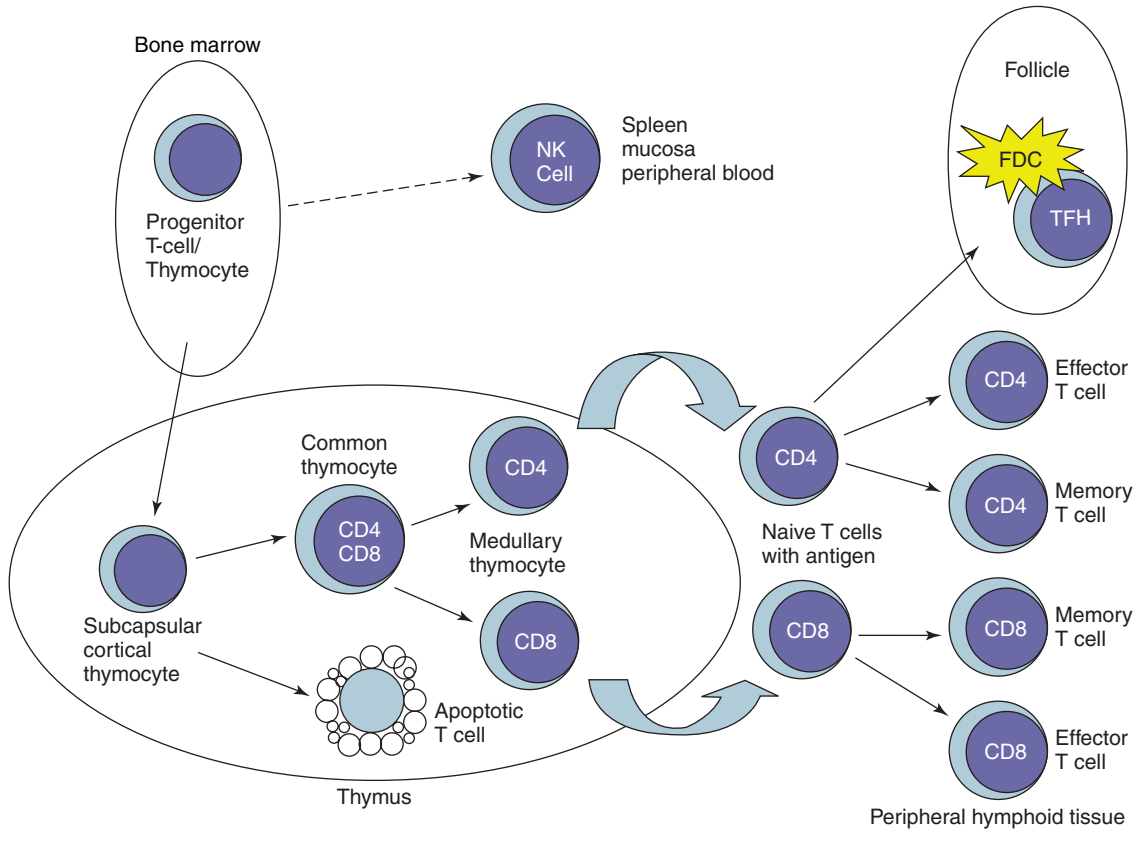

FIGURE 1.6 **Differentiation of T cells**. Progenitor T cells may develop into NK cells in the periphery, or they may enter the thymus where they develop into supcapsular cortical thymocytes and continue on to common thymocytes or apoptotic cells. Common thymocytes are CD4+ and CD8+, which then develop into medullary thymocytes and naïve T cells, either as CD4+ or CD8+. The naïve T cells are stimulated by antigens and transform into effector and memory T cells. T helper cells (TFH) develop from CD4+ naïve T cells within the follicle with the follicle dendritic cell (FDC).

show detached nuclear fragments (Hernandez, 1980; Bain, 2002). Some mature neutrophils in women have a drumstick- or club-shaped nuclear appendage attached to the nuclear lobe by a single fine chromatin strand containing the inactivated X chromosome (Barr body). Females typically have six or more drumsticks per 500 PMNs (Davidson, 1954). In males with Klinefelter syndrome (XXY), drumsticks occur but are fewer in number (Bain, 2002).

The myeloblast is an immature cell with a large oval nucleus, sizable nucleoli, and few or no granules. The promyelocyte stage contains primary (azurophilic or nonspecific) large peroxidase-positive granules that stain metachromatically (reddish-purple) with a polychromatic stain such as the Wright stain. During the myelocyte stage of maturation, secondary (specific) granules are formed that are peroxidase negative. After the myelocyte stage, the primary granules lose their intense staining properties and are no longer evident by light microscopy (DeSantis, 1997). Mature segmented neutrophils contain primary (peroxidase-positive) granules and specific (peroxidase-negative) granules in a 1:2 ratio. The granules cannot be distinguished individually but are responsible for the pink background color of the neutrophil cytoplasm seen during and after the myelocyte stage. Primary granules contain lysozyme, myeloperoxidase, acid phosphatase, elastase, defensins, and cathepsin G. Secondary granules contain lysozyme, collagenase, lactoferin, B_{12}-binding protein, NADPH oxidase, and cytochrome b. A third type (tertiary) granule identified by electron microscopy has been documented.

When evaluating the granularity of neutrophils, it is important to be aware of possible artifacts such as may arise from prolonged storage and suboptimal staining. Toxic granulation (increased granulation) refers to activated neutrophils that contain large purple or dark blue primary granules (Figure 1.7 and Figure 1.8). With toxic granulation the cytoplasmic

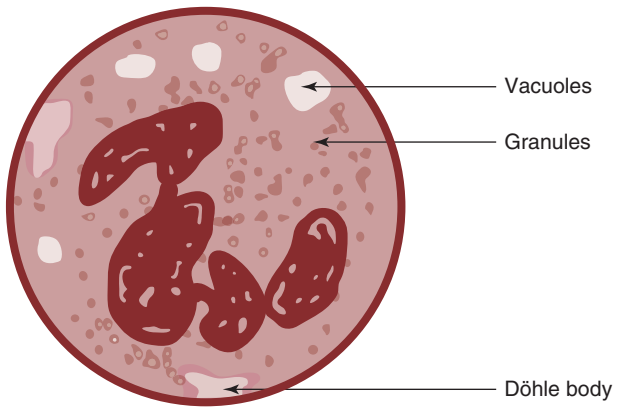

FIGURE 1.7 Schematic diagram of a toxic segmented neutrophil. The key features are prominent cytoplasmic granules, clear vacuoles, and Döhle bodies located peripherally adjacent to the cell membrane.

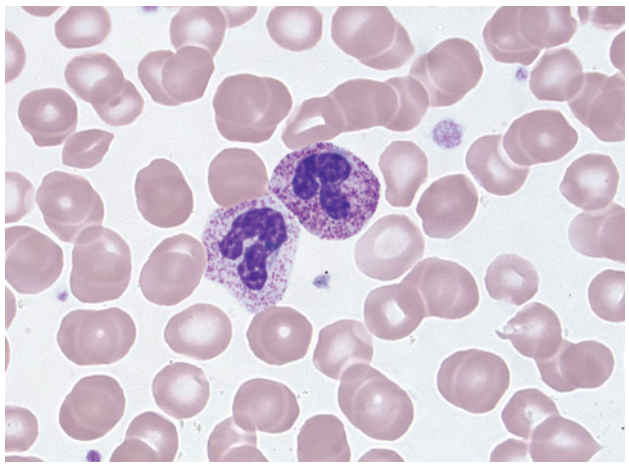

FIGURE 1.8 Neutrophils with toxic granulation seen in a patient with known infection.

granules enlarge and stain darker than normal granules (Schofield, 1983). Neutrophils with toxic granules may resemble eosinophils (which have larger granules), basophils, monocytes, or the inclusions of Alder–Reilly anomaly (see later). Activated neutrophils may in addition contain multiple round, empty cytoplasmic vacuoles and Döhle bodies. Blood stored for prolonged periods can artifactually cause vacuoles. Cytoplasmic vacuolation can also be caused by autophagocytosis (e.g., following chloroquine or sulfonamide therapy). Döhle bodies are blue-gray inclusions seen in the cytoplasm that represent areas of rough endoplasmic reticulum. They may be single or multiple and of varying size. Döhle-like bodies can also be found in patients with May–Hegglin anomaly, burns, myelodysplasia, and in pregnancy (see later). In May–Hegglin anomaly these bodies correspond to amorphous cytoplasmic areas devoid of organelles. Increased granulation is also a characteristic of G-CSF therapy (Schmitz, 1994). Compared to toxic granulation, however, hypergranulation induced by G-CSF therapy has a higher density of granules, which stain more red and often obscure the nucleus (Nguyen, 2000). Other changes that may be encountered in patients receiving growth factor therapy include a neutrophilia with a prominent left shift, Döhle bodies, nuclear segmentation abnormalities (hyposegmentation, hypersegmentation, ring nuclei), leukoerythroblastosis, and rarely a monocytosis, transient lymphocytosis, and eosinophilia. Alder–Reilly anomaly (see later), when present in granulocytes, can also mimic toxic granulation. Alder–Reilly bodies, however, tend to be larger than normal granules. Finally, nuclear projections and cytoplasmic pseudopodia may be observed as rare alterations in toxic neutrophils.

Table 1.1 lists several of the alterations and abnormalities that may be seen in neutrophils. Apoptotic neutrophils may be seen in association with infection, diabetes (Figure 1.9), glucocorticoid administration, and neoplastic diseases (Sudo, 2007; Shidham 2000), but may also occur if blood is left at room temperature for a long time. These are important to recognize as they may mimic nucleated RBCs on low-power examination. Neutrophils may also contain a variety of phagocytosed material such as bacteria, fungi, cryoglobulin, and malarial pigment. (Figure 1.10).

Normal Neutrophil Cytochemistry

The most reliable method for identifying azurophilic granules on blood films is staining the cells for peroxidase with a myeloperoxidase stain. Production of this enzyme by leukemic cells has been the hallmark for distinguishing lymphoblastic from myeloid leukemia. Chloroacetate esterases appear early in maturation and can be used to detect the origin of immature cells.

