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Leukocyte Disorders

BASIC LEUKOCYTE CONCEPTS

Leukogram

Leukocyte responses are evaluated by component parts of the leukogram, including total leukocyte (white blood cell [WBC]) count, differential leukocyte count, absolute numbers of specific leukocytes per microliter of blood, and examination of leukocyte morphology on the Romanowsky's-stained (e.g., Wright's-stained) blood smear.

Hematologic techniques are described in Chapter 2. The leukocyte differential count is the relative percentages of various leukocyte types (i.e., segmented neutrophils [segs], band neutrophils, lymphocytes, monocytes, eosinophils, basophils) in the stained blood smear. Absolute leukocyte counts are the numbers of each type of leukocyte per microliter of blood.

The leukogram is used to monitor the patient's health status, to construct a differential diagnosis, to evaluate a patient's response to treatment, or to suggest a prognosis. Although changes in the leukogram are seldom pathognomonic for a given disease, they do identify and characterize several disease processes and indicate trends suggesting development or resolution of illness (Latimer, 1995). The leukogram does not normally document sepsis, identify specific causative agents, or pinpoint location of inflammation. However, characteristic changes in the leukogram identify presence of inflammatory disease and characterize its severity. Toxic changes and severity of inflammation may suggest sepsis, which may be confirmed and localized by cytology, microbiologic culture, serum chemistries, radiographs, ultrasonography, surgical biopsy, or serology. Any one or a combination of these tests may localize the disease process to a tissue or organ system or provide a definitive diagnosis.

This chapter is concerned with understanding and interpreting leukogram abnormalities. Basic leukocyte concepts are reviewed to facilitate understanding the leukocyte response and to avoid common interpretive errors. Supplemental information regarding leukogram interpretation may be found in other textbooks (Duncan, Prasse, and Mahaffey, 1994; Latimer, 1995; Meyer and Harvey, 1998; Feldman, Zinkl, and Jain, 2000).

Absolute Versus Relative Leukocyte Values

Use of absolute WBC numbers allows more consistent evaluation of leukogram responses than using relative percentages (Figure 4-1). For example, a WBC count of 10,000 leukocytes/μl with 65% segs has 6500 segs/μl. The 6500 segs/μl is normal, but 65% segs are not always normal. A WBC count of 1000 leukocytes/μl with 65% segs indicates severe neutropenia (i.e., 650 segs/μl). A WBC count of 50,000 leukocytes/μl with 65% segs indicates neutrophilia (i.e., 32,500 segs/μl).

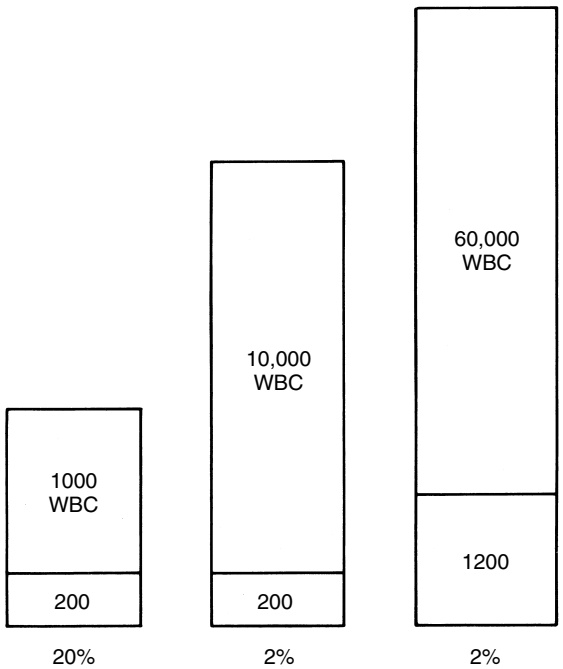

FIGURE 4-1. Relative and absolute leukocyte counts. The bottom chamber of each bar indicates the absolute number of band neutrophils, and the percentage below the bars indicates the relative percentage of band neutrophils. The relative change between the first and second bar (i.e., 20% to 2%) seems great; however, no change exists in the absolute number of band neutrophils in the blood (i.e., 200 bands/μl of blood). The relative percentage of band neutrophils between the second and third bars seems identical. However, a normal number of band neutrophils exist in the dog with 200 bands/μl of blood, but a true increase (left shift) in band neutrophils occurs in the dog with 1200 bands/μl in the third bar. *WBC,* White blood cell.

For initial leukogram evaluation, absolute cell counts for each leukocyte type should be interpreted individually. Subsequent changes in the leukogram can be summarized by a few hematologic terms indicating an increase or decrease in a given leukocyte type. For example, leukocytosis with mature neutrophilia, lymphopenia, and monocytosis indicates succinctly that the complete blood count (CBC) had an increased WBC count, an increase in the number of mature segs, and no increase in the number of band

neutrophils; that the lymphocyte count was decreased; and that the monocyte count was increased, respectively.

> NOTE: Absolute cell numbers rather than percentages are used to evaluate the leukogram.

Blood Leukocyte Production, Circulation, and Emigration

To interpret the concentration of leukocytes in the blood, one must consider rate of leukocyte production in bone marrow and release into blood, distribution and circulating half-life of leukocytes, and rate of emigration of leukocytes from blood into tissues.

Leukocyte Production

Granulocytes (i.e., neutrophils, eosinophils, basophils) and monocytes are produced in the bone marrow. Although the bone marrow produces some lymphocytes, most are derived primarily from the peripheral lymphoid tissues (i.e., thymus, lymph nodes, spleen, tonsil, bronchial-associated lymphoid tissue, gut-associated lymphoid tissue). Leukocytes develop in the bone marrow from pluripotent and committed stem cells influenced by interleukins and colony-stimulating factors (Raskin, 1996). Pluripotent stem cells provide a reserve compartment for hematopoietic cell (i.e., leukocyte, erythrocyte, platelet) production if bone marrow has been severely damaged by toxins, drugs, infectious agents, or radiation (Figure 4-2). Pluripotent stem cells are found in both bone marrow and blood. They may affect repopulation of the bone marrow after a severe insult, provided proper stromal cells and growth factors are present. The number of pluripotent stem cells decreases with age, so younger animals generally have a better chance of restoring bone marrow function than do aged adults.

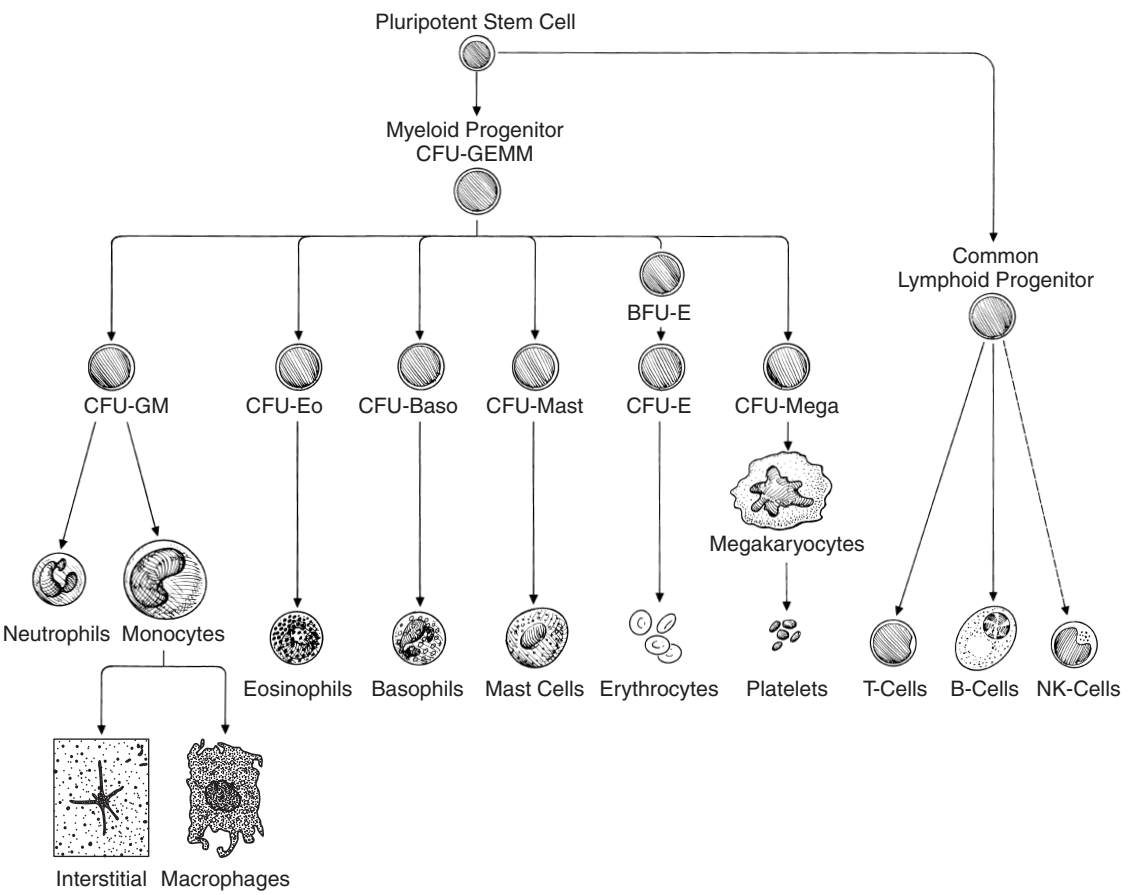

FIGURE 4-2. Differentiation of the bone marrow pluripotent stem cells. *Dotted lines* reflect uncertainty in derivation. *CFU*, Colony forming unit; *GEMM*, granulocyte-erythroid-monocyte-megakaryocyte; *GM*, granulocyte-macrophage; *BFU*, burst-forming unit; *E*, erythroid; *Mega*, megakaryocytic; *Eo*, eosinophil; *Baso*, basophil; *DC*, dendritic cell; *NK*, natural killer.

The following discussion of bone marrow leukocyte production emphasizes production of neutrophils. Cellular "pools" are used to conceptualize the "location" of neutrophils within the bone marrow and blood and to simplify interpretation of bone marrow and CBC data (Figure 4-3). Bone marrow is divided into two pools: The first, the mitotic pool of myeloblasts, promyelocytes, and myelocytes, provides a steady supply of neutrophils to meet tissue demand for these cells. The second pool is maturation and storage, which consists of metamyelocytes, bands, and segmenters that lack mitotic ability. These cells undergo progressive maturation and provide a reserve of segs to meet increased tissue demands for neutrophils until the mitotic pool increases neutrophil production. The maturation and storage functions are combined in Figure 4-3. Neutrophil release from the bone marrow into the blood is age ordered according to maturity (segs, bands, metamyelocytes, myelocytes, and promyelocytes, in that order). As bone marrow stores of segs are depleted, nonsegmented neutrophils (nonsegs) (e.g., bands, metamyelocytes, younger neutrophils) are released into the blood, and a left shift occurs.

The maturation and storage pool constitutes 80% of the myeloid cell population, whereas the mitotic pool usually accounts for 20% of the myeloid series. In contrast with neutrophils, promonocytes and monocytes are released into blood at a relatively young age. This lack of monocyte maturation and storage in the bone marrow explains why monocytes are observed infrequently in most bone marrow aspirates, unless severe neutropenia is present.

Leukocyte production within the bone marrow can be evaluated by bone marrow aspiration for cytology and core biopsy for histopathology. Romanowsky-stained aspirates of marrow allow qualitative and quantitative observations on leukocyte morphology and maturation. Core biopsy provides the best estimation of bone marrow cellularity and detects stromal reactions (e.g., myelofibrosis, granulomatous osteomyelitis). If the bone marrow fails to produce sufficient leukocytes, erythrocytes, or platelets, then leukopenia, anemia, or thrombocytopenia (or a combination thereof) results. In contrast, accelerated production of these cellular elements produces leukocytosis, polycythemia, thrombocytosis, or a combination thereof.

> NOTE: The production of segs from bone marrow myeloblast takes approximately 6 days in the dog and cat but shifts from the marginal pool, or increased release from the storage pool can influence the number of blood neutrophils within minutes to hours.