Reference Range

The normal range for neutrophils is $2.5–7.5 \times 10^9$/L. However, the normal range can vary. People of African and Middle Eastern descent may have lower counts, which are still normal. At birth, the mean neutrophil count rises rapidly to a peak at 12 hours of age, but then drops by 72 hours of age. Thereafter the neutrophil count slowly decreases so that the lymphocyte becomes the predominant cell at two to three weeks of age (DeSantis, 1997). Several perinatal factors may significantly alter neutrophil dynamics including bacterial disease, maternal hypertension, maternal fever

TABLE 1.1 Alterations of Neutrophil Morphology

Abnormality	Condition
Neutrophil nuclei	
Left shift	Pregnancy, infection, shock, hypoxia
Hypersegmentation	Megaloblastic conditions, iron deficiency, uremia, infection, hereditary neutrophil hypersegementation, myelokathexis
Hyposegmentaion	Pelger–Huet anomaly, lactoferin deficiency, MDS, AML
Botryoid (grape-like) nucleus	Heatstroke, hyperthermia, burns
Neutrophil cytoplasm	
Hypogranulation	Lactoferin deficiency, MDS
Hypergranulation	Toxic granulation, pregnancy, infection, inflammation, G-CSF therapy, aplastic anemia, hypereosinophilic syndrome, Alder–Reilly anomaly, chronic neutrophilic leukemia, MDS
Abnormal granules	Chediak-Higashi syndrome, Alder–Reilly anomaly, MDS, AML
Vacuolation	Infection, G-CSF therapy, acute alcohol poisoning, Jordan's anomaly, carinitine deficiency, kwashiorkor, myelokathexis
Döhle bodies/inclusions	Infection, inflammation, burns, pregnancy, G-CSF therapy, May–Hegglin anomaly, Fechtner syndrome, kwashiorkor, MDS, AML

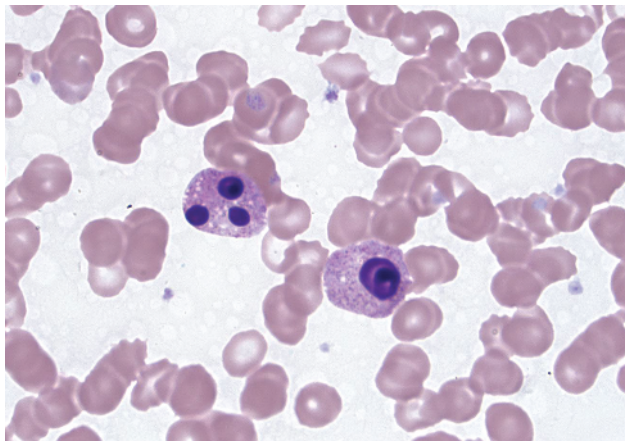

FIGURE 1.9 Neutrophil apoptosis in the peripheral blood of a patient with diabetes mellitus. Apoptotic cells have round, dense pyknotic nuclei.

prior to delivery, hemolytic disease, and periventricular hemorrhage (Manroe, 1979). A diurnal variation of neutrophil counts has been observed in adults, but not infants. Both neutrophilia and neutropenia are defined using the absolute neutrophil count (ANC). The ANC is equal to the product of the WBC count and the percentage of polymorphonuclear cells (PMNs) and band forms noted on the differential analysis:

$$\mathbf{ANC} = \mathbf{WBC\ (cells/microL)} \times \%(\mathbf{PMNs} + \mathbf{bands}) \div \mathbf{100}$$

The ANC is reported to be as sensitive but more specific than the WBC count as an indicator of occult bacteremia (Gombos, 1998).

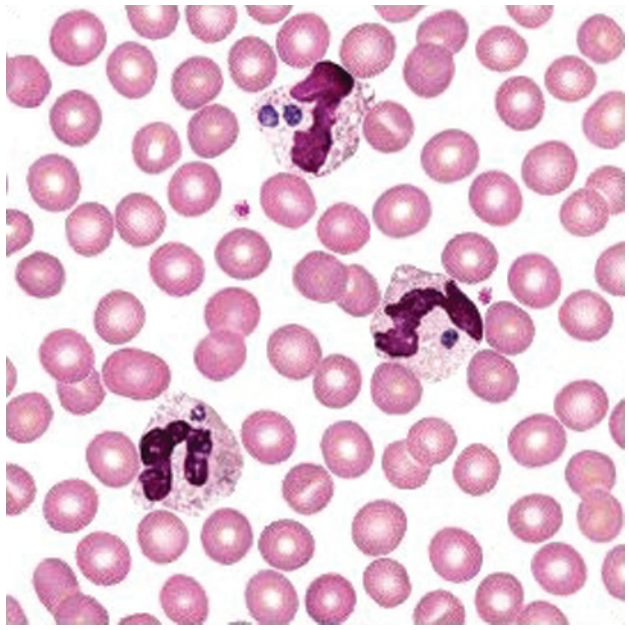

FIGURE 1.10 Peripheral blood smear from a patient with Ehrlichiosis (human granulocytic anaplasmosis due to infection with the HGA agent *E. phagocytophila*). The neutrophils contain characteristic intraleukocytic morulae. These may resemble Döhle bodies. Such intracytoplasmic inclusions may be seen in the cytoplasm of neutrophils in many (20–80%) patients with human granulocytic ehrlichiosis and in mononuclear cells in a minority (1–20%) of patients with human monocytic ehrlichiosis.

Neutrophilia

Definition. Neutrophilia is the presence of more than $20.0 \times 10^3/mm^3$ neutrophils in the circulating blood. In infants with neutrophilia the ANC is

$>10.0 \times 10^3/mm^3$, in children it is $>8.0 \times 10^3/mm^3$, and in adults it is $>7.0 \times 10^3/mm^3$. The term *granulocytosis* has sometimes been used interchangeably with neutrophilia. In strict terms, granulocytes include neutrophils, eosinophils, and basophils. Total granulocyte count (TGC) is the product of the WBC count and the percentage of PMNs, bands, metamyelocytes, myelocytes, and promyelocytes.

ICD-10 Code D72.8

Pathophysiology. Neutrophilia can be due to a reactive or neoplastic process (Table 1.2). Significant causes of neutrophilia in the neonate can be due to maternal factors (smoking, fever, prolonged oxytocin, and dexamethasone administration) and/or fetal factors (stressful delivery, hypoxia, crying, physiotherapy, pain, hypoglycemia, seizures, infection, hemolysis, intraventricular hemorrhage, meconium aspiration, and hyaline membrane disease).

Clinical Approach. Reactive causes of neutrophilia are usually part of an inflammatory or infectious course, or can be drug induced. Pharmaceuticals that are commonly associated with neutrophilia are glucocorticoids, growth factors, and psychiatric medications. The reactive causes can have an associated left shift, meaning that the granulocytes in question have more immature forms circulating in the peripheral blood. However, the presence of immature granulocytes can also suggest a neoplastic process. Therefore a substantial history is required to help differentiate the possible source of neutrophilia. Morphologic features that characterize a reactive neutrophilia with or without a left shift are toxic granulation, cytoplasmic vacuolation, and Dohle bodies. The absence of these changes and associated basophilia raises the possibility of neoplasia, particularly myeloproliferative neoplasms. A leukocyte alkaline phosphatase (LAP) score can be used that is high in infection (as well as polycythemia vera) but low in CML and PNH. The LAP score, however, may be normal in polycythemia vera and (juvenile) CML.

Differential Diagnosis. Chronic neutrophilic leukemia, essential thrombocythemia and polycythemia vera are usually associated with an absolute neutrophilia without a left shift. Primary myelofibrosis and chronic myeloid leukemia are usually associated with an absolute neutrophilia and left shift. Performing molecular genetic studies on blood for the bcr/abl and JAK2V617F mutation can help differentiate between myeloid neoplasms and a reactive cause of mature neutrophilia. The differentiation from an acute myeloid leukemia depends on both the cell proliferation and maturity of the cell population. If blasts constitute greater than 20% of the differential or abnormal promyelocytes are identified, further workup for acute leukemia should be pursued.

Neutropenia

Definition. Neutropenia is defined as an ANC below $2.5 \times 10^3/mm^3$ in infants an ANC below $1.5 \times 10^3/mm^3$ in adults. However, it is important to be aware that neutrophil counts can be naturally lower

TABLE 1.2 Major Causes of Neutrophilia

Acute Neutrophilia	
Physical or emotional stress	Cold, heat, convulsions, pain, labor, panic, depression, infarction, exercise, postoperative period, following seizures, frequent blood transfusion, acute hemorrhage
Infections	Localized and system bacterial, rickettsial and spirochetal infections
Inflammation or tissue necrosis	Burns, electric shock, trauma, vasculitis, antigen-antibody reaction, complement activation
Drugs, hormones, and toxins	Smoking, glucocorticoids, epinephrine, venoms, colony-stimulating factors
Chronic neutrophilia	
Infections	Persistence of infections that cause acute neutrophilia
Inflammation	Acute inflammation involving any organ or systemic such as colitis, nephritis, gout, Sweet's syndrome
Tumors	Carcinoma, lymphoma, brain tumors, melanoma, multiple myeloma, paraneoplastic reaction
Drugs	Continued exposure to drugs that cause acute neutrophilia, lithium, rarely drug reaction
Metabolic and endocrine disorders	Eclampsia, thyroid storm, Cushing's disease, gout, diabetic ketoacidosis
Benign hematologic disorders	Sickle cell disease, hemorrhage, recovery from agranulocytosis, asplenia
Hematologic neoplasms	Myeloproliferative neoplasms
Hereditary and congenital	Down syndrome

TABLE 1.3 Major Causes of Neutropenia

Acquired	
Infection	Any overwhelming infection
Autoimmune disease	Felty syndrome, systemic lupus erythematosus
Complement activation	Hemodialysis, filtration leukapheresis acute respiratory distress syndrome
Drug-induced neutropenia	Clozapine, thionamides, sulfasalazine, Chemotherapeutic agents
Toxins	Alcohol, benzene
Non-neoplastic hematologic disorders	Aplastic anemia, marrow replacement, megaloblastic anemia
Hematologic neoplasms	Myelodysplastic syndrome, primary idiopathic myelofibrosis, acute leukemia, T-large granular lymphocytic leukemia, lymphomas with bone marrow involvement
Congenital	
Constitutional	Shwachman–Diamond–Oski syndrome, cyclic neutropenia, Chediak–Higashi syndrome, Kostman syndrome, Fanconi anemia, dyskeratosis

in some ethnic groups such as Africans, African Americans, and Yemenite Jews (Tefferi, 2005). An ANC below $0.5 \times 10^3/mm^3$ is considered to represent severe neutropenia.

ICD-10 Code D70

Pathophysiology. Neutropenia can be caused by decreased production, increased destruction, hereditary disorders, medications, or infections (Table 1.3). The susceptibility to infection in neutropenic patients is related to the ANC. Neutropenia can be classified as follows:

- **Acquired neutropenia:** Postinfection, drug-induced (e.g., penicillin, chloramphenicol, ibuprofen, phenytoin, propylthiouracil, procainamide, chlorpropamide, phenothiazine), autoimmune (e.g., Felty syndrome, lupus erythematosus), isoimmune (e.g., alloimmune neonatal neutropenia), chronic splenomegaly, benign familial neutropenia, benign neutropenia of childhood, chronic idiopathic neutropenia, and nutritional deficiency.
- **Intrinsic defects:** Cyclic neutropenia, Kostmann syndrome (severe infantile agranulocytosis), myelokathexis (neutropenia with tetraploid or cloverleaf nuclei), Schwachman–Diamond–Oski syndrome, Chédiak–Higashi syndrome, reticular dysgenesis, and dyskeratosis congenita.