Neutrophil Circulation

When granulocytes and monocytes are released into the blood, they distribute between circulating and marginal cell pools, circulate for a brief period of time, and emigrate from blood vessels into tissues. In the blood, the total blood neutrophil pool (TBNP) is subdivided into circulating and marginal cell pools. Neutrophils (and other leukocytes) in the total leukocyte count are collected from the circulating pool, which encompasses the mainstream or central axial flow of blood within vessels. The marginal neutrophil pool is a "hidden" population associated with the

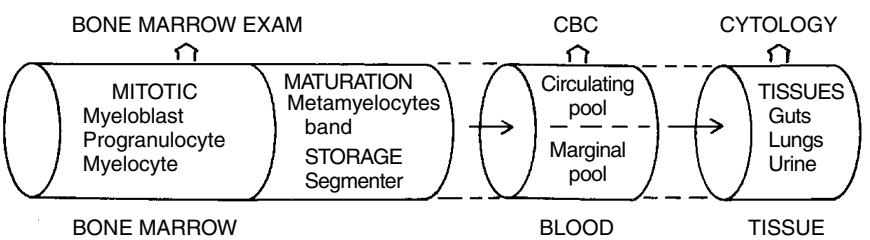

FIGURE 4-3. Neutrophil compartments in the body. Neutrophils in various areas of the body are grouped into pools for evaluation. The bone marrow cells are divided into the mitotic, maturation, and storage pools (see text). Neutrophils in blood are either in the circulating pool, which is sampled by a complete blood count (CBC), or the marginal pool, which is hidden from sampling via the CBC. Neutrophils move in one direction into the tissues, where they can be evaluated by cytology or histopathology. *(Modified from Boggs DR, Winkelstein A:* White cell manual*, ed 3, Philadelphia, 1975, FA Davis.)*

TABLE 4-1. Total Blood Neutrophil Pool, Circulating Neutrophil Pool, and Marginal Neutrophil Pool in Dogs and Cats

	DOG	CAT
TBNP $\times 10^8$/kg	10.2	28.9
CNP $\times 10^8$/kg	5.4	7.8
MNP $\times 10^8$/kg	4.8	21.0

Total blood neutrophil pool *(TBNP)* in cats is larger than in dogs because of a very large marginal neutrophil pool. The relatively large feline marginal neutrophil pool *(MNP)*, compared with that of the dog, allows a larger potential shift of neutrophils into the circulating neutrophil pool *(CNP)* with more dramatic leukocytosis during fear, excitement, or strenuous exercise.

endothelial lining of capillaries, especially the lungs and spleen. As cells continually shift between these pools, the leukocyte count changes. In dogs, circulating and marginal pools are about equal. In cats, the marginal pool is two to three times the size of the circulating pool (Table 4-1). Therefore if neutrophils are mobilized from the marginal pool to the circulating pool in response to fear, excitement, or strenuous exercise, the neutrophil count might double in dogs and triple in cats.

Neutrophil Emigration into Tissues

Neutrophils normally spend about 10 hours in the vascular system before emigrating from the blood vessels into the tissues. Emigration is a random (i.e., nonage ordered) and unidirectional event (these cells do not return to the circulation). In health, neutrophils primarily migrate into the respiratory, digestive, and urinary tracts at a low rate in response to bacteria and other stimuli. Although neutrophils may be visible in respiratory cytologic samples and urine sediment, they are quickly lysed in the septic environment of the lumen of the bowel. In disease, the circulating half-life of neutrophils may be shortened considerably, accompanied by increased cell migration into tissues. In inflammation, excessive tissue neutrophils may be visible as exudate or pus. In diseases such as enteritis, tissue neutrophils may be hidden from cytologic or gross observation; however, increased tissue demand for neutrophils usually is reflected in the leukogram.

The CBC allows quantitative and qualitative observations about leukocytes freely circulating in peripheral blood. The leukogram represents balance of leukocyte production in bone marrow, distribution in the vascular system, and emigration from blood vessels into tissues. One uses cytologic preparations such as smears of exudate; bone marrow, lymph node, and other tissue aspirates; and urine sediment to evaluate leukocytes and infectious agents in tissues.

LEUKOCYTOSIS AND NEUTROPHILIA

Leukocytosis is usually synonymous with neutrophilia. For example, in 232 CBC with a leukocytosis of greater than 17,000 WBCs/μl, 226 (97.4%) had neutrophilia.

Differential Diagnosis of Neutrophilic Leukocytosis

The differential diagnosis of neutrophilic leukocytosis includes inflammation, stress and corticosteroids, exercise and epinephrine, or leukemia (discussed later). An algorithm (Figure 4-4) differentiates the initial three possibilities. Inflammation is most specifically

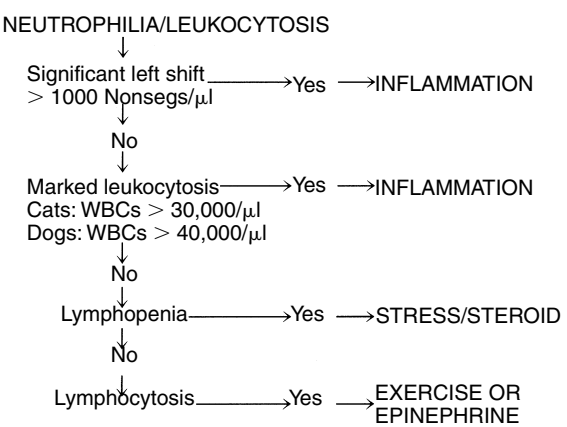

FIGURE 4-4. Evaluation of leukocytosis and neutrophilia. The common causes of neutrophilia and subsequent leukocytosis usually may be differentiated based on the immaturity of the neutrophils, the magnitude of the neutrophilia in the absence of leukemia, and the tendency of the lymphocytes to increase or decrease. Granulocytic leukemia is rare and not considered here. When the laboratory finding is present *(Yes)*, the conclusion to the right is made. When the laboratory finding is absent *(No)*, one moves down to the next differentiating feature. Inflammation may be responsible for total white blood cell *(WBC)* counts of less than 30,000 to 40,000 leukocytes/μl that are associated with lymphopenia because of concurrent stress. Some leukograms lack features to clearly indicate the causes of disease.

identified by the presence of a left shift (an absolute increase in nonsegs). Inflammation may also be suggested by the magnitude of leukocytosis (if it exceeds that expected with corticosteroid or epinephrine-associated conditions). When mild neutrophilia is present without a left shift, the specific cause of leukocytosis may be unclear. In such instances, the absolute lymphocyte count may be useful. Lymphopenia usually indicates an endogenous stress-associated or exogenous glucocorticoid effect, whereas transient lymphocytosis implies an epinephrine or exercise-associated effect. Processes may often occur concurrently, such as both inflammation and stress or corticosteroid treatment.

NOTE: Leukocytosis has several causes: inflammation, glucocorticoid response from stress or treatment, epinephrine response from fear or exercise, and leukemia.

Inflammation

Inflammation is a common and important laboratory diagnosis. Acute inflammation usually causes neutrophilia and is the major consideration for neutrophilic leukocytosis. Neutrophils predominate in suppurative or exudative diseases but may be admixed with other inflammatory cells (e.g., lymphocytes, plasma cells, monocytes and macrophages, eosinophils). Although bacterial infection (i.e., sepsis) commonly causes neutrophilic exudation, purulent to pyogranulomatous inflammation also may occur with certain mycotic, protozoal, and viral infections (especially feline infectious peritonitis). Inflammation also may occur from nonseptic processes such as necrosis (e.g., pancreatitis, pansteatitis), chemical exposure (e.g., turpentine is an experimental method of abscess formation), immune-mediated diseases (e.g., systemic lupus erythematosus, immune-mediated hemolytic anemia [IMHA]), and toxins (e.g., endotoxin, snakebite). Neoplasms may cause inflammation in five ways: (1) predisposing the patient to bacterial infection, (2) damaging normal tissue, (3) outgrowing or damaging the blood supply with subsequent necrosis, (4) ulceration, or (5) producing a paraneoplastic effect wherein tumor products stimulate the bone marrow to produce neutrophils.

NOTE: Inflammation is recognized by a left shift in neutrophils or marked nonneoplastic leukocytosis.

Left Shift

A significant left shift is denoted by the finding of greater than 1000 nonsegs/µl in the presence of a normal neutrophil count or neutrophilia and is diagnostic for the presence of inflammatory disease (see Figure 4-4). Milder left shifts (i.e., 300 to 1000 nonsegs/µl) occur in hemorrhagic, chronic, or granulomatous diseases. Absolute number of nonsegs and their state of immaturity indicate the severity of the left shift. Immature nonsegs observed in blood include bands (stabs), metamyelocytes (juveniles), myelocytes, and promyelocytes (see Color Plate 2F). Bands usually constitute most of the left shift because release of neutrophils from bone marrow is an age-ordered process. Neutrophils younger than bands indicate an increasingly severe left shift associated with increasingly intense inflammation. Classification of immature neutrophils on blood smears is very subjective; therefore one should not overinterpret small changes from reference values or from day to day. If several myelocytes and metamyelocytes are found, one should report the number of metamyelocytes, myelocytes, and promyelocytes (not simply group them under the heading of *nonsegs*) to indicate severity of the left shift. Detectable numbers of blast cells (i.e., myeloblasts) or irregular maturation patterns may suggest granulocytic leukemia (discussed later).

NOTE: A left shift is an absolute and clinically significant increase in immature neutrophils and is the most specific indicator of inflammation.

Leukogram Changes in Inflammation

Figure 4-5 gives a basic pattern of leukogram changes to expect during an inflammatory disease. Individual animals will vary from this pattern in response to drug administration or to variations in disease intensity. The greatest left shift would be expected in early stages of the disease process. As the preexisting bone marrow maturation and storage pool is depleted of segs, then neutrophil bands

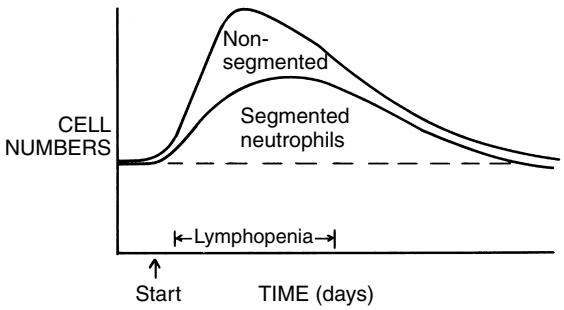

FIGURE 4-5. Expected leukocyte changes with resolving inflammation. The greatest leukocytosis and left shift are expected early in acute inflammation. This period is also accompanied by the lymphopenia of stress. During later phases of inflammation, a more mature form of neutrophilia is expected, because bone marrow hyperplasia and marrow production of neutrophils should be adequate to allow maturation of neutrophils before release into blood. Tissue demand for neutrophils also tends to decrease during recovery.

and metamyelocytes are released. Over ensuing days, myeloid hyperplasia within the bone marrow expands neutrophil production. If neutrophil production and maturation time are sufficient, mainly mature cells are released into the blood and the severity of the left shift should diminish. If tissue inflammation stabilizes at a persistent low-to-moderate level, the bone marrow should reach a production rate sufficient for most neutrophils to mature before release. Thus chronic inflammation may be characterized by little to no left shift and minimal to absent leukocytosis. This form of chronic inflammation is the hardest to identify by CBC data alone. Cytology of the affected tissue may detect low-grade purulent inflammation or exudation.

NOTE: The second most common diagnosis from a leukogram is that inflammation is present in the animal. The leukogram also characterizes the type and severity of the inflammatory disease.

A normal leukogram appearance need not exclude inflammatory diseases, especially if inflammation is mild, chronic, or only involves a surface (e.g., cystitis). No test is 100% sensitive. Increased rouleaux (see Chapter 2) in canine blood smears or fever suggests inflammation. Increased rouleaux formation usually is associated with production of acute phase proteins such as fibrinogen.

Bone Marrow Response During Inflammation

Myeloid hyperplasia of the bone marrow is expected with inflammation of greater than 2 to 3 days' duration. Leukocytosis and left shift are usually present, so a bone marrow sampling is not needed to prove the marrow is active. Myeloid hyperplasia in bone marrow aspirates usually is identified while one is investigating another hematologic problem, such as nonregenerative anemia, thrombocytopenia, or suspected leukemia (see Chapter 2). In these situations, concurrent inflammation and infection, necrosis, or generalized bone marrow stimulation may cause myeloid hyperplasia.

Prognosis

Magnitude of the neutrophilic leukocytosis or neutropenia during inflammation reflects the balance of bone marrow production and tissue demand for these cells. If the bone marrow is responding typically to an inflammatory process with a mild-to-moderate left shift, prognosis is relatively good. Leukocytosis in dogs is usually less than 40,000 WBCs/μl. In 182 canine CBC with leukocytosis, 151 CBC (83%) had 17,500 to 39,990 WBCs/μl. Leukocytosis in this range suggests a favorable prognosis. Only 5% had marked to extreme leukocytosis of 61,050 to 127,500 cells/μl. Leukemoid reactions in this range indicate a poor prognosis. The magnitude of feline leukocytosis is usually less than in dogs (i.e., 70% of cases are < 30,000 WBCs/μl). Obviously, other factors not derived from the CBC data, such as the cause and site of inflammation, also affect the prognosis.

Criteria suggesting a poor prognosis are summarized in Table 4-2. A degenerative left shift, leukopenia and neutropenia, leukemoid reaction, or a combination thereof is an atypical, unexpected response to inflammatory disease indicating severe disease, inadequate bone marrow production, problems interfering with an effective response, or a combination of these. *Lymphopenia* indicates stress. Severe or persistent lymphopenia indicates severe or persistent stress. The most common, current definition of a degenerative left shift is that nonsegs exceed the number of segmenters, regardless of total leukocyte count. Finding *more nonsegs than circulating* segs indicates that the bone marrow cannot produce neutrophils at a rate sufficient for

TABLE 4-2. Leukogram Findings Considered Poor Prognostic Indicators

FINDING	REASON FOR POOR PROGNOSIS
Degenerative left shift	Tissue demand exceeds bone marrow's production of neutrophils or causes inadequate time for maturation of neutrophils
Leukopenia	Tissue demand exceeds bone marrow's production of neutrophils
Leukemoid reaction	Even excessive neutrophils cannot correct the cause
Significant toxic neutrophils	Moderate-to-many, moderately to severely toxic neutrophils indicate toxemia, often gram-negative sepsis
Severe or persistent lymphopenia	Indicates severe stress or lack of relief from stress

them to mature properly. Either cell production is decreased, tissue demand for neutrophils has escalated dramatically, or both; a guarded prognosis is indicated. Severity of change and trend over daily hemograms are important in assessing prognosis. A degenerative left shift, severe neutropenia and leukopenia, lymphopenia, and *marked toxic change* in most neutrophils suggest a poor prognosis and usually gram-negative sepsis.

In *severe leukopenia* (e.g., < 1000 WBCs/µl), an increase in the absolute number of immature neutrophils may not be observed. Both leukopenia and neutropenia are unfavorable prognostic signs. These findings suggest that bone marrow is incapable of producing sufficient numbers of neutrophils, that tissue consumption of neutrophils is overwhelming, or both. Neutropenia, either primary or secondary, severely predisposes the patient to infection and septicemia.

A *leukemoid reaction* is a marked leukocytosis (≥ 50,000 to 100,000 WBCs/µl) as the result of inflammation. The magnitude of leukocytosis is leukemia-like. A leukemoid reaction indicates a poor prognosis, because despite abundant (and actually excessive) neutrophils, the inflammatory disease is not being corrected. Causes of leukemoid reactions include severe localized infections (e.g., pyometra, abscess), IMHA, paraneoplastic syndromes with bone marrow stimulation (e.g., metastatic fibrosarcoma, renal carcinoma, rectal adenoma), rare parasitism (e.g., *Hepatozoon canis* infection), and neutrophil functional defects (canine leukocyte adhesion protein deficiency [CLAD] of Irish setters) (Latimer, 1995; Latimer, Campagnoli, and Danilenko, 2000). With pyometra and some walled off abscesses, there is an anatomic problem. Infection and pus cannot drain from the body, and antibiotics may not penetrate the lesion. With CLAD, dysfunctional neutrophils are incapable of correcting common infections even in high numbers. The leukemoid reaction in IMHA seems an exception, but acute destruction and phagocytosis of erythrocytes with hypoxic tissue damage is a strong stimulus for an inflammatory reaction.

Differentiation of a leukemoid reaction from chronic granulocytic leukemia is difficult. The presence of one of the typical inflammatory diseases associated with a leukemoid reaction should be documented initially. Leukocytosis should resolve if the cause of inflammatory disease is discerned and treated appropriately. A guarded prognosis is indicated until the possibility of granulocytic leukemia is excluded and the leukocytosis begins to resolve after treatment. Leukemoid reactions lack the blast cells or atypia that occurs in acute granulocytic leukemia (see the discussion of myeloproliferative disease later in this chapter). Left shifts in leukemoid reactions usually involve bands and metamyelocytes (perhaps even with myelocytes and promyelocytes). Signs of toxicity are common.

NOTE: A guarded to poor prognosis is indicated by the presence of a degenerative left shift, leukopenia, leukemoid reaction, or moderate-to-marked neutrophil toxicity.

Leukoerythroblastic Reaction

The leukoerythroblastic reaction is uncommon but mimics erythroleukemia. A leukoerythroblastic reaction occurs when immature erythroid and myeloid cells are released into circulation in various diseases (e.g., bone marrow necrosis, severe hemolytic or blood loss anemia, hemangiosarcoma, extramedullary hematopoiesis, bone marrow metastatic disease) that disrupt the bone marrow-blood barrier, resulting in premature hematopoietic cell release into the blood.

Toxic Neutrophils

Toxic neutrophils (see Chapter 2) indicate toxemia and commonly accompany severe inflammation and infection. Bacterial toxins cause the most severe toxic changes in neutrophils; however, nonbacterial toxins (e.g., chemotherapeutic agents) also may be responsible.

TABLE 4-3. Leukocyte Changes in a Dog Treated with Dexamethasone

	FRIDAY	SATURDAY	MONDAY	TUESDAY	WEDNESDAY
WBCs/μl	12,200	22,200	19,600	31,100	29,300
Segs/μl	9525	18,648	10,976	26,433	25,491
Bands/μl	0	0	196	0	0
Lymphocytes/μl	1905	1998	5096	1866	879
Monocytes/μl	635	1554	2156	2799	3132
Eosinophils/μl	635	0	1176	0	0

Hematologic data are from an apparently normal dog treated daily with dexamethasone (except on Sunday) to illustrate the corticosteroid and stress response. Data for Friday, the first day before treatment, should be used for baseline (reference) values. Exceptions to the classic pattern occurred every day except Wednesday.
WBCs, White blood cells.

Classification of toxemia is based on the number of neutrophils affected (percentage; or few, moderate, many) and severity of morphologic change (1+ to 4+ toxic change). A few (1+) toxic neutrophils are of minimal importance, but moderate-to-many (2+ to 4+) toxic neutrophils are a poor prognostic sign (see Color Plate 2F). For example, severely toxic neutrophils in a young dog with bloody diarrhea suggest parvovirus infection. Abundant bacterial toxins absorbed from the damaged bowel promote toxic neutrophil changes. Toxic changes in neutrophils sometimes are the only indicator of disease (the rest of the leukogram may appear normal) and are one reason why one should always look at the blood smear.

Stress and Corticosteroid Response

Stress and corticosteroid administration are common causes of neutrophilia. The classic, acute leukogram pattern is moderate leukocytosis with mature neutrophilia, lymphopenia, and eosinopenia. In dogs, mild-to-moderate monocytosis also may occur (e.g., 2500/μl). Leukograms that indicate changes secondary to endogenous or exogenous steroids are common. Leukocytosis from corticosteroid treatment in dogs may reach 30,000 to 40,000 cells/μl, with a predominance of neutrophils. The typical response is 15,000 to 25,000 WBCs/μl (Duncan, Prasse, and Mahaffey, 1994).

Neutrophilia develops in 4 to 12 hours and returns to baseline values in less than 24 hours. In cats, leukocytosis usually is a little weaker (e.g., 22,000 WBCs/μl with 18,000 neutrophils/μl), and monocytosis is not present. The chronic hematologic pattern from hyperadrenocorticism and long-term corticosteroid treatment varies (including normal), but expected changes include lymphopenia, eosinopenia, and a normal neutrophil count (Latimer, 1995; Moore, Mahaffey, and Hoeing, 1992). Coexisting processes may have opposing stimuli (e.g., a dog may have a disease stimulating eosinophilia and be stressed or treated with corticosteroids and thus have a variable eosinophil count).

> NOTE: Lymphopenia of stress and corticosteroid treatment is the most common alteration in the leukogram of dogs and cats.

The effects of corticosteroid treatment on the canine leukocyte response are better understood by considering Tables 4-3 and 4-4. After corticosteroid exposure, the TBNP expands because of increased release of neutrophils from the bone marrow into the blood and decreased emigration of neutrophils from the blood into the tissues (see Table 4-4). In addition, neutrophils are shifted from the marginal pool (where they cannot be counted) to the circulating pool (where they are quantitated by the WBC count). A left shift is not expected with stress or corticosteroid treatment. Nuclear hypersegmentation of neutrophils (called a *right shift*) is more likely because corticosteroids decrease emigration of and prolong the circulating half-life of neutrophils in the blood (see Table 4-4). As neutrophils age, progressive nuclear hypersegmentation or lobulation develops. Hypersegmented neutrophils have five or more nuclear lobes. Corticosteroids also increase the bone marrow release rate of neutrophils, which is a major cause of the leukocytosis. However, this effect is usually too mild to stimulate release of bands and metamyelocytes in the presence of a normal bone marrow storage pool of neutrophils. A left shift indicates a depleted bone marrow

TABLE 4-4. Effects of Cortisone on Canine Granulocytes

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storage pool of neutrophils and significant tissue demand for neutrophils secondary to concurrent inflammation.

All expected corticosteroid-induced changes may not be present in every leukogram. A "best fit" approach is used to classify leukograms. For example, Table 4-3 presents hematologic data from a healthy dog treated repeatedly with dexamethasone. One day after initial treatment (Saturday), five of the expected steroid and stress features occurred (i.e., leukocytosis, neutrophilia, no left shift, eosinopenia, monocytosis). Lymphopenia was not present, although usually this is the most consistent change. On day 3 (Monday), lymphocytosis, monocytosis, and eosinophilia best resemble physiologic leukocytosis. Only on day 5 (Wednesday) is the classic corticosteroid or stress pattern observed. Some of the variation may be the result of the time between treatment with dexamethasone and collecting the blood sample. Maximal leukocyte changes occur at 4 to 12 hours and may be normalized by 24 hours.

NOTE: Corticosteroid treatment will cause a neutrophilia, lymphopenia, eosinopenia, and (in dogs) monocytosis. The magnitude of changes will vary during a 24-hour period after treatment.

Exercise and Epinephrine Response

Transient physiologic leukocytosis is noted mainly in young, healthy cats during epinephrine release from fear or after strenuous exercise (e.g., struggling during venipuncture). The TBNP remains unchanged, but a shift of cells occurs from the marginal to the circulating neutrophil pool. Subsequently, neutrophilic leukocytosis is detected. No increased release of neutrophils from the bone marrow, nor decreased emigration of neutrophils from the capillary beds is seen. Physiologic leukocytosis in cats is greater in magnitude than in dogs because cats have a larger marginal neutrophil pool (three neutrophils in the marginal pool for every neutrophil in the circulating pool; see Table 4-1). These pool shifts in cats are significant; the WBC count often reaches 20,000/µl, and neutrophilia may be overshadowed by lymphocytosis (6000 to 15,000/µl) (Duncan, Prasse, and Mahaffey, 1994). Dogs have such weak physiologic leukocytosis that it is not recognized clinically because values likely remain within reference ranges. It is best seen in research dogs that are routinely bled and have a record of consistent hemograms and then are suddenly exercised or frightened so that the mild increase in WBCs, neutrophils, lymphocytes, and packed cell volume (PCV) can be detected.

NOTE: Physiologic release of epinephrine (fear or vigorous activity) causes an immediate, mild-to-moderate increase in neutrophils and lymphocytes that should subside gradually over 30 to 60 minutes.

LEUKOPENIA AND NEUTROPENIA

Leukopenia and neutropenia occur infrequently in dogs and cats and constitute a poor prognostic sign. The two most likely causes of neutropenia are (1) excessive tissue consumption of neutrophils during severe inflammation and (2) disrupted bone marrow production (Table 4-5). (See also the discussion of bone marrow in Chapter 2

TABLE 4-5. Major Causes of Neutropenia

	ANIMALS AFFECTED
Consumption of Neutrophils	
Overwhelming sepsis/endotoxemia (important)	Dogs/cats
Parvovirus enteritis (important)	Dogs/cats
Salmonellosis	Dogs/cats
Immune-mediated destruction (rare)	Dogs
Bone Marrow Suppression	
Feline leukemia virus (FeLV) (important)	Cats
Feline immunodeficiency virus (FIV)	Cats
Parvovirus (important)	Dogs/cats
Ehrlichiosis	Dogs
Bone marrow toxicity	Dogs/cats
Estrogen (endogenous/exogenous)	Dogs
Phenylbutazone*	Dogs/cats
Cancer chemotherapy	Dogs/cats
Irradiation	Dogs/cats
Leukemia (important)	Dogs/cats
Myelophthisis/myelonecrosis	Dogs/cats
Immune-mediated destruction of neutrophil precursors (rare)	Dogs/cats

*Incomplete list of other drugs is in text.
Courtesy of Dr. M.D. Willard.

and the section on myeloid hypoplasia, discussed later.) A third cause of neutropenia, only documented in experimental settings, is a temporary shift of neutrophils from the circulating to the marginal pool where they cannot be counted. Endotoxin can cause this change. This transient form of neutropenia is actually "pseudoneutropenia," because the TBNP is unchanged. Rarely, immune-mediated or "steroid-responsive" neutropenia occurs with lupus or some drugs.