Neutropenia is often seen accompanying qualitative neutrophil disorders. Neutropenia is also common in several primary immunodeficiency diseases such as CD40L deficiency, WHIM syndrome (warts, hypogammaglobulinemia, immunodeficiency and myelokathexis), X-linked hyper-IgM, X-linked agammaglobulinemia and Chediak-Higashi syndrome (Rezaei, 2009). Congenital neutropenia includes nonsyndromic variants (caused by mutations in ELA2, HAX1, GFI1, or WAS) and syndromic variants (due to mutations in genes controlling glucose metabolism, e.g., SLC37A4 and G6PC3, or lysosomal function, e.g., LYST, RAB27A, ROBLD3/p14, AP3B1, VPS13B) (Klein, 2009). Defects in genes encoding ribosomal proteins (SBDS, RMRP) and mitochondrial proteins (AK2, TAZ) are also associated with some congenital neutropenia syndromes.

Clinical Approach. In some cases there may be telltale signs that will help you make a diagnosis. For example, vitamin B_{12} or folate deficiency results in atypical neutrophils that are hypersegmented, whereas aplastic anemia displays a decrease in bone marrow hematopoiesis. However, the underlying diagnosis resulting in neutropenia will typically require a complete history with additional lab testing. Cyclic neutropenia has a very characteristic history of recurrent episodes of fever and neutropenia in a young child. Neutrophils can also have dysfunctional problems, as are discussed later. A bone marrow evaluation can help determine the cellularity of the bone marrow, presence of malignant cells, chromosomal abnormalities suggesting malignant clones, and myeloid nuclear abnormalities. Also, because certain acquired neutropenias may be associated with the presence of antineutrophil antibodies (directed against neutrophil-specific antigens, e.g., NA1, NA2, NB1, ND1, and NE1 and non–neutrophil-specific HLA antigens), their detection (e.g., by immunofluorescence or agglutination assay) may be helpful. Overall, neutropenia is a

worrisome occurrence because patients become susceptible to infections when they do not have adequate numbers of neutrophils to respond to an inflammatory and/or infectious assault.

Disorders of Lymphocytes

Normal Lymphocyte Physiology

Lymphocytes differentiate from lymphoblasts into two major types of lymphocytes:

- **T lymphocytes (T cells)**: These cells identify foreign substances in your body and begin an immune response;
- **B lymphocytes (B cells)**: These cells produce antibodies to foreign substances.

Lymphocytes differentiate further after exposure to an antigen. Upon exposure, lymphocytes become effector or memory lymphocytes (Cianci, 2010). The effector B cells release antibodies and effector T cells release cytotoxic granules or send a signal for helper cells (Malaspina, 2007). The memory cells remain in the peripheral blood and retain the ability to respond to the same antigen in the future.

Normal Lymphocyte Morphology

One cannot reliably distinguish functional and immunological lymphocyte subsets by morphology alone. While normal circulating lymphocytes vary in size and shape, they can arbitrarily be divided into small (''mature'') and large (''granular'') lymphocytes (Nguyen, 2000). Mimics of reactive lymphocytes include lymphoma cells, lymphoblasts, monocytes, plasma cells, and nucleated red blood cells.

Mature (Small) Lymphocytes. Mature lymphocytes are approximately the same size as red blood cells (i.e., 7 microns in diameter). They have a prominent nucleus with regular nuclear contours and dense chromatin, and only have a small rim of cytoplasm. Nuclear clefts in small lymphocytes may normally be seen in children. Benign binucleated lymphocytes have been documented in some individuals (Troussard, 1997) and also in association with radiation (Bain, 2002). Rare childhood storage disorders can manifest with prominent cytoplasmic vacuoles in lymphocytes. Inclusions within lymphocytes can be seen in Chédiak–Higashi syndrome, Alder–Reilly anomaly, and Tay–Sachs disease (Bain, 2002). Lymphocyte vacuolation can occur in I-cell disease, the mucopolysaccharidoses, Jordan's anomaly, Niemann–Pick disease, Wolman's disease, Pompe's disease, Tay–Sachs disease, Batten–Speilmeyer–Vogt disease, type II sialidosis, and galactosemia (Bain, 2002).

Reactive Lymphocytes. The term *reactive* is used to describe transformed benign lymphocytes, and should not be used interchangeably with *atypical* which should be used to describe malignant-appearing cells (Marty, 1997). Several reactive lymphocyte forms have been described (e.g., Downey classification) (Glassy, 1998). Reactive lymphocytes tend to be larger (9–30 μm) than mature (8–12 μm) lymphocytes. They may also have more abundant basophilic, slightly foamy, or vacuolated cytoplasm. Reactive cells often have an indented surface, which appears scalloped at the edges (Figure 1.11). The nucleus ranges in shape from round to reniform in appearance. Unlike resting small lymphocytes, reactive lymphocytes may have nucleoli. Their nuclear chromatin is typically finer than normal small lymphocytes. Plasmacytoid reactive lymphocytes (sometimes called ''plymphs'' or Turk cells) resemble plasma cells (Figure 1.12). Plymphs often have a large eccentric nucleus with prominent chromatin clumping, and occasionally a perinuclear hoff may be seen. Reactive lymphocytes that have a plasma cell-like appearance are usually seen as part of a heterogeneous mix of reactive lymphoid cells. They likely represent intermediate B lymphocytes differentiating into plasma cells. Reactive lymphocytes enlarge due to antigen stimulation. More recent studies suggest that reactive lymphocytes are activated T lymphocytes produced in response to infected B lymphocytes (Thomas, 2008). They act as normal lymphocytes in sites of local

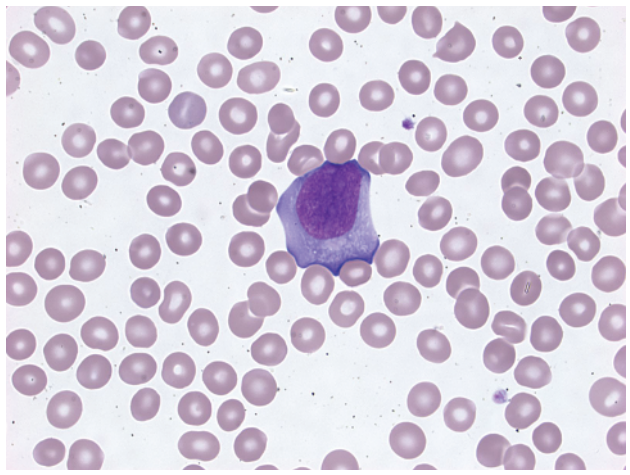

FIGURE 1.11 **Reactive lymphocyte in a patient with infectious mononucleosis (monospot test positive)**. The large reactive lymphocyte shown corresponds to a so-called Downey type II cell. These cells have an abundant pale gray-blue amoeboid cytoplasm that partially surrounds adjacent erythrocytes. The curled edges against the RBCs and radiating cytoplasm are slightly darker staining.

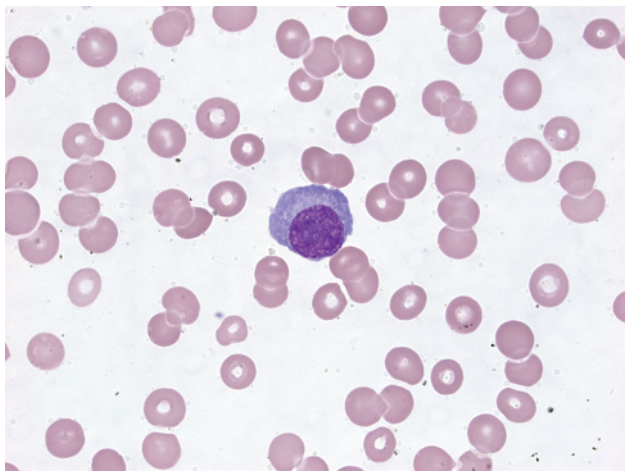

FIGURE 1.12 **Plasmacytoid appearing reactive lymphocyte with coarse chromatin and an eccentric nucleus**.

inflammation, playing a role in the primary cellular immune or helper T-cell response (Simon, 1997).

Large Granular Lymphocytes (LGLs). LGLs are large (T-cell phenotype) lymphocytes with azurophilic granules that contain proteins involved in cell lysis, such as perforin and granzyme B. LGLs normally comprise 10 to 20% of the total lymphocyte population (Nguyen, 2000). Monocytes by comparison contain smaller granules and have a ground-glass cytoplasm.

Natural Killer (NK) Cells. NK cells are a third type of lymphocyte; they are similar to cytotoxic T cells and LGLs, which cause cell lysis of tumor cells and virus-infected cells by releasing granzyme B. NK cells were named as such because they do not require antigen priming to destroy abnormal self-cells. Therefore the cytoxic affect occurs naturally (Morice, 2007). These cells have a large granular lymphocyte appearance; however, NK cells do not display T-cell receptors or pan-T markers (CD3).

Normal Lymphocyte Immunophenotype

In general, T lymphocytes are CD3 positive and individually display CD4 or CD8 staining. Few lymphocytes display both CD4 and CD8 positivity. T-cell LGLs exhibit CD8 and variable CD11b, CD56, and CD 57 positivity. Granzyme B can also be used for T-cell LGL identification (Table 1.4). B lymphocytes display CD20 and CD79a, and are also positive for CD19 on flow cytometry. Plasma cells do not express many surface antigens and are negative for the B cell markers CD19 and CD20 (Table 1.5). They are identified by CD38 and CD138 staining. Natural killer cells display CD16 (FcγRIII), CD56, CD57, CD2, and granzyme B.

Lymphocytosis

Definition. Lymphocytosis is defined as the presence of more than $4 \times 10^3/mm^3$ lymphocytes in the circulating blood in adults, more than $7 \times 10^3/mm^3$ in children, and more than $9 \times 10^3/mm^3$ in infants.

ICD-10 Code D72.8

TABLE 1.4 T-Cell Phenotype During Stages of Maturation

	CD7	CD1a	CD2/CD5	CD3	CD4/CD8	TdT
Prothymocyte	+	−	−	−	−	+
Subcapsular thymocyte	+	−	+	Cytoplasmic +	Double +	+
Cortical thymocyte	+	+	+	Surface +	CD4+ or CD8+	+
Medullary thymocyte	+	−	+	Surface +	CD4+ or CD8+	−
Peripheral T cell	+	−	+	Surface +	CD4+ or CD8+	−

TABLE 1.5 B-Cell Phenotype During Stages of Maturation

	Antigen	TdT	CD79a	PAX5	CD20
Pro-B	Independent	+	−	−	−
Pre-B	Independent	+	+	+	−
Immature B	Independent	+	+	+	+
Mature naïve B	Independent	−	+	+	+
Germinal center B	Dependent	−	+	+	+
Memory B	Dependent	−	+	+	+
Plasma cell	Dependent	−	+	−	−

TABLE 1.6 Major Causes of Lymphocytosis

Reactive causes of lymphocytosis	
Acute viral infections	Infectious mononucleosis (Epstein Barr virus), cytomegalovirus, HIV, hepatitis, adenovirus, chickenpox, herpes simplex and zoster, influenza, mumps, measles
Acute bacterial infections	Pertussis (whooping cough), brucellosis, tuberculosis, typhoid fever, paratyphoid fever
Protozoan infections	Toxoplasmosis, Chaga's disease
Chronic bacterial infections	Tuberculosis, brucellosis, syphilis
Autoimmune disease	Rheumatoid arthritis, idiopathic thrombocytopenic purpura, systemic lupus erythematosus, autoimmune hemolytic anemia
Drug and toxic reactions	Dilantin, dapsone, lead, organic arsenics
Endocrine causes	Stress, Addison's disease, glucocorticoid deficiency, thyrotoxicosis
Miscellaneous	During recovery from acute infections (especially in children), allergic reactions, malnutrition, rickets
Neoplastic causes of lymphocytosis	
Acute lymphoblastic leukemia	
Non-Hodgkin lymphoma	
T-cell large granular lymphocytic leukemia (T-LGL)	

Pathophysiology. Lymphocytosis can be divided into reactive and neoplastic etiologies. Table 1.6 lists some common causes of lymphocytosis. Reactive lymphocytosis is most commonly associated with acute viral illnesses such as infectious mononucleosis due to Epstein Barr virus (EBV) (Nkrumah, 1973; Marty, 1997; Peterson, 1993). Lymphocytosis can also occur with other infections like whooping cough (Kubic, 1990). Reactive lymphocytosis secondary to stress (e.g., myocardial infarction, sickle cell crisis, and trauma) may be more transient (Groom, 1990; Karandikar, 2002).