During excessive tissue utilization of neutrophils, neutropenia is severe; this stimulates release of very immature neutrophils, even myelocytes and promyelocytes, from the bone marrow. A clinically important left shift is present when 10% or more of the total neutrophil population are bands or younger forms of neutrophils. The left shift is degenerative when bands and younger forms of neutrophils outnumber the segmenters. An inflammatory process involving a large surface area, such as septic peritonitis, enteritis, or septicemia, tend to cause severe neutropenia and leukopenia. Gram-negative bacterial infections are frequently associated with consumptive neutropenia. When a severe left shift, marked toxic change, and developing leukopenia occur, gram-negative sepsis should be suspected. In contrast, localized infections (abscess or pyometra) with pyogenic bacteria usually cause leukocytosis.

Hemograms may differentiate leukopenia of overwhelming infection from that of primary bone marrow disease. Neutrophils have a short life span in blood, and their numbers should decrease before anemia or thrombocytopenia develop. This is usually the pattern in severe inflammation or overwhelming infection. Pancytopenia or bicytopenia suggests primary bone marrow disease, but neutropenia of overwhelming inflammation may coexist with anemia or thrombocytopenia of other causes (e.g., hemorrhage, disseminated intravascular coagulation, or both). When the clinician is unsure of the problem, bone marrow aspiration and core biopsy are indicated to evaluate hematopoiesis. Time course of the disease process may assist in differentiating these two causes of neutropenia. Primary bone marrow disease may develop insidiously, whereas consumptive neutropenia develops rapidly. A left shift, toxic changes in neutrophils, and rouleaux formation suggest neutropenia as the result of severe inflammation or infection.

Severe, primary neutropenia can predispose to septicemia. Neutropenia and leukopenia occur with myelotoxicity during cancer chemotherapy. Neutrophil counts are often lowest 5 to 7 days after initiation of treatment. Guidelines may predict sepsis (Couto, 1985). Neutrophil counts of less than 2000 cells/µl require monitoring the patient for sepsis. Sepsis (probably from enteric bacteria) is presumed to be present if the patient has less than 500 neutrophils/µl and is febrile. Furthermore, chemotherapy with myelosuppressive agents should be discontinued if the neutrophil count is less than 2500 cells/µl or the platelet count is less than 50,000/µl.

Bone Marrow Diseases Causing Neutropenia

Persistent, unexplained neutropenia is an indication for bone marrow aspiration biopsy, core biopsy, or both. Possible causes of decreased myelopoiesis include decreased production of neutrophils (i.e., myeloid hypoplasia, ineffective granulopoiesis, bone marrow necrosis) or bone marrow lesions (i.e., myelofibrosis, disseminated granulomatous infection, leukemia) that displace normal hematopoietic tissue (see Chapter 2). A cause (e.g., estrogen or phenylbutazone toxicosis) is infrequently discovered by examination of bone marrow; however, one may determine whether myeloid hypoplasia or myelophthisic disease is responsible.

Ineffective granulopoiesis is suggested when leukopenia is accompanied by normal to increased numbers of developing myeloid cells in the bone marrow (in the absence of overwhelming infection). Cells are destroyed in the bone marrow ("recycled") before maturation and so are not released into the blood. This situation occurs to a small degree in healthy dogs (i.e., the myelocyte sink) but is exaggerated in diseases such as canine parvovirus infection and feline leukemia virus (FeLV) infection. Approximately 50% of FeLV-positive, neutropenic cats have marked granulocytic hyperplasia with a shift to immaturity, whereas 50% have myeloid hypoplasia suggesting viral destruction of hematopoietic tissue. Persistent neutropenia despite myeloid hyperplasia in FeLV-infected cats indicates ineffective granulopoiesis, provided increased tissue consumption of neutrophils is not responsible for the neutropenia. Ineffective granulopoiesis may also be a drug idiosyncratic reaction (e.g., phenobarbital).

Leukopenia and neutropenia may also be the result of damage or depletion of myeloid cells (i.e., myeloid hypoplasia) (see Chapter 2). Causes of myeloid hypoplasia include parvovirus infection, endogenous and exogenous estrogen toxicosis in dogs, *Ehrlichia canis* infection, cancer chemotherapy, irradiation, and idiosyncratic reactions to drugs such as phenylbutazone, trimethoprim-sulfadiazine, or chloramphenicol (see Chapter 3). Although immune-mediated neutropenias have not been well documented in dogs and cats, steroid-responsive neutropenias have occurred in both species. Artifactual neutropenia may occur from leukocyte aggregation after exposure to ethylenediaminetetraacetic acid (EDTA) anticoagulant or by obtaining diluted blood specimens from intravenous fluid administration lines.

Parvovirus Infection

Parvovirus infection may be associated with diarrhea, vomiting, and leukogram abnormalities that may include leukopenia, neutropenia with severe toxic changes, and lymphopenia. *Parvovirus* infects and destroys rapidly dividing cells (e.g., intestinal crypt epithelium, lymphoid tissue, hematopoietic cells). This tissue predilection may cause enteritis (with or without bloody diarrhea) or leukopenia. Massive neutrophil exudation into the damaged gut contributes to leukopenia. Leukopenia is transient and usually occurs early in canine parvovirus disease, so it may be missed without multiple CBC. Mast cells from the inflamed gut may be detected in blood smears. Neutrophilic leukocytosis is expected with recovery. During periods of intense granulopoiesis, a leukemoid reaction plus some disturbance of normal cell maturation may occur. With complete clinical recovery, baseline leukogram values are regained. Antemortem diagnosis of parvovirus enteritis is accomplished most easily by detection of virus in feces (see Chapter 9). Panleukopenia in cats has a similar hematologic response.

Feline Leukemia Virus Infection

FeLV infection causes various disorders (see Chapter 15) such as cytopenias, including neutropenia (Brown and Rogers, 2001). FeLV viremia is diagnosed mainly by enzyme-linked immunosorbent assay (ELISA) procedures and indirect fluorescent antibody (IFA) examination of blood smears or buffy coat smears. If ELISA testing for FeLV group-specific antigen is negative but FeLV infection is still suspected, bone marrow aspirates should be examined via IFA to exclude the possibility of sequestered virus.

Feline Immunodeficiency Virus Infection

Feline immunodeficiency virus (FIV) infection may be associated with cytopenias (i.e., neutropenia, anemia, thrombocytopenia) (Shelton, Linenberger, Abkowitz, 1991). Many diseases have been associated with FIV, including various infections, malignancies, lymphadenopathy, colitis, and central nervous system disorders. Diagnosis of FIV involves ELISA and Western blot procedures available at various laboratories (see Chapter 15).

Cyclic Hematopoiesis

Cyclic hematopoiesis (i.e., gray collie syndrome, cyclic neutropenia) is an autosomal recessive disease characterized primarily by cyclic neutropenia with 11- to 12-day cycles described originally in silver-gray collie pups. Neutropenia as severe as 0 to 400/µl predisposes affected collies to life-threatening bacterial infections. A stem cell defect causes cyclic decreases in production of platelets, other granulocytes and monocytes, and erythrocytes (i.e., reticulocytes). Because of a longer half-life of platelets and erythrocytes,

numeric change in these elements is less noticeable than for neutrophils. Cyclic hematopoiesis, often with a more irregular periodicity, also has been observed in other breeds of dogs, in rare cats with FeLV infection (Swenson et al, 1987), and after cyclophosphamide treatment of some dogs. Oscillations of neutrophils, other leukocytes, reticulocytes, and platelets occur at 8- to 29-day intervals.

Hemograms obtained at 2- to 3-day intervals should document cyclic neutropenia, but daily CBC may be required to document cycling of other cells and platelets.

MONOCYTOSIS AND MONOCYTOPENIA

Monocytosis occurs in about 30% of hospitalized dogs and 11% of cats. Blood monocytes replenish macrophages in tissues. Tissue macrophages include Kupffer's cells in hepatic sinusoids, pulmonary alveolar macrophages, dendritic cells in lymph nodes, and microglial cells in the brain (in addition to histiocytes and multinucleate giant cells occurring in response to inflammation). Maturation from blood monocytes to tissue macrophages is accompanied by changes in cellular morphology, metabolic activity, enzyme content, and synthesis of biologically active proteins.

Macrophages remove necrotic debris, kill fungi and some parasites, inactivate viruses, respond to foreign bodies, phagocytose senescent and abnormal red blood cells (RBCs), and destroy neoplastic cells. Monocytosis is expected in inflammatory diseases with a high need for macrophages. In IMHA, for example, RBCs coated with antibody or complement are being destroyed continuously. Necrotic cell debris also must be removed to allow tissue regeneration and healing. Although macrophages are a "late" component of most inflammatory processes, monocytosis may occur in both acute and chronic disease processes. Monocytosis also may accompany suppuration, pyogranulomatous and granulomatous inflammation, necrosis, malignancy, hemolytic or hemorrhagic disease, or immune-mediated diseases. Rarely, monocytosis is the only leukogram change in dogs with sepsis or bacterial endocarditis.

Differential diagnosis of monocytosis includes canine response to corticosteroids (see the discussion on stress and corticosteroid response, earlier) and disorders requiring macrophages. Extreme monocytosis may indicate leukemia. If concurrent lymphopenia and eosinopenia are present, a stress or corticosteroid leukocyte response is likely. If lymphopenia and eosinopenia are not present, chronic inflammation or tissue destruction should be suspected.

Monocytopenia is not significant. Low numbers of monocytes normally are present in the blood. Monocytes are unevenly distributed on the blood smear, so they may be undercounted on a 100-cell leukocyte differential count. Uneven monocyte distribution is a greater problem on glass wedge smears than on coverslip smears. Automated cell hematology analyzers often detect more monocytes than microscopic counts.

LYMPHOCYTOSIS

Lymphocytes uniquely retain the ability to divide and recirculate between blood and tissues. Lymphocytosis may be transient (15 to 30 minutes in duration) or persistent. Transient lymphocytosis occurs in physiologic leukocytosis (discussed earlier). Persistent nonneoplastic lymphocytosis usually signifies strong immune stimulation from chronic infection, viremia, immune-mediated disease, or recent immunization. Supportive laboratory evidence of chronic infection (in addition to history and physical findings) may include hyperproteinemia with polyclonal gammopathy; presence of "reactive" lymphocytes; CBC evidence of inflammation; or cytologic or histologic documentation of inflammation, lymph node hyperplasia, or both. Differentiation of persistent extreme lymphocytosis from chronic lymphocytic leukemia (CLL) is discussed later in the chapter.

Reactive Lymphocytes and Blast-Transformed Lymphocytes

Reactive lymphocytes are large, immune-stimulated lymphocytes with dark-blue cytoplasm and irregular, scalloped, or cleaved nuclei. They are also called *immunocytes*. In contrast, blast-transformed lymphocytes have a large nucleus with light, dispersed chromatin pattern with prominent nucleoli or nucleolar rings. Rare reactive lymphocytes are visible in blood smears from healthy animals, whereas a few to several reactive lymphocytes may occur in blood smears from sick or recently vaccinated animals. Reactive lymphocytes are not of special diagnostic significance. The number

of reactive lymphocytes does not consistently reflect the degree of immune stimulation, nor is it pathognomonic for any specific disease. Reactive blast-transformed lymphocytes may appear unusually numerous or active (especially in young animals) and have been mistaken for both lymphoid leukemia and presumed lymphoma with a leukemic blood picture.

LYMPHOPENIA AND EOSINOPENIA

Severe stress or exogenous corticosteroid administration (see Stress and Corticosteroid Response) usually causes lymphopenia and eosinopenia. Lymphopenia is expected early in acute, severe, stressful disease (see Figure 4-5), and the return of the lymphocyte count to normal is a good prognostic sign indicating recovery from the stress of disease. Loss of lymphocyte-rich lymph may cause lymphopenia. Examples include chylothorax in dogs and cats, protein-losing enteropathy and lymphangiectasia in dogs, and disruption of normal lymphatic circulation by inflammatory, infectious, or neoplastic diseases in both species (Latimer, 1995). Lymphopenia occurs in some viral diseases by direct viral damage of lymphoid tissue and through lymphocyte redistribution from stress or antigen exposure. Canine viral diseases causing lymphopenia include distemper, infectious canine hepatitis, parvoviral enteritis, and coronavirus enteritis. Cats may experience lymphopenia in panleukopenia and FeLV infection.