Clinical Approach

Lymphocytosis is particularly common in children with infection. In viral-induced lymphocytosis there is typically a mixed population of reactive cells, including small lymphocytes, plasmacytoid cells, and few larger lymphocytes. The minimal morphologic criteria for the diagnosis of infectious mononucleosis are (1) 50% or more mononuclear cells (lymphocytes and monocytes) in a blood smear, (2) at least 10 reactive lymphocytes per 100 leukocytes, and (3) marked lymphocyte heterogeneity (Peterson, 2006). Increased numbers of apoptotic lymphocytes are often seen with viral infections. In the elderly population, lymphocytosis is more concerning to be secondary to a neoplastic or lymphoproliferative disorder such as leukemia or lymphoma. Large granular lymphocytes will occur in increased number in large granular lymphocytic leukemia (T-LGL), which can mimic a reactive process and is thus frequently underdiagnosed. T-LGL is known to be associated with several autoimmune and hematologic conditions including myelodysplastic syndromes. Therefore it is important to keep T-LGL in the differential diagnosis of a reactive lymphocytosis. Lymphocytosis after splenectomy is usually mild, and will be accompanied by the presence of Howell–Jolly bodies (Juneja, 1995). Morphological criteria alone may be insufficient to distinguish reactive from malignant lymphocytes. In such cases immunophenotyping (e.g., flow cytometry) and molecular studies (e.g., PCR for IgH gene rearrangement) may be needed.

Lymphocytopenia

Definition. Lymphocytopenia is defined as the presence of less than $1.0 \times 10^3/mm^3$ lymphocytes in the circulating blood in adults. Children have higher normal levels of lymphocytes, and lymphocytopenia is defined as less than $2.0 \times 10^3/mm^3$.

ICD-10 Code D72.8

Pathophysiology. Lymphocytopenia can be secondary to congenital immunodeficiency disorders or a reactive process to underlying disease (Table 1.7).

Clinical Approach. Lymphocytopenia is more commonly a reactive etiology that is associated with infectious disease (e.g., AIDS), autoimmune disorders (e.g., rheumatoid arthritis, SLE, myasthenia gravis), nutritional deficiencies (e.g., zinc), systemic diseases (e.g., sarcoidosis, protein-losing enteropathy, renal insufficiency, Hodgkin lymphoma, carcinoma), congenital immunodeficiency disorders, iatrogenic causes (e.g., chemotherapeutic agents, radiation therapy), and idiopathic (idiopathic CD4+ T lymphocytopenia) (Laurence, 1993; Schoentag, 1993, Buckley,

TABLE 1.7 Major Causes of Lymphopenia

Reactive causes of lymphocytopenia	
Infectious diseases	HIV, influenza, hepatitis, tuberculosis, babesiosis, pneumonia, sepsis
Autoimmune disorders	Rheumatoid arthritis, myasthenia gravis, systemic lupus erythematosus
Nutritional disorders	Zinc deficiency, protein malnutrition
Systemic diseases	Renal insufficiency, sarcoidosis, carcinoma, Hodgkin lymphoma
Iatrogenic causes	Radiation therapy, burns
Medications	Chemotherapy, glucocorticoid therapy
Congenital immunodeficiency disordes	
Wiskott–Aldrich syndrome	
Severe combined immunodeficiency disease	
Congenital thymic aplasia (DiGeorge syndrome)	

2000; Datta, 2009). Congenital disorders have classic presentations and dysfunctions. These disorders include severe combine immunodeficiency disease (SCID), DiGeorge syndrome and Wiskott–Aldrich syndrome.

Severe combined immunodeficiency disease (SCID) is also known as Bubble Boy syndrome, alymphocytosis, and Glanzmann–Riniker syndrome. Most cases of SCID are secondary to mutations of the common gamma chain (γ_c). The common gamma chain is a protein of the interleukin receptors, including IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21. A nonfunctional common gamma chain leads to defects of interleukin signaling, development of and differentiation of T and B cells. Consequently this disease is characterized by a dysfunctional immune system with markedly decreased or absent T cells and NK cells and nonfunctional B cells. Patients are commonly affected by recurrent opportunistic infections, ear infections, chronic diarrhea, and oral candidiasis. SCID is severe, and newborns can die within a year if they are not diagnosed and treated early on in the disease process (Gaspar, 2001).

DiGeorge syndrome (congenital thymic aplasia) is characterized by lymphocytopenia and aplasia of the thymus (Figure 1.13) and parathyroid glands. This syndrome is likely secondary to a defect of the third and fourth pharyngeal pouches. These patients have a deletion at q11.2 on chromosome 22 (Hay, 2007; Kobrynski, 2007). They have multiple birth defects, which include congenital heart disease, palate neuromuscular problems (velo-pharyngeal insufficiency), learning disabilities, atypical facial features, and hypocalcemia secondary to parathyroid aplasia. DiGeorge syndrome results in recurrent infections due to the immune system's inability to mediate a T-cell response due to thymic aplasia.

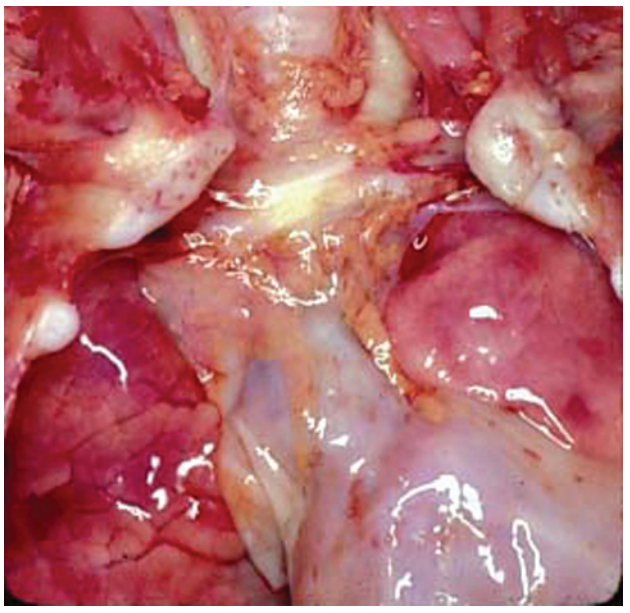

FIGURE 1.13 Gross image of an opened chest after removal of the breast plate in a patient with complete DiGeorge syndrome. Note the total absence of any thymic elements, exposing the innominate vein. (Image courtesy of Dr. Ronald Jaffe, Pittsburgh Children's Hospital).

Wiskott–Aldrich syndrome is an X-linked recessive disorder that is characterized by eczema, thrombocytopenia, lymphocytopenia, recurrent infections, and bloody diarrhea. A mutation of the Wiskott–Aldrich syndrome protein (WASP) gene leads to WAS protein dysfunction on the X chromosome (Ochs, 2006). This leads to decreased antibody production and IgM levels are reduced, IgA and IgE levels are elevated, and IgG levels can be reduced or elevated. Initially the patient has relatively normal lymphocyte function, which decreases with time. Eventually the patient has an impaired immune system and hence is subject to recurrent infections.

Disorders of Plasma Cells

Normal Plasma Cell Physiology

While in the spleen or lymph node, T lymphocytes can stimulate B lymphocytes in the germinal centers to differentiate into more specialized cells: plasma cells or memory B lymphocytes. Plasma cells stem form plasmablasts, which divide rapidly and preserve the B-lymphocyte capability of presenting antigens to T cells. A plasmablast will progress to form a differentiated plasma cell. Plasma cells have an indeterminate life span. They can live for days to months after the process of affinity maturation in germinal centers. Plasma cells secrete large amounts of antibodies, but they can only produce a single class of immunoglobin. Each plasma cell can create hundreds to thousands of antibodies per second, which is more than plasmablasts. Plasma cells play a key role in the humoral immune response, even though they cannot switch antibody class or act as antigen-presenting cells like their precursors.

Normal Plasma Cell Morphology

Plasma cells differ morphologically from lymphocytes and granulocytes and are easily recognized. Plasma cells are oval in shape and range in size from 5 to 30 μm. They have basophilic cytoplasm and an eccentrically placed nucleus, which has a characteristic cartwheel or clock-face appearance. Their nuclear structure is due to heterochromatin arrangement. Nucleoli are absent. They also have a characteristic paranuclear hoff, which is a cytoplasmic clearing that contains the golgi apparatus. The remaining cytoplasm contains an abundant rough endoplasmic reticulum. The cytoplasmic constituents make plasma cells well suited for secreting large amounts of immunoglobulins. Plasma cells may contain crystals. Cells with globular cytoplasmic inclusions constipated with immunoglobulins have been called Mott cells or morular cells.

Plasmacytosis

Definition. Increase in circulating plasma cells.

ICD-10 Code D72.8

Pathophysiology. Plasma cells are mature and specialized B lymphocytes. They are not seen in peripheral blood in healthy persons. Increased circulating plasma cells may occur secondary to a reactive process (Moake, 1974; Glassy, 1998) or with malignancy. Conditions and diseases that may be associated with plasmacytosis include chronic infections (viral, bacterial, fungal, and parasitic infection), inflammatory states, immunization, autoimmune disorders, alcoholic liver disease, cirrhosis, drug reactions, serum sickness, hypersensitivity reactions, sarcoidosis, granulomatous disease, and plasma cell disorders (monoclonal gammopathy of undetermined significance, myeloma, plasma cell leukemia, gamma heavy chain disease, and rarely Waldenstrom's macroglobulinemia) (Bain, 2002).