Age affects the lymphocyte count. Younger animals usually have higher counts. For example, the minimal lymphocyte counts expected in dogs of various age categories are 2000/µl from 3 to 6 months of age; 1500/µl from 8 to 24 months of age; and 1000/µl over 24 months of age. Thus one uses different lymphocyte counts to indicate lymphopenia in young animals.

Eosinopenia may be difficult to document by routine WBC counts. These cells may not be observed in feline leukocyte differential counts in health and may account for only 2% of healthy canine leukocyte differential counts. Variation expected with a 100-cell, manual differential leukocyte count when 2% of a cell type is present is 0% to 8% (95% confidence interval). The clinical impression of true eosinopenia in a sick animal is solidified if concurrent lymphopenia is present. The most common causes of eosinopenia are severe stress associated with illness and response to corticosteroid administration. Fictitious eosinopenia of greyhounds and other dogs may result from misidentification of "vacuolated or gray" eosinophils. Instead of having red-orange granules, affected eosinophils appear to have clear vacuoles.

EOSINOPHILIA

General Comments

Eosinophilia usually indicates eosinophilic inflammation somewhere in the body, though intense tissue infiltration with eosinophils may be unaccompanied by detectable eosinophilia because eosinophils have a much shorter half-life in blood than in tissues. Eosinophils kill parasites, regulate the intensity of hypersensitivity reactions mediated by immunoglobulin E (IgE) antibodies, and may promote inflammation and tissue damage (Center et al, 1990; McEwen, 1992). Eosinophils kill parasites by attaching to them and forming a digestive vacuole between the eosinophil and parasite. Eosinophils degranulate potent molecules such as major basic protein and eosinophil peroxidase, which damage the wall of the parasite or ova. More intense eosinophilic response occurs with parasites within tissues (e.g., heartworms, strongyles, migrating lung flukes such as *Paragonimus kellicotti)*. Endoparasites, such as *Giardia* species or tapeworms that do not invade tissue generally do not incite eosinophilia. Some parasites may not stimulate eosinophilia until they die and expose previously hidden antigens. Production of eosinophilia is an immune response. The first exposure to a parasite produces a modest, delayed eosinophilia. The second exposure to the same parasite results in an intense, dramatic eosinophilia.

The inflammatory response to certain allergens is similar to that for tissue-invasive parasites. Lymphocytes respond to the allergen by producing an IgE-type immune response. Eosinophils stimulated to degranulate may destroy normal tissue. For example, eosinophilic inflammation from inhaled allergens in feline asthma may cause damage to respiratory epithelium.

Eosinophils are attracted to inflamed tissue by mast cell products and lymphokines. Eosinophils may be visible in mast cell tumors and lymphoma. The interaction among lymphocytes, mast cells, and eosinophils is extensive. Although mediators of each cell type act locally or on the bone marrow,

T lymphocytes play a major role in eosinophil production and maturation via interleukin-5 production.

Diagnostic Approach

One should consider parasitic and allergic processes first when identifying the cause of eosinophilia (Figures 4-6 and 4-7) (Center et al, 1990; Latimer, 1995). Less frequent causes include neoplasia (e.g., mast cell tumor, lymphoma, mucinous carcinomas, fibrosarcomas, eosinophilic leukemia), fungal infection (e.g., zygomycosis, cryptococcosis), viral infection (e.g., FeLV), or bacterial infection (e.g., *Streptococcus* or *Staphylococcus* species). The hypereosinophilic syndrome can produce severe, persistent circulating and tissue eosinophilia.

Mast cells are numerous near the surface of the skin, digestive tract, respiratory tract, and genitourinary tract; therefore eosinophilia can also be evaluated via a body system approach. The integumentary system is easily examined for ectoparasites, dermatitis, or masses. Flea bite allergy is a common cause of eosinophilia in cats. The respiratory tract may be evaluated cytologically by transtracheal wash, bronchial brushings, swabs, or collecting material adherent to endotracheal tubes. Heartworm disease and pulmonary

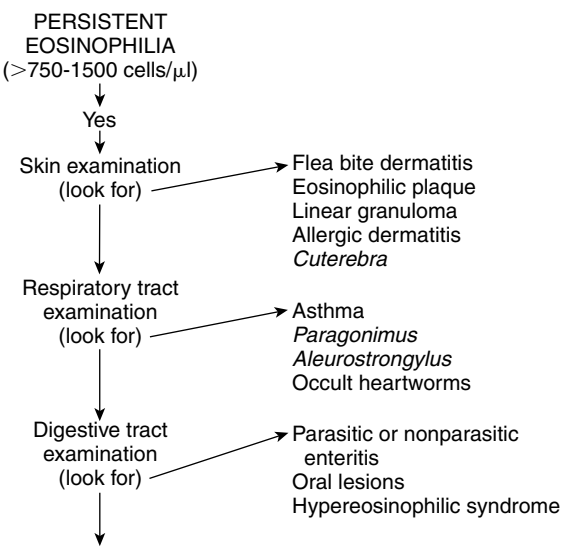

FIGURE 4-6. Evaluation of feline eosinophilia. An algorithm to diagnose common causes of eosinophilia in cats.

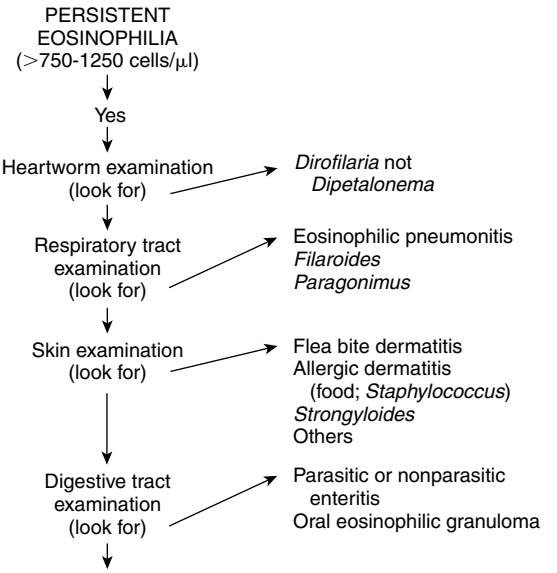

FIGURE 4-7. Evaluation of canine eosinophilia. An algorithm to diagnose common causes of persistent eosinophilia in dogs.

infiltrates with eosinophils (PIE) are common causes of eosinophilia in dogs. Fine-needle aspiration or surgical biopsy may aid in evaluating dermal or pulmonary masses. Examination of the upper alimentary tract should include visual inspection of the oral cavity for eosinophilic granulomas, especially the eosinophilic granuloma complex of cats and Siberian husky dogs. Fecal ova examination is inexpensive, though the presence or absence of ova is usually unrelated to the primary cause of eosinophilia. If eosinophilic gastroenterocolitis is suspected, consider endoscopic examination, surgical biopsy, and cytologic evaluation of any lesions. Estrus in dogs occasionally causes eosinophilia.

BASOPHILIA

Basophils are rare in normal dogs and cats. Basophilia is the presence of greater than or equal to 2% basophils or repeated detection of any basophils. Basophilia of 2% may be missed. Basophilia must be 3% to 6% to be consistently detected. Basophilia may also be missed because basophils are difficult to identify. Feline basophil granules are numerous, moderately sized, round to oval, and grayish beige to light lavender. This appearance

of feline basophils is unique, and these cells may be mistaken for eosinophils with faded granules or monocytes. Feline eosinophils have red-orange, rod-shaped granules, whereas monocytes have blue-gray cytoplasm that is devoid of distinct granules. Infrequently, feline basophils have one or two dark-staining purple granules that facilitate cell identification. Canine basophils have few, widely scattered purple (i.e., metachromatic) or poorly stained granules. These cells may be misidentified as monocytes. The scarcity of basophils in blood smears may contribute to the lack of confidence in identifying these cells. Automated hematology analyzers (i.e., flow cytometers: Advia 120, Cell Dyn 3500) have not been validated to determine accurately feline and canine basophils on automated differential counts.

Basophils are an integral component of hypersensitivity reactions, as are mast cells. Basophils are involved in hemostasis, lipid metabolism, parasite rejection (e.g., ticks), and tumor cell killing (Latimer, 1995). Basophilia has two major causes: (1) parasitism (especially *Dirofilaria immitis* infection) and (2) hypersensitivity reactions. Occasionally, basophilia is associated with lipemia, canine and feline mast cell tumors, or as an unusual component of granulocytic leukemia (Latimer, 1995; O'Keefe et al, 1987).

ABNORMAL NUCLEAR MORPHOLOGY AND CYTOPLASMIC INCLUSIONS

Hyposegmentation is decreased nuclear segmentation of granulocytes, indicating immaturity. Hyposegmentation most commonly indicates inflammation. Hypersegmentation presents with excessive lobulation, often more than five segments. This nuclear change reflects more mature neutrophils. Hypersegmentation may be the result of prolonged neutrophil time in circulation, which is commonly associated with corticosteroid administration. However, genetic developmental abnormalities, preleukemic or dysplastic conditions, and leukemia may be responsible for nuclear changes. Dysplasia and leukemia will be discussed later in this chapter.

Cytoplasmic inclusions may be associated with toxic change in neutrophils. Infectious agents such as bacteria (i.e., neutrophilic *Ehrlichia*), protozoa (i.e., *Hepatozoon canis*, *Leishmania* spp.), or yeast (i.e., *Histoplasma capsulatum*) are found within the cytoplasm of circulating leukocytes providing a definitive diagnosis. Rarely, genetic diseases are responsible for a variety of cytoplasmic inclusions.

Pelger-Huët Anomaly

Pelger-Huët anomaly (PHA) is an inherited disorder of leukocyte development characterized by nuclear hyposegmentation of neutrophils, other granulocytes, and monocytes. Almost all neutrophils resemble bands or metamyelocytes (i.e., absolute numbers look like a severe degenerative left shift). The characteristic WBC appearance should be recognized and reported to avoid undue concern to clinicians reading the CBC who may otherwise consider inflammation or dysplastic changes associated with leukemia. The nuclear chromatin pattern of the granulocytes appears coarse and mature, and the cytoplasm is clear and devoid of toxic changes. PHA presents as a lifelong "degenerative left shift" but is usually an incidental finding. Neutrophils function normally, and a predisposition to infection has not been demonstrated. PHA occurs in both dogs and cats and is presumed to be inherited in an autosomal dominant manner (Latimer, 1995; Latimer and Robertson, 1994). However, PHA may be inherited in an autosomal incompletely dominant pattern in Australian shepherds, suggesting that the expression of the anomaly is governed by two or more alleles (Latimer, Campagnoli, and Danilenko, 2000).

Bacterial infection, drug treatment, developing granulocytic leukemia, or FeLV infection may cause acquired nuclear hyposegmentation of neutrophils (i.e., pseudoPHA). Bona fide PHA anomaly can be confirmed by finding the anomaly in blood smears of parents, siblings, or other relatives or by proving inheritance of the trait by prospective breeding trials. Persistent nuclear changes on repetitive blood smear analysis after eliminating causes of pseudoPHA allows tentative diagnosis.

Genetic Disease with Cytoplasmic Inclusions

Chédiak-Higashi Syndrome

Chédiak-Higashi syndrome (CHS) occurs as an autosomal recessive trait in Persian cats with yellow-green eyes and a diluted smoke-blue haircoat (Latimer and Robertson, 1994). This anomaly is transmitted in an autosomal recessive pattern. This disorder is associated

with large pink lysosomal granules or cytoplasmic inclusions in neutrophils. Coarse clumping of melanin granules is associated with color dilution of the haircoat and irises. In addition, decreased choroidal pigment causes a red fundic reflex and photophobia in bright light. Blood smear examination and the cat's gross appearance confirm diagnosis of CHS. Neutrophil inclusions are positive on peroxidase and Sudan black B stains. In contrast with other animal species, cats with CHS have no predisposition to infection. Cats with CHS have an increased bleeding time secondary to mild platelet dysfunction, however. Hemostasis may be prolonged slightly after trauma, venipuncture, or elective surgery.

Lysosomal Storage Diseases

Storage diseases are rare inherited enzyme defects that cause accumulation of intermediate metabolites of complex molecules within cellular lysosomes. Depending on the nature of the metabolite and its affinity for Romanowsky-type stains, cellular inclusions may appear purple and stringy, as in mucopolysaccharidosis (MPS) VI (i.e., Maroteaux-Lamy syndrome) of cats or MPS VII of dogs, or may appear as clear vacuoles in leukocytes in cats with lysosomal acid lipase deficiency. The characteristic purple granules of MPS can be differentiated from toxic granulation, because cytoplasmic basophilia is absent and toxic granulation is uncommon in dogs and cats. Lysosomal storage diseases often are associated with progressive central nervous system or skeletal disease but may be identified on a CBC by observing characteristic inclusions or vacuoles in circulating leukocytes.