Clinical Approach. Any increase of plasma cells in a blood smear is abnormal and may be concerning for neoplasia, particularly advanced stages of myeloma. Circulating abnormal plasma cells can be a seen with multiple myeloma, monoclonal gammopathy of undetermined significance, and plasma cell leukemia. Large numbers of immature circulating plasma cells (20% of plasma cells in the differential) are present in plasma cell leukemia. Potential look-alikes include reactive plasmacytoid lymphocytes and lymphoma cells. Morphological features of malignant plasma cells include frequent binucleated and multinucleated forms, large nucleoli, and atypical mitotic figures. Rouleaux formation may be seen on a peripheral blood smear with increased quantities of an M protein. The blood smear may also show a faint purple background when the level of the M protein is very elevated. This background can be demonstrated using the ''scratch test,'' by making a scratch on the slide and comparing the abnormally stained proteinaceous material to the clean colorless glass slide (Pantanowitz, 2004) Circulating nucleated RBCs or a leukoerthrobastic pattern may be seen in some cases with myeloma. Correlation with clinical history, imaging (e.g., bone lesions, underlying chronic inflammatory disease or infection like syphilis, tuberculosis, HIV, or malaria), and where indicated, additional laboratory evaluation (serum protein electrophoresis, immunofixation, Bence–Jones proteins in urine, renal insufficiency, hypercalcemia, flow cytometry, bone marrow assessment, etc.) may be needed to exclude a neoplastic proliferation of plasma cells.

Disorders of Monocytes

Normal Monocyte Physiology

Monocytes originate from the same myeloid stem cell as neutrophils, basophils, and eosinophils. Promonocytes mature under of the influence of granulocyte–macrophage-colony stimulating factor (GM-CSF) or macrophage-colony stimulating factor (M-CSF). Approximately half of all monocytes are stored in the red pulp of the spleen. The bone marrow and the spleen respond to inflammatory signals by releasing monocytes into the peripheral

blood. Monocytes differentiate into macrophages and histiocytes as they migrate into the tissue. Monocytes have an important role in the inflammatory response, including phagocytosis with direct pathogen clearance and antibody-mediated cellular cytotoxicity (Silva, 2010). They also contribute to tissue repair and homeostasis.

Normal Monocyte Morphology

On a Wright–Giemsa stained blood film, the monocyte has a diameter of 12 to 15 μm. This is the largest normal cell seen in the peripheral blood smear. The nucleus is eccentrically placed, is reniform to round or irregular in shape, occupies approximately half the area of the cell (Figure 1.14), and has fine reticulated chromatin. Monocytes have a moderate to abundant cytoplasm that is often vacuolated, stains grayish-blue, and contains a variable number of fine, pink-purple granules (so-called ground-glass appearance). Occasionally these cells may contain phagocytosed material (Figure 1.15).

Normal Monocyte Cytochemistry

Nonspecific esterase (NSE) is frequently used as a marker for monocytes. The most useful cytochemical reaction to detect the esterase activity of monocytes is α-naphthyl acetate esterase (ANAE) activity at acid pH. Monocyte esterases are inhibited by sodium fluoride, whereas the esterases of the granulocytic series are not. Monocytes also give a weak but positive periodic acid–Schiff reaction (for polysaccharides) and variable Sudan black B reaction (for lipids) (Hayhoe 1980).

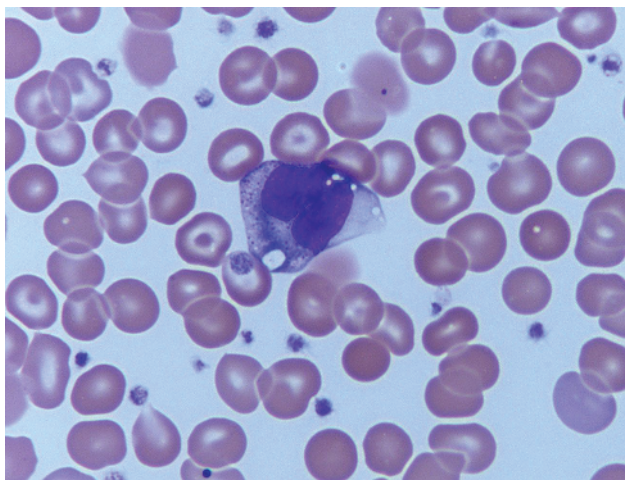

FIGURE 1.14 Mature monocyte characterized by an indented nucleus and abundant blue-gray cytoplasm containing vacuoles and sparse lilac granules.

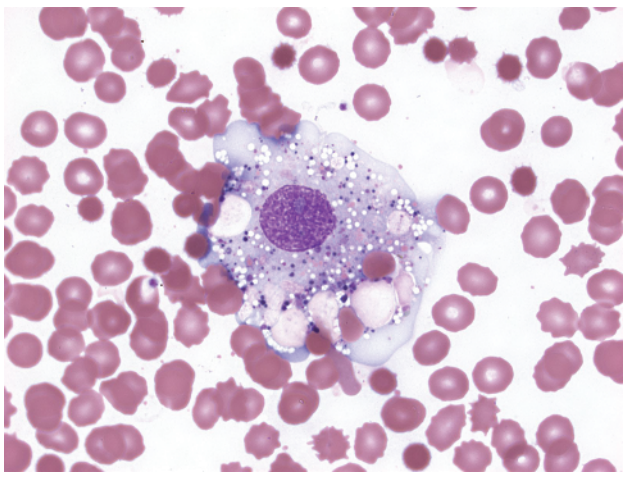

FIGURE 1.15 Monocyte showing marked erythrophagocytosis.

Normal Monocyte Immunophenotype

CD11b, an adhesion surface glycoprotein, and CD14, a receptor for endotoxin (LPS), are the most characteristic surface antigens of the monocyte lineage. The CD68 antigen is a specific marker for monocytes/macrophages that is routinely used in paraffin-embedded tissue. A variable percentage of blood monocytes express the CD4 receptor seen in T helper lymphocytes. HIV-1 utilizes CD4 receptors as an entry pathway for infection of monocyte/macrophages.

Monocytosis

Definition. Absolute monocytosis is defined as monocytes in excess of $1.0 \times 10^3/mm^3$ in adults and $1.2 \times 10^3/mm^3$ in neonates.

ICD-10 Code D72.8

Pathophysiology. Absolute monocytosis can be secondary to either neoplastic disorders or reactive immune responses (Table 1.8) (Maldonado, 1965). A mild monocytosis commonly accompanies reactive neutrophilia. In neutropenic patients, monocytosis represents a compensatory mechanism (Nguyen, 2000).

Clinical Approach. Reactive monocytes tend to have folded nuclei and vacuolated cytoplasm. Potential look-alikes include monoblasts, atypical lymphocytes, myelocytes, metamyelocytes, and even band neutrophils. In adults, if reactive causes are excluded, absolute monocytosis that is persistent for greater than three months should be considered as a marker of a myelodysplastic/myeloproliferative neoplasm (e.g., chronic myelomonocytic leukemia). The

TABLE 1.8 Causes of Monocytosis

Reactive Causes of Monocytosis	
Indolent infection	Bacterial: subacute bacterial endocarditis, tuberculosis, brucellosis, syphilis
	Rickettsial: Rocky Mountain spotted fever, typhus
	Protozoan: malaria, kala azar, trypanosomiasis
Chronic inflammation	Celiac sprue, inflammatory bowel disease, sarcoidosis, rheumatoid arthritis, SLE
Neoplasms	Hodgkin and non-Hodgkin lymphoma, multiple myeloma, cytokine producing carcinoma
Miscellaneous	Recovery from agranulocytosis, tetrachloroethane poisoning, post splenectomy, G-CSF and cytokine therapy, long-term hemodialysis
Neoplastic causes of monocytosis	
Chronic	Chronic myelogenous Leukemia
	Chronic myelomonocytic Leukemia
	Myelodysplastic syndrome
Acute	Acute monoblastic/monocytic leukemia[a]
	Acute myelomonocytic leukemia[a]
	Acute myeloid leukemia with inversion 16[a]

[a]In these disorders the monocytes tend to be immature.

threshold of monocytes greater than $1.0 \times 10^3/mm^3$ is low and does not help distinguish between reactive and neoplastic causes. A mild monocytosis usually accompanies neutrophilia in reactive conditions, and this may be useful in distinguishing reactive from a neoplastic process. Monocytosis accompanied by cytopenias and other features of myelodysplasia (e.g., hypolobate and hypogranular neutrophils, hypogranular platelets, and dimorphic red blood cells) suggests CMML.

Monocytopenia

Definition. Monocytopenia is a form of leukopenia associated with a deficiency of monocytes.

ICD-10 Code D72.9

Pathophysiology. The causes of monocytopenia include acute infections, stress, treatment with glucocorticoids, aplastic anemia, hairy cell leukemia, acute myeloid leukemia, and treatment with myelotoxic drugs.

Clinical Approach

In the setting of monocytopenia an underlying cause should be sought, particularly hairy cell leukemia.

Disorders of Eosinophils

Normal Eosinophil Physiology

Eosinophils are granulocytes that originate from the same myeloid stem cell precursor that can develop into neutrophils, basophils, or monocytes. Eosinophils are released from the bone marrow into the peripheral blood and tissues in response to interleukins: IL-1, IL-3, and especially IL-5. Degranulation of eosinophils releases major basic protein, histamine, peroxidase, and eosinophil-derived neurotoxin. They have two key roles in the immune system: destroying foreign substances and promoting an inflammatory response. Eosinopenia (reduction in eosinophil count) is usually a nonspecific finding, or it may be attributed to a physiological fall during pregnancy, acute stress, Cushing's syndrome, or drugs.

Normal Eosinophil Morphology

Eosinophils have a diameter of 12 to 17 μm and have a characteristic bilobed nucleus. A small number of normal eosinophils may be trilobed. Their cytoplasm contains a plethora of almost spherical granules that stain reddish orange (Figure 1.16). Immature eosinophils may have fewer granules.

Normal Eosinophil Cytochemistry

Normal eosinophils show positivity for myeloperoxidase and Sudan black and moderate reactivity with naphthol-AS or α-naphthyl esterase. They do not show toluidine blue metachromasia or positivity for alkaline phosphatase, chloroacetate esterase, or periodic acid–Schiff (Hayhoe, 1980).

Eosinophilia

Definition. Absolute eosinophilia is the presence of more than $0.6 \times 10^3/mm^3$ eosinophils in the circulating blood (Figure 1.17).

ICD-10 Code D72.1

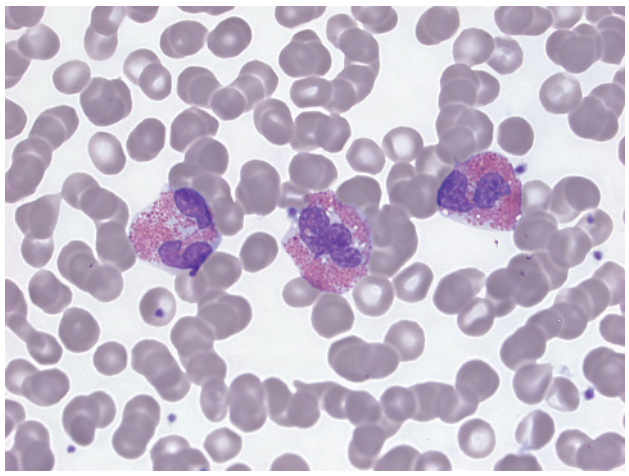

FIGURE 1.16 Mature eosinophils containing segmented nuclei and cytoplasm filled with coarse orange-red granules. Note that the central eosinophil has a trilobed nucleus.

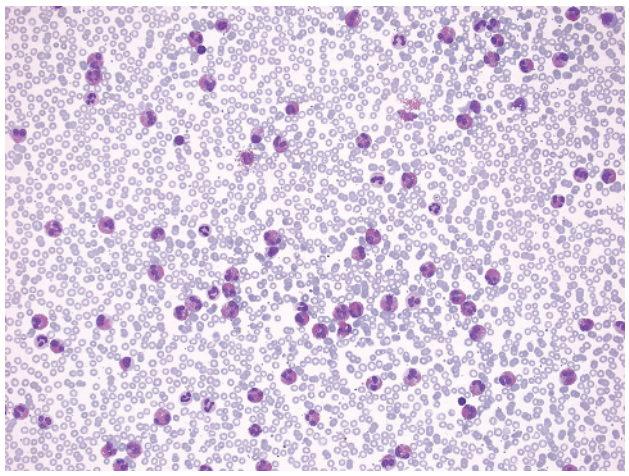

FIGURE 1.17 Blood smear showing eosinophilia.

Pathophysiology. Eosinophilia can be divided into mild ($<1.5 \times 10^3/mm^3$), moderate ($1.5–5.0 \times 10^3/mm^3$), or severe ($>5.0 \times 10^3/mm^3$). Absolute blood eosinophilia may be a primary or secondary phenomenon (Lombardi, 2003; Tefferi, 2006; Pardanani, 2008). Refer to Table 1.9 for a list of common causes of eosinophilia. Of note, tumors with tumor-associated tissue eosinophilia appear to have a better prognosis that those without such a response (Lowe, 1981).

- **Primary eosinophilia:** Primary eosinophilia is classified into two categories, clonal and idiopathic. Clonal eosinophilia requires the presence of either cytogenetic evidence or bone marrow histological evidence of an otherwise classified myeloid neoplasm such as acute leukemia or a myeloproliferative neoplasm. Genetic mutations involving the platelet-derived growth factor receptor genes (PDGFRA, PDGFR-B, and FGFR1) have been pathogenetically linked to clonal eosinophilia, and their presence predicts treatment response to imatinib. Clonal eosinophilia is also associated with chronic myeloid leukemia, chronic eosinophilic leukemia, mastocytosis, and AML with inv (16). Accordingly, cytogenetic and/or molecular investigations for the presence of a molecular/genetic abnormality should accompany the evaluation for primary eosinophilia. Idiopathic eosinophilia (hypereosinophilic syndrome or HES) is a diagnosis of exclusion (i.e., not secondary or clonal). HES refers to a heterogeneous group of disorders (Roufosse, 2003). The diagnosis of HES requires documentation of (1) sustained eosinophilia (i.e., absolute eosinophil count≥1500 cells/μL for at least 6 months), (2) no other etiology for eosinophilia, and (3) target organ damage from eosinophilic infiltration with mediator release (e.g., involvement of the heart, lung, skin, or nerve tissue). There are several variants of HES including myeloproliferative variants, T-lymphocytic variants, familial HES, idiopathic (unclassified) HES, overlap HES, and associated HES (Sheikh, 2007).
- **Secondary eosinophilia:** Causes of secondary (i.e., reactive) eosinophilia include tissue-invasive parasitosis, allergic or inflammatory conditions, drug reactions, and malignancies in which eosinophils are not part of the neoplastic process (Kano, 2009). The level of eosinophilia usually parallels the extent of tissue invasion by parasitic worms. Fungal infections usually associated with eosinophilia include aspergillosis (e.g., allergic bronchopulmonary aspergillosis) and coccidioidomycosis.

Clinical Approach. In a patient with blood eosinophilia, the possibility of secondary eosinophilia must be excluded first. For example, a detailed allergy, drug, and travel history along with stool for ova and parasites are a useful starting point (Checkley, 2010). HIV infection may also be associated with eosinophilia (Skiest, 1997). Once this is accomplished, blood and bone marrow studies should be obtained. The finding of many abnormal eosinophils (e.g., monolobated, hypersegmented, ring forms, hypogranulation, cytoplasmic vacuoles) is non-specific, but often associated with idiopathic HES. An increase in eosinophils with granules that have basophilic staining characteristics (so-called pre-eosinophilic granules or a hybrid

TABLE 1.9 Reactive Cause of Eosinophilia

Acquired eosinophilia	
Atopic/allergic diseases	Asthma, urticaria, eczema, rhinitis
Parasitic infestation (with tissue invasion)	Tricninosis, hookworms, Ascaris lumbricoides, schistosomiasis, filariasis, fascioliasis
Drug reaction	Dapsone, allopurinol, sulfa, recombinant human interleukins
Hematopoietic neoplasms	Hodgkin and non-Hodgkin lymphoma
Infectious diseases	Scarlet fever, cat scratch disease, chlamydia, fungi
Inflammatory disease	Colitis, celiac disease, vasculitis, sarcoidosis
Skin diseases	Dermatitis herpetiformis, bullous pemphigus, pemphigoid
Carcinoma	Squamous cell carcinoma, large cell lung carcinoma, adenocarcinoma, bladder transitional cell carcinoma
Miscellaneous	Adrenal insufficiency, atheroembolic disease, hyper-IgE syndrome (Job syndrome), Omenn syndrome
Primary eosinophilia	
Idiopathic	Hypereosinophilic syndrome (HES)
Clonal eosinophilia	Myeloid and lymphoid neoplasms with eosinophilia and abnormalities of PDGFRA, PDGFRB OR FGFR1
	Chronic eosinophilic leukemia, NOS
	Chronic myelogenous leukemia
	Mastocytosis

eosinophilic-basophilic granulocyte) favors leukemia (Weil, 1987; Bain, 2002). Blood studies should include serum tryptase (an increased level suggests systemic mastocytosis), T-cell receptor gene rearrangement analysis (positive test results suggest an underlying clonal T-cell disorder), and serum IL-5 (an elevated level requires careful evaluation of bone marrow studies and T-cell gene rearrangement studies for the presence of a clonal T-cell disease) (Ogbogu, 2009). Bone marrow examination should include cytogenetic studies, tryptase immunostains, and FISH or reverse transcriptase polymerase chain reaction (R-PCR) to screen for FIP1L1-PDGFRA. The last mentioned test can also be performed on peripheral blood. Positive genetic studies suggest a clonal/primary eosinophilic disorder. Persistent eosinophilia with target organ damage can be interpreted as a neoplastic process (chronic eosinophilic leukemia/hypereosinophilic syndrome) even in the absence of genetic abnormalities.

Inherited Abnormalities

Inherited abnormalities of eosinophils are rare. They include the following disorders:

- Absence of peroxidase and phospholipids in eosinophils: An autosomal recessive defect that produces no signs of disease.
- Chédiak–Higashi syndrome (described later): Almost all granulated cells, including eosinophils, contain large abnormal granules
- Neutrophil-specific granule deficiency (described later): This inherited abnormality also involves eosinophils.

Disorders of Basophils

Normal Basophil Physiology

Basophils are granulocytes that originate from the same myeloid stem cell precursor that can develop into neutrophils, eosinophils, or monocytes. Basophils are released from the bone marrow into the peripheral blood and tissues in response to interleukins: GM-CSF, IL-3, and IL-5. Reactive processes lead to degranulation, which releases heparin, histamine, aryl-sulfatase A, and eosinophil chemotactic factor. They have one main role in the immune system: degranulating and promoting an inflammatory response. Some causes of basopenia include acute stress, Cushing's syndrome, ACTH therapy, acute allergic reaction, hyperthyroidism, and progesterone therapy.

Normal Basophil Morphology

Basophils have diameters of 10 to 14 μm and have a characteristic lobated nucleus (Figure 1.18) that is usually obscured by the cytoplasmic granules, which are large and deeply basophilic (dark purple). Hypogranulation of basophils can be seen during an acute allergic attack, in myeloproliferative disorders, and may be an artifact due to the water solubility of these granules (Nguyen, 2000). Eosinophils and neutrophils with marked toxic granulation may mimic

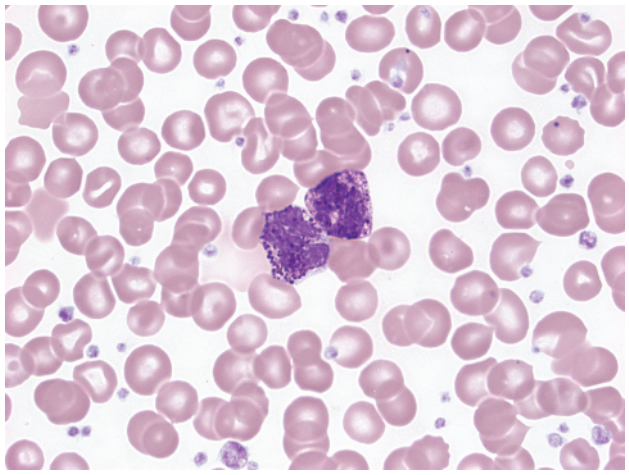

FIGURE 1.18 **Blood smear showing two basophils from a patient with basophilia**. Note that the basophilic granules in these cells overlay and therefore partially obscure the nuclei.

basophils. Mast cells with basophilic granules can also look like basophils. Mast cells are extremely rare in the peripheral blood of healthy individuals (Bain, 2002). Mast cells are larger than basophils, have a small round nucleus and more abundant cytoplasm, and harbor more tightly packed small dark granules.

Basophilia

Definition. Basophilia is the presence of more than $0.2 \times 10^3/mm^3$ basophils in the circulating blood.

ICD-10 Code D75.8

Pathophysiology. Basophilia can be divided into primary causes, which are associated with chronic myeloid leukemia and basophilic leukemia, and secondary causes, which are reactive. Common causes of basophilia are listed in Table 1.10.

Clinical Approach. Basophil morphology is unremarkable in reactive basophilia. However, basophils in some inherited conditions can have abnormal granules. Abnormal basophils (e.g., hypogranulation) may also indicate MDS or a myeloproliferative disorder. However, degranulation can also occur in acute allergic conditions and during postprandial hyperlipidemia. In patients with suspected primary basophilia, molecular genetic studies for BCR-ABL and JAK2V617F, among others, should be considered to exclude a myeloproliferative neoplasm.