LEUKOCYTE FUNCTION DEFECTS

Neutrophil dysfunction has been reviewed (Latimer, Campagnoli, and Danilenko, 2000). Neutrophil dysfunction is rare but may be suspected in animals with a history of recurrent infections in association with a normal to extremely elevated neutrophil count and lack of neutrophil migration into sites of infection as determined by cytology. Diagnosis of neutrophil dysfunction requires excluding common causes of immune deficiency and documenting abnormalities in neutrophil adherence, chemotaxis, phagocytosis, bactericidal activity, or a combination thereof. Tests of neutrophil function are labor intensive, expensive, and available only through a few specialists or research laboratories to which the animal must be referred for diagnosis.

Canine CD11/CD18 Adhesion Protein Deficiency

Canine adhesion protein deficiency (i.e., canine leukocyte adhesion molecule deficiency, CLAD, canine granulocytopathy syndrome) occurs infrequently in Irish setter and Irish setter-cross puppies. CLAD is an autosomal recessive disease. Affected puppies have recurrent bacterial infections before 12 weeks of age. Leukogram abnormalities often include a leukemoid reaction (e.g., up to 208,000 cells/μl). Fluids from sites of infection contain few neutrophils, because they are less able to immigrate from the microvasculature to the sites of infection. Neutrophils lack CD11/CD18 adhesion proteins on the plasma membrane surface, which facilitate phagocytosis of organisms and cell emigration from blood vessels (Trowald-Wigh et al, 1992).

Chronic Rhinitis and Pneumonia in Doberman Pinschers

Chronic respiratory disease in eight closely related Doberman pinschers has been attributed to impaired neutrophil bactericidal activity (Breitschwerdt et al, 1987). Neutrophils phagocytize bacteria normally but are unable to kill them. Immunoglobulin concentrations, complement concentrations, and mitogen-stimulated lymphocyte transformation are normal.

Acquired Neutrophil Dysfunction

Acquired neutrophil dysfunction has been reported in a few dogs with poorly regulated diabetes mellitus, pyoderma, demodicosis, protothecosis, and lead toxicosis. In cats, neutrophil dysfunction has been documented infrequently in FeLV infection and feline infectious peritonitis (Latimer et al, 2000).

NEOPLASTIC AND DYSPLASTIC DISEASES OF BLOOD LEUKOCYTES

General Comments

The proliferative responses of leukocytes previously discussed arise from the purposeful need to replace missing circulating cells primarily related to increased utilization or consumption. One evaluates the bone marrow

and interprets these changes as hyperplasia. Other proliferations may be characterized as poorly regulated with abnormal cell development, termed *dysplasia*, or as nonpurposeful, unregulated new growth, termed *neoplasia*. Dysplastic changes may be observed in hematopoietic cells with or without neoplasia. These changes may occur as *preleukemia* (i.e., before the onset of overt leukemia) or be observed as an inheritable condition without consequence.

Inherited dysplastic conditions exist in breeds such as toy and miniature poodles with enlarged erythrocytes and their precursors or cavalier King Charles spaniels with abnormally large platelets related to an autosomal recessive defect. These dogs have no clinical signs and the dysplastic findings are usually incidental. Secondary myelodysplasia is more often associated with drug-induced and nutritional conditions that may be reversible once the inciting cause is removed or associated with certain infections, malignancies, and immune-mediated disease (Table 4-6). Idiopathic or primary myelodysplastic syndrome (MDS) is a clonal disease, indicating it originates from a single transformed stem cell that affects multiple nonlymphoid lineages such as erythroid, granulocytic, and megakaryocytic precursors. Any of the primary or secondary forms of myelodysplasia may progress to leukemia.

Leukemia, literally meaning white blood, is a term that reflects an abnormal population of hematopoietic cells found within the blood or bone marrow as opposed to solid tissues, such as lymph node, spleen, or liver. Lymphoid or myeloid (nonlymphoid) cells may be involved in leukemia. Five causes of leukemia are (1) virus infections, such as FeLV that may affect progenitor cell development; (2) genetic abnormalities inherited or acquired that lead to altered cell growth; (3) defective immune systems, such as with FIV infection that does not permit normal defense mechanisms against leukemia; (4) chemicals (e.g., benzene, therapeutic drugs), which have rarely been associated with leukemia; and (5) radiation that may alter progenitor cells.

Clinical signs of leukemia vary. Common signs include lethargy, pale mucous membranes, anorexia, weight loss, fever, frequent infections, icterus, and abnormal bleeding such as petechiae. Physical findings often include hepatosplenomegaly, lymphadenopathy, or tonsillar enlargement.

Strong laboratory indicators of leukemia include marked leukocytosis of a monomorphic cell type, the presence of many blast cells, or morphologic irregularities in shape or size of late-stage forms. A large buffy coat may be noted when measuring the PCV because of marked leukocytosis or thrombocytosis. Blast cells have a large round nucleus, fine chromatin pattern, and one or more prominent nucleoli. Reactive and blast-transformed lymphocytes are often present in small numbers (e.g., 1 to 5/blood smear) and are not diagnostic. Numerous reactive and blast-transformed lymphocytes may occur in ill, nonleukemic animals (e.g., 1% to 5% of WBC) and may mimic leukemia. Rare mitotic figures do not indicate leukemia because mitotically active cells such as rubricytes, promyelocytes, myelocytes, monocytes, and reactive lymphocytes are encountered in nonleukemic animals. Morphologic irregularities may include developmental changes of *dysplasia*, such as megaloblastic erythroid precursors that have an excessive amount of cytoplasm and unusual nuclear chromatin pattern, enlarged segs with many nuclear lobes (i.e., macropolycytes), enlarged highly vacuolated platelets, or dwarf megakaryocytes, and *asynchronous maturation* wherein maturity of nucleus and cytoplasm differ.

TABLE 4-6. Forms of Myelodysplasia and Associated Conditions

GENERAL CATEGORY	ASSOCIATED CONDITIONS
Congenital dysplasia	Toy and miniature poodles (erythrocytes and precursors) Cavalier King Charles spaniels (platelets) Giant schnauzers (neutrophilic precursors)
Secondary MDS	Drugs: azathioprine, cyclophosphamide, cytosine arabinoside, vincristine, chloramphenicol Infectious agents: FeLV, FIV Nutritional deficiencies: folate, cobalamin receptor defect in giant schnauzers Immune-mediated disease: immune-mediated anemia/thrombocytopenia Malignant diseases: lymphoma, chronic myeloproliferative disease, plasma cell myeloma
Primary MDS	Idiopathic clonal defects

MDS, Myelodysplastic syndrome; *FeLV*, feline leukemia virus; *FIV*, feline immunodeficiency virus.

White cell counts in leukemia are variable from leukocytosis, leukopenia, or even within normal limits. Anemia is often nonregenerative but rarely hemolysis is found in some forms of leukemia. Thrombocytopenia or less commonly thrombocytosis is associated with the other hematologic changes. Laboratory tests used to diagnose hematopoietic neoplasia or myelodysplasia and evaluate the extent of involvement include bone marrow evaluation, biochemical profiles, cytology, serology, and urinalysis, in addition to hematology. If changes in the blood are not diagnostic, bone marrow examination may confirm leukemia. Blast cells are often found in much higher numbers in marrow than in blood. Bone marrow evaluation should involve optimally an aspirate and core biopsy with concurrent peripheral blood evaluation. Cytology of effusions or solid tissue masses help to determine a primary source. Serology is indicated for evaluation of leukemia-causing viruses. Protein evaluation of the serum and urine is helpful in determining the presence of gammopathies associated with lymphoproliferative conditions (see Chapter 12).

NOTE: Leukemia is less common than other causes of alterations in leukocyte numbers and morphology. Stress, inflammation, and reactive lymphocytes are much more common.

When the diagnosis of leukemia is confident, without knowledge of the specific type, many veterinarians stop the workup and recommend euthanasia. The following information is for those choosing to treat leukemic cases and who need a more specific diagnosis for treatment choices. Definitive diagnosis of specific leukemias usually requires a veterinary clinical pathologist. Slide review from routine CBC with Wright's-stained smears of EDTA anticoagulated blood may allow differentiation of the cellular origin, such as lymphoid from myeloid (i.e., nonlymphoid). Evaluation of the bone marrow is often necessary to classify the leukemia, because the blood may not be representative of the degree of leukemic involvement. If cell morphology in a leukemic patient is insufficient to determine the cell lineage, one may use specialized tests (i.e., cytochemistry, electron microscopy, immunocytochemistry) usually conducted at academic institutions or referral laboratories. Cytochemistry involves nonimmunologic cytoplasmic markers for enzymes, lipids, or glycogen within the cell (Raskin and Valenciano, 2000). Enzymes include peroxidase found in certain granulocytes and nonspecific esterases found in monocytes or T lymphocytes (see Table 4-7).

TABLE 4-7. Selected Cytochemical Stains for Identification of Cell Types in Canine and Feline Leukemias

STAIN	CELLS THAT STAIN
Peroxidase	Neutrophils—all stages positive Monocytes—weak positive when present Eosinophils—positive in dog, negative in cat
Sudan black B	Neutrophils—all stages positive Monocytes—weak positive when present Eosinophils—positive in dog, negative in cat
Leukocyte alkaline phosphatase	Neutrophils—myeloblasts, strong Monocytes—monoblasts, weak or rare in dogs Lymphocytes—subset of B cells Eosinophils—between the granules in cat Basophils—weak or occasional staining
Chloroacetate esterase	Neutrophils—strong positive Basophils—moderately positive Mast cells—variably positive Megakaryocytes—weak positive when present
Alpha-naphthyl butyrate esterase	Monocytes—diffuse positive Lymphocytes—focal positive in T-cells Megakaryocytes—diffuse positive
Nonspecific esterase + fluoride*	Lymphocytes—positive in dog, variable in cat Megakaryocytes—weak positive in dog
Periodic acid-Schiff	Neutrophils—all stages positive Megakaryocytes—positive in dog, variable in cat Mast cells—variably positive Lymphocytes—plasma cells Eosinophils—weak or variable staining Basophils—weak or variable staining Monocytes—weak or variable staining
Acid phosphatase	Neutrophils—all stages positive Lymphocytes—focal positive in T-cells Eosinophils—positive Basophils—positive in cat, variable in dog Monocytes—diffuse positive Megakaryocytes—diffuse positive Mast cells—positive

*Inhibition of nonspecific esterase with fluoride is used to inhibit or reduce the staining of monocytes.

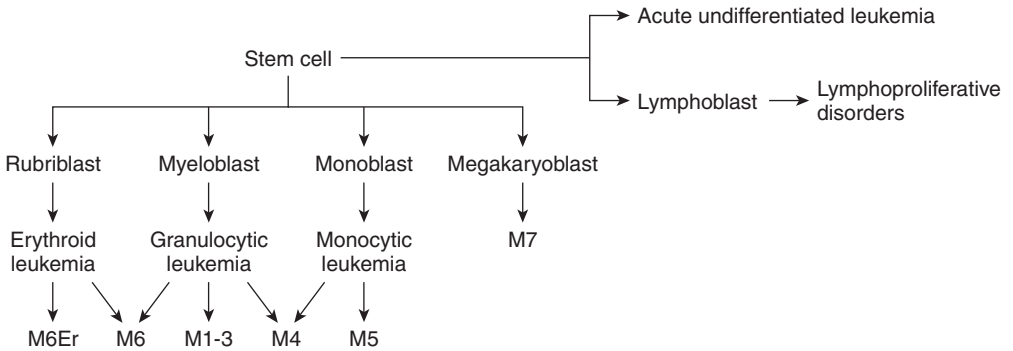

FIGURE 4-8. Diagram depicting origin of the most common neoplastic disorders of hematopoietic cells. *M6Er*, Erythroleukemia with erythroid predominance; *M6*, erythroleukemia; *M1-3*, myeloblastic and promyelocytic leukemias; *M4*, myelomonocytic leukemia; *M5*, monoblastic leukemia; *M7*, megakaryoblastic leukemia.

Electron microscopy is helpful to study the ultrastructural features of the cytoplasm and nucleus (Grindem, Perman, and Stevens, 1985a and 1985b). Immunocytochemistry involves staining of specific cell surface antigens by the use of antibodies such as monoclonal antibodies against CD3 (T cells), CD4 (helper cells), CD8 (cytotoxic or suppressor cells), CD14 (monocyte progenitors), CD21 (B cells), and CD79 (B cells) (Moore and Vernau, 2000). Blood and bone marrow specimens for these special procedures require specific instructions in handling, preparation, and fixation. Therefore the clinical pathologist should be consulted to avoid delay in diagnosis caused by improper sample or sample handling.