QUALITATIVE DISORDERS OF WBCS

Congenital Disorders of Leukocytes

In addition to the aforementioned quantitative WBC disorders, leukocytes can also have qualitative disorders. There are many constitutional/congenital conditions that may result in dysfunction of neutrophils (Table 1.11). Genetic dysfunctions are rare, but can be severe. Acquired defects are more common, but much less severe. Most of the disorders that will be discussed are associated with neutrophil defects or dysfunction; however, there is a congenital disorder of NK cells that leads to hereditary lymphohistiocytosis (HLH). Familial HLH is an autosomal recessive disease and has a possible gene etiology on chromosomes 9 and 10 (Ohadi, 1999). Secondary HLH occurs in the setting of a strong immunologic response associated with viral (particularly EBV), bacterial, fungal, or parasitic infections and collagen-vascular diseases, as well as T-cell lymphomas. This disease has characteristic findings of fever, splenomegaly, and jaundice. The patient presents with pancytopenia, which is secondary to profound hemophagocytosis within the bone marrow, spleen, and liver (Henter,

TABLE 1.10 **Causes of Basophilia**

Reactive causes of basophilia	
Allergic disease	Chronic sinusitis, hypersensitivity reactions
Infectious disease	Chickenpox, smallpox, tuberculosis
Hematopoietic Neoplasms	Chronic myeloid leukemia, Waldenström's macroglobulinemia, basophilic leukemia, Hodgkin lymphoma, polycythemia vera
Miscellaneous	Radiation, myxedema, chronic hemolytic anemia, post splenectomy, diabetes, increased at onset of menses
Neoplastic causes of basophilia	
Chronic	Chronic myeloid leukemia
Acute	Acute basophilic leukemia

TABLE 1.11 Congenital Disorders of Leukocytes

Adhesion defects	Leukocyte adhesion deficiency (types 1, 2, 3)
Granule defects	Neutrophil-specific granule deficiency
	Chediak–Higashi syndrome
	Myeloperoxidase deficiency
Chemotaxis defects	Chediak–Higashi syndrome
	Hyper-IgE (Job) syndrome
	Neutrophil-specific granule deficiency
	Down syndrome
	Neutrophil actin deficiency
	A–mannosidase deficiency
	Severe combined immunodeficiency disease (SCID)
	Wiskott–Aldrich syndrome
	Complement disorders
	Lazy leukocyte syndrome
Phagocytic defects	Chediak–Higashi syndrome
	Neutrophil-specific granule deficiency
	Myeloperoxidase deficiency
	Chronic granulomatous disease
	Immunodeficiency disorders with decreased immunoglobins
	Complement disorders

1991). Hemophagocytosis is observed by the presence of NK cells with perforin release and activated histiocytes that have engulfed erythrocytes, leukocytes, platelets, their precursors, and cellular fragments (Favara, 1992).

The major categories of these neutrophil dysfunctions are adhesion defects, defects in granule structure/function, mobility and chemotaxis defects, and phagocytic or microbicidal defects. Neutrophils have three techniques for combating microorganisms, including phagocytosis, degranulation, and setting neutrophil extracellular traps. The process of phagocytosis consists of the neutrophil internalizing a microbe and killing the microorganism by producing reactive oxygen species (Segal, 2005). As mentioned earlier, neutrophils contain three types of granules. When neutrophils degranulate, the proteins from the three types of granules are able to kill neighboring microbes (Hickey, 2009). Neutrophil extracellular traps are a combination of granule proteins and a chromatin that forms extracellular fibers (Haslett, 1992). The traps bind up microbes and degrade virulence factors. The neutrophils can then phagocytize the microbes or kill them with high concentration degranulation. A breakdown of any of these functions can lead to an increased risk of infection.

There are several laboratory tests that may be used to evaluate neutrophil function (Bogomolski-Yahalom, 1995; Elloumi, 2007). However, most of these are not routinely available in clinical labs. They include the bactericidal killing assay, chemiluminescence assay, superoxide assay, nitroblue tetrazolium (NBT) slide test, and neutrophil chemotaxis assays. Most of these tests rely on the respiratory burst in neutrophils.

Chédiak–Higashi Syndrome

ICD-10 Code E70.3

Chédiak–Higashi is an autosomal recessive disease that is caused by a gene mutation of the CHS1 or LYST protein (part of the BEACH family of vesicle trafficking regulatory proteins), that results in cellular dysfunction and fusion of cytoplasmic granules. Afflicted patients have characteristic giant cytoplasmic granules (Figure 1.19) that result in large secondary lysosomes, which functionally contain less proteinases, elastase, and cathepsin G and, as a result, have slower bactericidal function (Kaplan, 2008; Rezaei, 2009). The giant lysosomal granules are more evident in bone marrow cells than the blood smear. These patients have moderate neutropenia, due to myeloid precursor death while in the marrow and splenic sequestration. They also have thrombocytopenia and perforin-deficient natural killer cells. So these individuals have an increased risk of bacterial and fungal infections due to defects in neutrophil chemotaxis, degranulation, and bactericidal activity. Moreover they are at an increased risk of viral infections due to dysfunctional NK cells. EBV is a classic infection that can lead to EBV-associated lymphoproliferative disorders in this setting (Nargund, 2010).

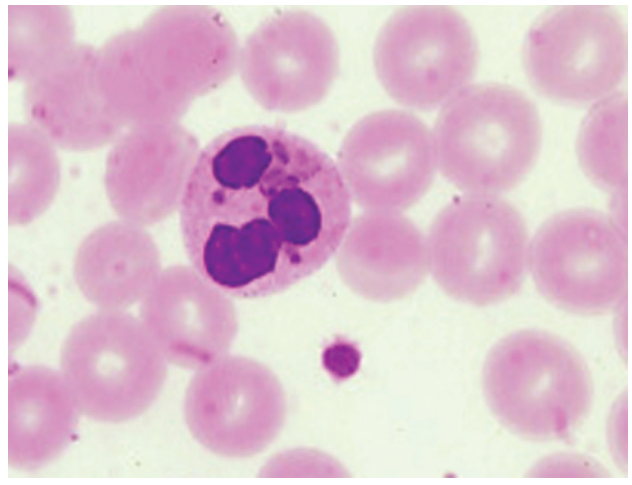

FIGURE 1.19 Segmented neutrophil from a patient with Chédiak-Higashi syndrome. Note the large cytoplasmic granules of varying size. (Image courtesy of Dr. Ronald Jaffe, Pittsburgh Children's Hospital.)

Patients with Chédiak–Higashi syndrome also have giant melanosomes that prevent an even distribution of their melanin. Phenotypically they have light silvery hair as well as pale skin and minimal pigment in their iris and optic fundus. They suffer from photophobia and can have horizontal nystagmus. These patients have variable peripheral and cranial neuropathy, ataxia, autonomic dysfunction, muscle weakness, and sensory deficits. They can have prolonged bleeding times with normal platelet counts secondary to impaired platelet aggregation function. Chédiak–Higashi syndrome has an accelerated phase that is characterized by lymphocytic proliferation in the liver, spleen, and bone marrow, and this can result in hepatosplenomegaly, worsening pancytopenia, and an increased susceptibility to infection (Dinauer, 2007). EBV infection in the accelerated phase can display viral-mediated hemophagocytic syndrome, tissue necrosis, and organ failure.

Neutrophil-Specific Granule Deficiency

ICD-10 Code D72.0

Neutrophil-specific granule deficiency (SGD) is an autosomal recessive disorder that is caused by a loss-of-function mutation of CCAAT/enhancer-binding protein ε (Gombart, 2001). The disorder leads to a lack of gelatinolytic activity in the tertiary granules, vitamin B_{12}-binding protein, lactoferrin, as well as a lack of collagenase in specific granules and defensins in primary granules. These neutrophils have a characteristic appearance of absent granules with a bilobed, hyposegmented nucleus (pseudo–Pelger–Huet anomaly). Eosinophils can also be affected and may lack major basic protein, eosinophilic cationic protein, and eosinophil-derived neurotoxin proteins (Rosenberg, 1993). These patients have recurrent pulmonary and cutaneous infections. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are the most commonly involved pathogens.

Chronic Granulomatous Disease

ICD-10 Code D72.0

Chronic granulomatous disease (CGD) is an X-linked (2/3 patients) and autosomal recessive (1/3 patients) disease with defects in expression of glycoproteins (gp91) in the phagocyte membrane. CGD is a primary immunodeficiency disorder of phagocytic cells (neutrophils, monocytes, and macrophages). It comprises five genetic defects impairing one of the five subunits of phagocyte NADPH oxidase (Phox). Phox normally generates reactive oxygen species (ROS) engaged in intracellular and extracellular microbial killing and resolving accompanying inflammatory processes. The defect can be associated will multiple membrane proteins including p22, p47, and p67. Affected leukocytes are also deficient in C3b receptors. Red blood cells (often acantholytic) bear the McLeod phenotype with absence of Kell antigens Kx and Km. Functionally, the neutrophils cannot activate the respiratory burst process and are not able to kill catalase positive organisms once phagocytosed (Holland, 2010). Consequently these patients have recurrent catalase positive infections with granuloma formation that keep the organisms localized (Heyworth, 2003). The diagnosis is made by neutrophil function testing and mutation analysis. The X-linked recessive inheritance may be confirmed by studying the family history.

Complement Disorders

ICD-10 Code D84.1

Abnormalities in antibodies and complement can result in neutrophil signaling dysfunction (Tedesco, 2008). Affected neutrophils have a break down in their ability to normally apply opsonins and bind chemotatic factors, which results in decreased microbicidal function. C3 deficiency is an autosomal recessive disorder that results in decreased opsonins and consequently can display motility/chemotaxis defects and phagocytic and microbicidal dysfunctions. The severe form of the disease is characterized by recurrent pyogenic infections, and it appears in homozygotes that have undetectable levels of serum C3. Heterozygotes have some functional C3 and can be asymptomatic.

Leukocyte Adhesion Deficiency

ICD-10 Code D72.0

There are two types of genetic leukocyte adhesion deficiency (LAD): type 1 (LAD-1) and II (LAD-II) (Etzioni, 2007). These are autosomal recessive disorders that have adhesion defects that impair cell migration, phagocytosis, and complement- or antibody-dependent cytotoxicity (Arnaout, 1990).

Leukocyte Adhesion Deficiency Type 1. LAD-1 results from an inability of neutrophils to leave the circulation during an infection due to abnormal leukocyte integrins. In LAD-1 there is dysfunction of the CD11/CD18 protein. Patients can have decreased

levels of CD11/CD18 cell surface molecules and moderate disease. Patients with complete absence of surface expression of CD11/CD18 proteins have more severe impairment of neutrophil and monocyte adhesion-dependent functions (Foucar, 2006). Although these patients display neutrophilia during infection, due to the decrease in adhesion function they suffer from recurrent sino-pulmonary, skin, and soft-tissue infections, delayed separation of the umbilical cord, poor wound healing, and severely impaired pus formation.

Leukocyte Adhesion Deficiency Type II. LAD-2 results from an inability to appropriately glycosylate another leukocyte adhesion molecule. These individuals have severe cognitive impairment, small stature, and abnormal facies. They have similar but less severe infections than those seen with LAD-1.

Pelger–Huet Anomaly

ICD-10 Code D72.0

Pelger–Huet anomaly (PHA) is an autosomal dominant disorder with mutations in the lamin β-receptor and results in neutrophils that fail to have normal segmentation (Speeckaert, 2009). It was described by Pelger in 1928. Huet recognized the familial nature of this condition in 1931. True PHA is seen in 1 out of 6000 individuals. The characteristic morphology of the neutrophils is of a nonsegmented or a bilobed nucleus, forming a characteristic spectacle or ''pince-nez'' (dumbbell) shape (Figure 1.20*A* and 1.20*B*). Pince-nez refers to a style of spectacles that are supported without earpieces, by pinching the bridge of the nose. The two lobes are connected by a thin bridge of chromatin. In rare homozygotes with this anomaly, all the neutrophils have round-oval nuclei. The cytoplasm is usually unremarkable in these neutrophils, but it may be accompanied by hypogranulation. Patients with this anomaly tend to be asymptomatic, with no functional deficits of granulocytes (Johnson, 1980) and no other lineage abnormalities. Individuals may show some reduced lobulation of eosinophils and basophils. Pelger–Huet cells can develop multiple lobes with folate or vitamin B_{12} deficiency. The acquired Pelger–Huet anomaly (pseudo–Pelger–Huet) may be induced by drugs (colchicine, sulfonamides), or represent a dysplastic feature seen in myelodysplastic syndrome or other hematologic neoplasms (e.g., AML). The finding of the same anomaly in family members can help confirm the diagnosis. Pelger–Huet cells need to also be distinguished from immature cells observed with a left shift, which have less dense chromatin.

May–Hegglin Anomaly

ICD-10 Code D72.0

May–Hegglin anomaly is a rare autosomal dominant disorder that is associated with a mutation of the MYH9 gene that encodes for nonmuscle myosin heavy chain IIA (Saito, 2008). This mutation results in large, basophilic cytoplasmic inclusions that are

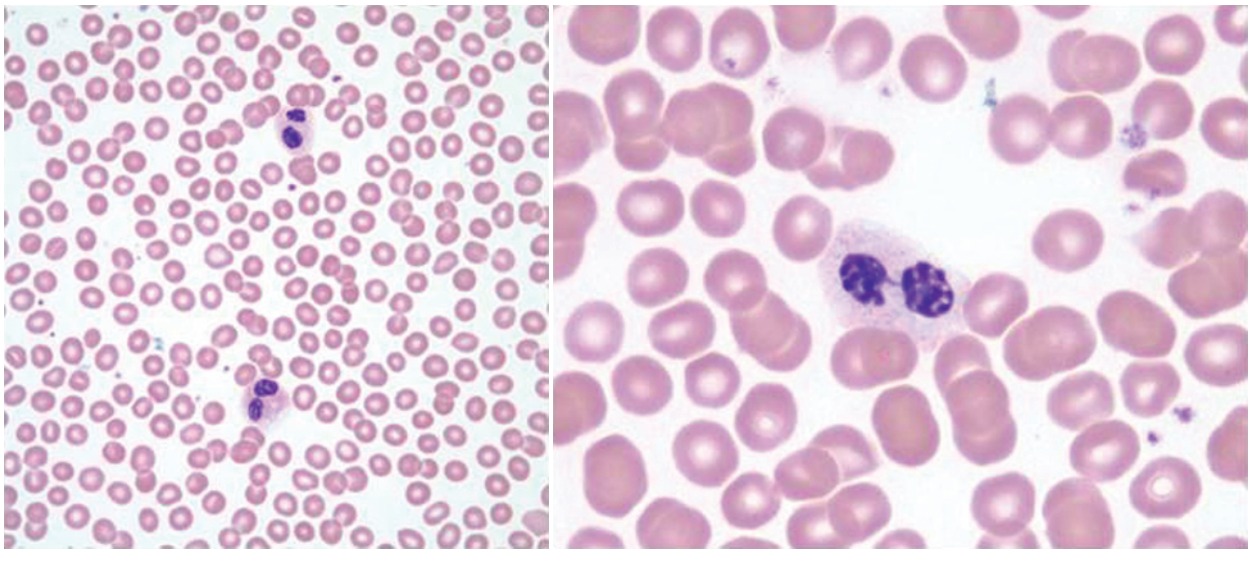

FIGURE 1.20 Blood smear from a heterozygous patient with Pelger–Huet anomaly. (**A**) Neutrophils show a characteristic bilobed appearance. (**B**) Neutrophil showing a classic bilobed Pelger–Huet nucleus with a ''pince-nez'' conformation.

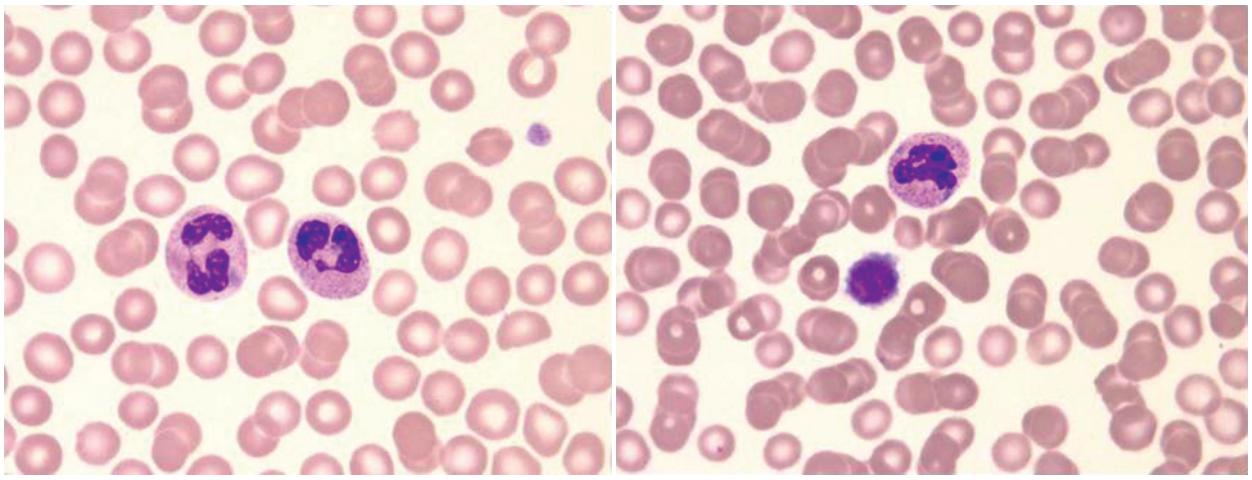

FIGURE 1.21 **Blood smear from a patient with May-Hegglin anomaly**. (**A**) Neutrophils are shown containing light blue Döhle bodies distributed both peripherally and between the nuclear lobes. (**B**) The finding of large cytoplasmic inclusions within a nontoxic neutrophil in association with a giant platelet is pathognomonic.

Döhle-body like (Figure 1.21*A*) (Cawley, 1971; Jenis, 1971). The inclusions are classically identified within neutrophils, but can also be seen in eosinophils, basophils, monocytes, and lymphocytes. The Döhle-like bodies can be abolished by addition of ribonuclease (Mais, 2005). The cytoplasm of neutrophils in this condition do not show other changes (e.g., granules, vacuoles) encountered with toxic or activated neutrophils. They may display other lineage abnormalities, including thrombocytopenia, giant poorly granulated platelets (Figure 1.21*B*), and neutropenia. Thrombocytopenia may be associated with bleeding and purpura (Norris, 1998). Platelet aggregation studies are usually normal.

Alder-Reilly Anomaly

ICD-10 Code D72.0

Alder–Reilly anomaly is an autosomal recessive disorder associated with several genetic mucopolysaccharidoses (i.e., Hurler and Hunter syndromes) (Presentey, 1986). These patients lack the lysozymal enzymes necessary to break down mucopolysaccharides, and they display deeply azurophilic granules in the cytoplasm of all leukocytes (Figure 1.22). The cytoplasmic inclusions are composed of precipitated mucopolysaccharide. Compared to toxic granulation, these bodies are larger and stain positive with metachromatic stains. The abnormality is easier to identify in the bone marrow. Patients often have no functional deficits of their leukocytes or other lineages.

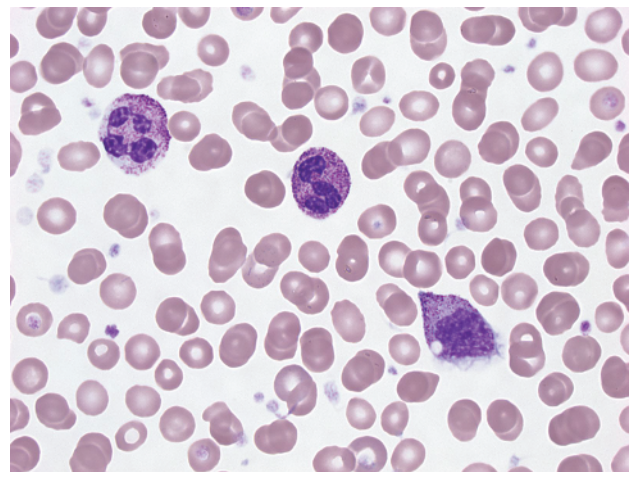

FIGURE 1.22 **Blood smear from an individual with Alder–Reilly anomaly**. Note the segmented neutrophils and a monocyte having many large granules (Alder–Reilly bodies).

Hereditary Hypersegmentation and Giant Neutrophils

ICD-10 Code D72.0

Hereditary hypersegmentation of neutrophils and hereditary giant neutrophils are autosomal dominant inherited disorders of neutrophils. As the name implies, in hereditary hypersegmentation neutrophil nuclei have more than five lobes, but these nuclei display no other abnormalities. Hereditary giant neutrophils have enlarged and hypersegmented nuclei, but not all neutrophils are affected. These

patients are asymptomatic and have no abnormalities of other lineages.

Acquired Disorders of Leukocytes

Acquired disorders of leukocytes are more common than congenital disorders. The major categories of neutrophil disorders are the same as the congenital disorders and include adhesion defects, defects in granule structure/function, mobility and chemotaxis defects, and phagocytic and microbicidal defects (Table 1.12). The symptomatology is variable and is dependent on the severity of each patient's defect. Megaloblastic myelopoiesis is a reversible change that can be observed in granulocyte morphology. It arises when DNA synthesis is reduced but RNA synthesis, and hence protein synthesis, remains unaltered. This is seen mainly with vitamin B_{12} and folic acid deficiency, or secondary to alcoholism or a drug-induced effect (e.g., anti-metabolite therapy). The dyssynchrony between nuclear and cytoplasmic development creates large precursors (i.e., giant metamyelocytes and giant bands) and occasionally large neutrophils (macropolyctes). Moreover there is hypersegmentation of neutrophils (Edwin, 1967). Hypersegmentation is defined as exceeding 5% of peripheral blood neutrophils with 5 nuclear lobes, or any neutrophils with 6 or more lobes (Nguyen, 2000). Hypersegmented neutrophils (so-called right shift) can also be seen with infection, uremia, and iron deficiency (Westerman, 1999; Düzgün, 2005), and may resemble dysplastic neutrophils. In the inherited condition known as myelokathexis, neutrophils are also hypersegmented with long chromatin filaments separating the lobes.

TABLE 1.12 Acquired Disorders of Leukocytes

Adhesion defects	Medications (epinephrine, corticosteroids)
	Diabetes
	Renal disorders
	Paraproteinemias
	Sickle cell anemia
Granule defects	Thermal injury
	Trauma/surgery
	Hematologic neoplasms (CML, AML, myelodysplasia)
Chemotaxis defects	Thermal injury
	Medications (colchicine, anti-inflammatory drugs)
	Periodontal disease
	Autoimmune disease (SLE, RA)
	Diabetes
	Malnutrition
	Cirrhosis
	Sepsis
	Viral infections (influenza, HSV, HIV)
	Hematologic/myeloid malignancies
Phagocytic defects	Thermal injury
	Autoimmune disease (SLE, RA)
	Diabetes
	Malnutrition
	Cirrhosis
	Sepsis
	Viral infections-(HIV)
	Sickle cell anemia
	Hematologic neoplasms

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