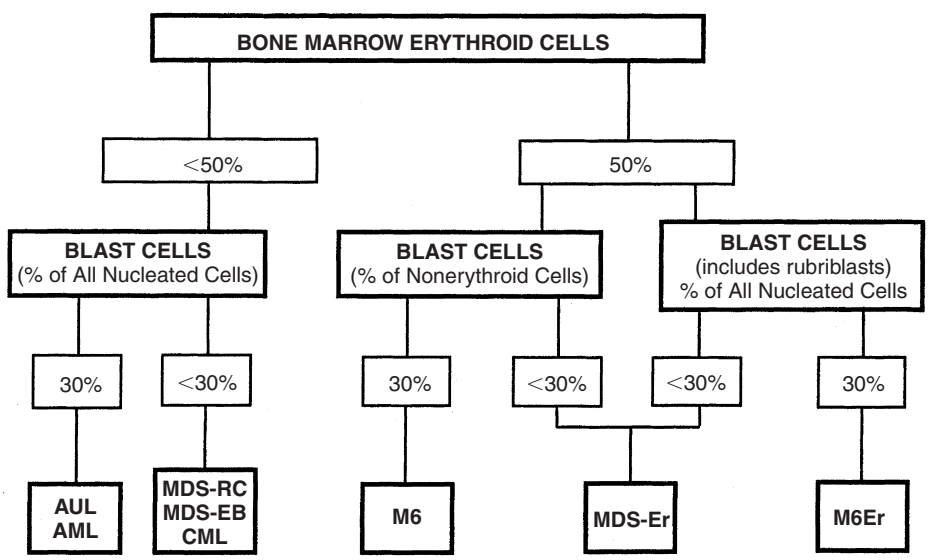

FIGURE 4-9. A scheme to classify myeloid leukemias in dogs and cats using the bone marrow erythroid cells from all nucleated cells *(ANC)*. ANC is a group that excludes lymphocytes, plasma cells, macrophages, and mast cells. Nonerythroid cells are calculated as ANC minus erythroid cells. Blast cells include myeloblasts, monoblasts, and megakaryoblasts. *AUL*, Acute undifferentiated leukemia; *AML*, acute myeloid leukemias M1-M5 and M7; *CML*, chronic myeloid leukemias; *MDS*, primary myelodysplastic syndrome; *RC*, refractory cytopenia; *EB*, excess blasts; *MDS-Er*, myelodysplastic syndrome with erythroid predominance; *M6*, erythroleukemia; *M6Er*, erythroleukemia with erythroid predominance. (Reprinted with permission from Raskin RE, *Vet Clin North Am Small Anim Pract* 26:1023, 1996.)

BOX 4-1. Summary List of Important Lymphoproliferative and Myeloproliferative Disorders

LYMPHOPROLIFERATIVE DISORDERS

Preneoplastic:

Persistent Lymphocytosis

Leukemic/Disseminated Forms:

Lymphoblastic Leukemia (B-, T-, Null-Cell Types)
Lymphocytic Leukemia (B-, T-Cell Types)
Granular Lymphocyte Leukemia (T-, NK-Cell Types)
Plasma Cell Myeloma
Mycosis Fungoides/Sézary Syndrome

Neoplastic Solid Tissue Forms:

Lymphoma (B- and T-Cell Types of Nodal and Extranodal Sites)
Plasmacytoma (Extramedullary Sites)

MYELOPROLIFERATIVE DISORDERS*

Preneoplastic:

Myelodysplastic Syndrome (Primary)

Leukemic/Disseminated Forms:

Acute Myeloid Leukemia (Types M1-M7)

Chronic Myeloproliferative Disease†

Chronic myelogenous leukemia (neutrophils)
Eosinophilic leukemia
Basophilic leukemia
Chronic myelomonocytic leukemia
Polycythemia vera
Essential thrombocythemia
Myeloid metaplasia/myelofibrosis or idiopathic myelofibrosis

Mast Cell Leukemia/Mastocythemia†

Malignant Histiocytosis or Disseminated Histiocytic Sarcoma (Dendritic Cells)

Neoplastic Solid Tissue Forms:

Cutaneous Histiocytoma (Dendritic Cells)
Localized Histiocytic Sarcoma (Dendritic Cells)

*The term *myeloproliferative* is used in its broadest sense to include nonlymphoid dysplastic and neoplastic conditions.
†Uncommon disease states.

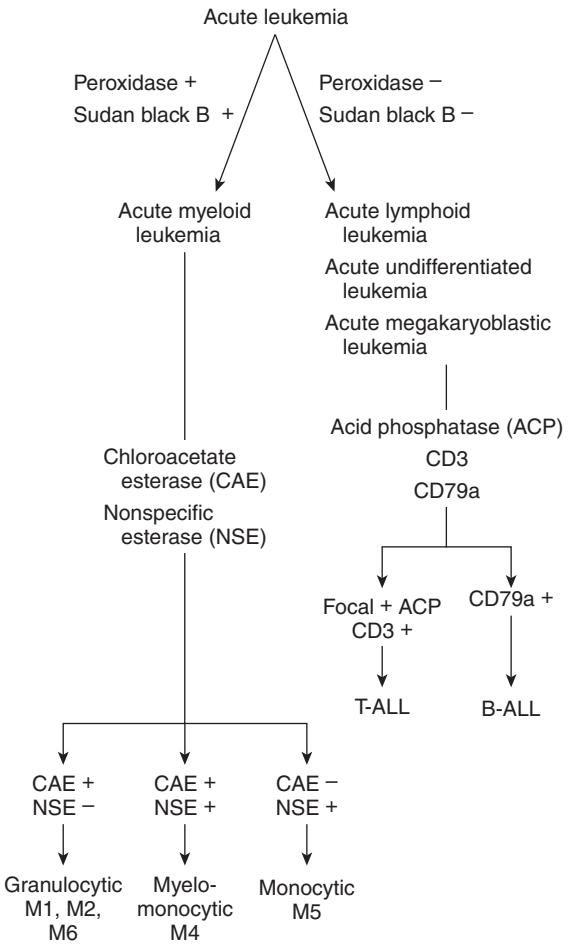

FIGURE 4-10. Simple classification of acute leukemia using cytochemical stains and immunocytochemistry.

Classification Schemes

Classification schemes for hematopoietic neoplasms are based primarily on cell lineage as determined by examination of Romanowsky-type (e.g., Wright's-) stained blood and bone marrow smears (Figures 4-8 and 4-9). Initially, hematopoietic neoplasms are separated into lymphoproliferative and myeloproliferative disorders (Box 4-1). The lymphoproliferative disorders involve lymphocytes, whereas myeloproliferative disorders involve the remaining nonlymphoid leukocytes, erythroid cells, and megakaryocytes or platelets singly or in combination. The distinction may be determined by cytochemical staining (Figure 4-10).

Various classification systems also have been used to classify lymphoid neoplasms. Most of these systems, such as the National Cancer Institute Working Formulation and updated Kiel System, rely on morphologic and immunologic features. Recently a system based on the World Health Organization classification of human lymphoid tumors has been suggested for animals (Valli et al, 2002). This veterinary system separates lymphoid neoplasms by immunophenotype and tumor site location primarily supported by morphologic criteria and may provide better prognostic information than previous classification schemes. Histopathology plays a key role in using this system. The lymphoid leukemic forms are

classified as acute or chronic. An abundance of blast cells or immature cells characterizes *acute* leukemia. *Chronic* leukemia has a proliferation of differentiated cells that may be difficult to distinguish from hyperplasia (e.g., reactive response). Generally, chronic leukemias have a more favorable prognosis than acute leukemias and therefore survive for longer periods of time.

Morphologic criteria to classify acute myeloid leukemias (AMLs) in dogs and cats have been developed by the American Society for Veterinary Clinical Pathology (ASVCP) (Jain et al, 1991). The percentage of blast cells was suggested as equal to or greater than 30% of nonlymphoid and nonhistiocytic cells in the bone marrow for AML, whereas chronic myeloid leukemias, MDSs, and leukemoid reactions would be expected to have less than 30% blast cells (see Figure 4-9). The AML are further separated and subtype is assigned (M1-M7) depending on the cell type (see Figure 4-10). Adjectives (e.g., granulocytic or myelogenous, monocytic, lymphocytic; see Figure 4-8) are used to designate the cell lineage. If the cell type cannot be determined, it is simply called an *acute undifferentiated leukemia*.

NOTE: In general, lymphoproliferative disorders are more responsive to treatment than acute myeloproliferative disorders.

Lymphoproliferative Disorders

Lymphoproliferative disorders are lymphoid conditions originating from solid tissues, such as the lymph nodes (i.e., nodal), alimentary tract, thymus, spleen, eye, and skin or from the bone marrow, such as plasma cell myeloma or some forms of leukemia. A persistent increase in differentiated lymphocytes over time without reaction to known infectious agents or antigenic stimulants suggests neoplasia in lieu of immune stimulation. Lymphoid neoplasms arranged in descending frequency of occurrence are lymphoma, plasma cell myeloma, CLL, and acute lymphoblastic leukemia (ALL). The approximate annual incidence rate for leukemias in dogs and cats is 31 and 224 cases per 100,000 animals, respectively. Lymphoid malignancies, including lymphoma, are responsible for over 85% of cat leukemias and over 65% of the dog leukemias. Cats have a sixfold greater incidence of lymphoma and a sixteenfold greater incidence of myeloproliferative disease than dogs, primarily the result of induction of neoplasia by FeLV infection.

Nodal Lymphoma

Lymphoma, formerly lymphosarcoma, is the most common lymphoid neoplasm of dogs and cats. Lymphoma usually originates in peripheral lymphoid tissues but may develop within extranodal sites (e.g., skin) or from precursor cells in the bone marrow. Multicentric disease is more common in dogs and usually involves the peripheral lymph nodes, spleen, and liver. In descending frequency, other forms of canine lymphoma include alimentary, thymic, and cutaneous disease. Thymic lymphoma is most common in young cats, whereas alimentary lymphoma is more common in older cats. Cats may also develop multicentric, renal, and cutaneous lymphoma. About 60% to 80% of cats with the thymic or multicentric forms of lymphoma are positive for FeLV group-specific antigen, whereas only 30% of cats with alimentary lymphoma are positive.

The CBC is not sensitive in identifying canine lymphoma; 21% of affected dogs have lymphocytosis and 25% have lymphopenia. At the time of diagnosis, up to 57% of dogs may be leukemic. Approximately 30% of cats with lymphoma have a leukemic blood profile. The CBC may document concurrent cytopenias, including anemia, thrombocytopenia, and leukopenia. Bone marrow involvement is used in staging lymphoma and determining treatment. Bone marrow evaluation is needed, because CBC results may not reflect marrow involvement and vice versa. Bone marrow core biopsies are most sensitive in identifying leukemic involvement (e.g., 97% of canine cases compared with 50% by blood smear analysis alone or compared with 60% using bone marrow aspirate biopsies alone) (Raskin and Krehbiel, 1989). Hypercalcemia (i.e., > 12 mg/dl), a factor most associated with T-cell lymphomas (see Chapter 8) is helpful in directing diagnostic efforts toward a lymphoid malignancy. Monoclonal gammopathy may occur infrequently with B-cell lymphoma.

Lymphoma is diagnosed daily by cytopathologists (see Chapter 16). Fine-needle aspirates of lymph nodes or other affected tissues and organs usually disclose a homogeneous population of lymphoblasts. If cytology is equivocal in distinguishing

extreme lymphoid hyperplasia from early lymphoma, a surgical biopsy may provide a definitive diagnosis based on alterations of normal tissue architecture. Surgical or endoscopic biopsies of deep tissues also may be diagnostic.

Immunophenotyping is useful for prognostic evaluation of lymphoma cases; dogs with T-cell lymphoma are at significantly higher risk of relapse and early death compared with B-cell lymphoma. Diagnostic immunocytochemistry of fine-needle aspirate material (or by flow cytometry) and immunohistochemistry of tissue sections are performed currently at certain academic institutions (e.g., flow cytometry at University of California at Davis and North Carolina State University).

In contrast to the mediastinal or thymic lymphoma that is composed of neoplastic lymphocytes and most associated with young cats and dogs, a neoplasm of thymic epithelium, termed *thymoma*, contains a variable numbers of mature lymphocytes and is usually observed in older dogs and cats. Both neoplasms will present as a cranial mediastinal mass, often with evidence of coughing, dyspnea, or thoracic effusion, including chylothorax. Thymic tumors are associated with paraneoplastic hypercalcemia. Thymoma may involve a concurrent mature lymphocytosis exceeding 25,000/µl blood and may be confused with CLL. Thymomas are potentially treatable. Surgical biopsy is the preferred method of diagnosis for thymoma as cytology may be confusing because of secondary chylothorax or the presence of many mast cells.

Plasma Cell Myeloma

Plasma cell myeloma (PCM) is a lymphoproliferative disorder of bone marrow plasma cells. Extramedullary plasmacytoma occurs in soft tissues (e.g., liver, kidney, spleen, skin). Four diagnostic features of PCM are (1) hyperproteinemia with a monoclonal gammopathy, (2) osteolytic lesions in spine, (3) greater than 15% to 20% plasma cells in bone marrow aspirates, and (4) Bence Jones proteinuria (see Chapters 7 and 12). Bence Jones proteinuria is infrequent in dogs and cats, and these light chains of antibodies are best identified by electrophoresis of urine with *detectable* proteinuria. Ehrlichiosis may mimic plasma cell neoplasia in having strong plasmacytosis in the bone marrow and an oligoclonal gammopathy in serum resembling a monoclonal gammopathy.

Histologic or cytologic evaluation of osteolytic areas or soft tissue masses is most diagnostic of PCM (see Chapter 16). Neoplastic plasma cells may exhibit anisocytosis, anisokaryosis, and a finely dispersed chromatin pattern. Binucleate plasma cells occur frequently in PCM, but binucleated plasma cells are normally visible in hyperplastic lymphoid tissue (e.g., lymph node aspirate). Plasma cell leukemia (i.e., neoplastic plasma cells in blood) occurs infrequently.

Acute Lymphoblastic Leukemia

ALL is less frequent than lymphoma. This rapidly progressive condition occurs mostly in middle-aged dogs (mean 6 years) and frequently in FeLV-infected cats. Because leukemia originates in the bone marrow, ALL may be associated with variable nonregenerative anemia, neutropenia, and thrombocytopenia in any combination. As the condition advances, lymphoblasts are found in enlarged visceral organs such as the liver and spleen. In ALL, lymphocyte size on the stained blood smear varies, with many lymphoblasts. CBC usually allows diagnosis of ALL; however, bone marrow is more consistently abnormal (i.e., dense infiltration by lymphoblasts). Lymphoblasts are difficult to distinguish from poorly differentiated myeloid precursors without cytochemical stains, cell surface markers, or PCR clonality tests, currently used as a research tool (see Figure 4-10). Most feline ALL cases have the T-cell immunophenotype, but canine cases may be T-, B-, or null-cell phenotypes. Hypercalcemia is an infrequent laboratory finding.

Reactive or blast-transformed lymphoid cells on blood smears, especially in young animals undergoing strong immune stimulus (e.g., infections), may confuse the diagnosis of ALL or other leukemias. In many nonneoplastic conditions (e.g., immune stimulation by illness or vaccination), a few to several reactive lymphocytes and blast-transformed lymphocytes (perhaps five per blood smear) may be observed. In contrast, ALL has a predominance of lymphoblasts and perhaps five lymphoblasts per oil immersion field. The use of buffy coat preparations to concentrate the leukocytes to find a few lymphoblasts is not advised, because these preparations often have several blast-transformed lymphoid cells in normal animals.

Chronic Lymphocytic Leukemia

CLL has a relatively favorable prognosis of continued life for about 12 months after diagnosis. It occurs mostly in middle- to old-aged dogs (mean 10 years) with a higher frequency in females. Cats rarely have this form of leukemia; most are FeLV negative. Differentiation of CLL from ALL or lymphoma with a leukemic blood profile is based on the relatively mature appearance of lymphocytes in CLL versus finding lymphoblasts in the other conditions. Lymphocytes in CLL are larger than normal and more homogeneous in appearance than in normal animals. Diagnosis of CLL is aided greatly by magnitude of the lymphocytosis, which may exceed 40,000 to 100,000 lymphocytes/µl. Maximum lymphocytosis in extreme immune reactions (e.g., chronic canine rickettsial infections) seldom exceeds 25,000 lymphocytes/µl and usually is less than 15,000 lymphocytes/µl. Lymphocytosis in cats with strong persistent, immune stimulus in nonleukemic diseases may reach higher maximum lymphocyte counts (e.g., 40,000/µl). Reactive lymphocytosis in young, asymptomatic cynomolgus monkeys can reach 70,000 lymphocytes for 1 or 2 weeks. These extreme changes may be called a lymphocytic leukemoid reaction. CLL is the first entity to rule out when lymphocytosis exceeds 25,000 to 40,000 lymphocytes/µl. The likelihood of CLL increases proportionally to lymphocytosis; counts greater than 50,000 to 100,000/µl are particularly diagnostic. Mild normocytic, normochromic nonregenerative anemia is common with variable thrombocytopenia.

Fine-needle aspirates of lymph nodes, spleen, and liver in CLL document lymphoid proliferation, but differentiation of hyperplasia from CLL may be difficult. CLL has a more homogeneous population, whereas hyperplasia has a more diverse population of lymphocytes, plasma cells, and lymphoblasts. Biopsy and histopathology may better document infiltration by neoplastic lymphocytes with loss of tissue architecture. In bone marrow aspirates, greater than 15% to 20% lymphocytes suggests CLL.

Immunophenotyping has demonstrated many of these cases to be T-cell in origin in the dog. Dysproteinemia may occur in dogs with B-cell chronic lymphocytic leukemia related to monoclonal gammopathy (most often IgM, but IgA and IgG gammopathies also occur). Another diagnostic test, currently available as a research tool, involves analysis of gene rearrangements to determine clonality and whether the lymphocyte proliferation is malignancy or hyperplasia.

Granular Lymphocyte Leukemia and Lymphoma

A subtype of lymphoid leukemia and lymphoma involves mostly medium-sized cells having abundant light-blue to clear cytoplasm that contain several small red or purple granules, termed *azurophilic granules*. These cells are called large granular lymphocytes (LGL) and are normally present in the blood of many animal species; specifically in the dog, LGL are present in 0% to 5% of leukocytes or in 0% to 19% of lymphocytes. The LGL may be increased as a result of immune-stimulation such as seen in canine ehrlichiosis. However neoplastic proliferations of LGL are common. In the cat, granular lymphocytes associated with intestinal lymphoma have prominent large coarse purple cytoplasmic granules. In the dog, LGL malignancies may appear as leukemia in the blood or as lymphoma in tissues such as the lymph nodes and spleen. In these tissue forms, blood lymphocyte counts can be normal, however neutropenia may be evident. The granules do not stain well with aqueous-based Wright's stains, such as those found in commercial quick stains. However, the granules appear readily with Wright's-Giemsa stain. Immunophenotyping of these cells indicate most are cytotoxic T cells expressing CD3 and CD8.

In the dog, LGL leukemia may be first recognized as a benign nontransitory proliferation of granular lymphocytes in the peripheral blood, termed *persistent lymphocytosis*. These cells are thought to arise from the red pulp of the spleen. As disease progresses, splenomegaly is commonly noted and cytologic aspirates demonstrate a marked increase in the LGL population. Typically, the bone marrow appears normal or contains minimal LGL involvement. Most dogs with LGL leukemia behave like CLL with a slow indolent course over several years, whereas a small group may have a more aggressive course, similar to ALL (McDonough and Moore, 2000).

Cutaneous Lymphoma

Mycosis fungoides is an uncommon form of epitheliotrophic cutaneous lymphoma (ECL) usually beginning in the skin and progressing to involve lymph nodes, spleen, and

bone marrow. A diagnostic feature of mycosis fungoides is focal accumulation of lymphoid cells within the epidermis forming *Pautrier's microabscesses*. *Sézary syndrome* is a rare variant of ECL associated with a leukemic blood profile characterized by large T cells with markedly convoluted nuclei (Latimer and Rakich, 1996). The neoplastic cells of ECL are cytotoxic T-cells that express CD3 and CD8.

In comparison to the epitheliotrophic mycosis fungoides, nonepitheliotrophic lymphoma is less frequent. This form was previously believed to be of B-cell origin; however, recent findings indicate this is exclusively T-cell lymphoma, although the morphologic and immunologic characteristics differ from mycosis fungoides. Both forms are aggressive and respond sporadically or poorly to treatment (Moriello, 2000).

Myeloproliferative Disorders

Characteristics of selective myeloproliferative disorders are described under the following headings. Myeloproliferative disorders include a preleukemic condition with dysplasia and neoplasia of mature and poorly differentiated nonlymphoid cells in the form of leukemia (see Box 4-1). Generally, AMLs are severe, rapidly progressive diseases, essentially unresponsive to treatment. In chronic myeloid leukemia, relatively mature cells are released from the bone marrow and the clinical course may be long. In myeloproliferative disorders, the leukemic cell type may vary over time in the same animal; therefore the diagnosis may reflect a specific point in time.

Blast cells of various cell types often lack identifiable characteristics, and the initial impulse is to call an undifferentiated blast a lymphoblast. Blasts in myeloproliferative disease are distinguished by sequential differentiation into more mature myeloid cells, if present, or cytochemical staining of a poorly differentiated leukemia (see Table 4-7).

NOTE: Myeloproliferative disorders occur infrequently or rarely, in contrast to lymphoproliferative disorders, which are more commonly observed.

Myelodysplastic Syndromes

Primary MDS is an irreversible acquired clonal disorder of multipotential hematopoietic cells in contrast to secondary MDSs, which are related to concurrent disease, nutritional deficiency, or drug-induced toxicosis (see Table 4-6). MDS is sometimes referred to as preleukemia, because patients having this syndrome often suffer from chronic debilitation that may remain unchanged or evolve into AML. Characteristic preleukemic changes in hematopoietic cells include cytopenias of one or more cell lines in peripheral blood and morphologic evidence of dysplasia. MDS has less than 30% myeloblasts of the nonlymphohistiocytic cells in the bone marrow, whereas AML has greater than 30% (see Figure 4-9). Several morphologic types exist relative to the numbers of myeloblasts present (e.g., MDS-RC [refractory cytopenia] or MDS-EB [excess blasts]) and erythroid cells present (e.g., MDS-Er [erythroid predominance]) (Raskin, 1996). Cases of MDS-EB having 5% or greater marrow myeloblasts, demonstrate shorter survival and poor response to treatment.

Bone marrow evaluation should be performed to document dysplasia if laboratory findings reveal macrocytic nonregenerative anemia, multiple cytopenias, or a persistent anemia despite attempted regeneration. Dysplastic changes vary among individuals. The erythroid line may have excessive numbers of rubriblasts and prorubricytes (apparent maturation arrest); megaloblastic rubricytes; nuclei with abnormal chromatin patterns, lobulation, or multinucleation; sideroblasts; or combinations thereof. Normal feline marrow lacks sideroblasts or stainable hemosiderin. The myeloid line may have excessive myeloblasts and promyelocytes (apparent maturation arrest), abnormal granulation, bizarre hypersegmented neutrophils (macropolycytes), nuclear hyposegmentation (acquired Pelger-Huët change), giant neutrophils, monocytoid neutrophils, or combinations thereof. The megakaryocytic line may have dwarf megakaryocytes, megakaryocytes with hypolobulated or multiple round nuclei, megakaryocytes with hyperlobulated nuclei with blue cytoplasm, megaplatelets, hypogranular platelets, hypergranular platelets, or combinations thereof. Multiple CBC over

NOTE: Preleukemic changes in hematopoietic cells include cytopenias of one or more cell lines in peripheral blood and morphologic evidence of dysplasia in blood or bone marrow.

time are needed to monitor the cytopenia or to document conversion to leukemia.

Acute Myeloid Leukemia

AML refers to a collection of neoplastic disease affecting precursors of nonlymphoid cells. Subtypes M1-M7 are defined by the cell of origin and the degree of differentiation (Raskin, 1996). Cytochemical staining (see Figure 4-10 and Table 4-7) or immunocytochemistry is used to distinguish the subtypes. For example, *acute myeloblastic (myelogenous) leukemia* (AML-M2) usually consists of neutrophils, but coproduction of basophils and eosinophils may occur. Total leukocyte counts are variable, but extreme leukocytosis may occur. Blast cells are often found in circulation. Severe anemia and thrombocytopenia may accompany AML. Myeloblasts, promyelocytes, and atypical cells denote subtypes M1 to M3. Myeloblasts may have a moderately basophilic cytoplasm with few small azurophilic granules. A disorderly maturation sequence also suggests neoplasia but may occur during repopulation of the bone marrow after cellular destruction (e.g., toxin, parvovirus). Repopulation of the bone marrow begins with immature cells (i.e., myeloblasts, progranulocytes, myelocytes), without the expected predominance of mature bands and segmenters; this appearance initially resembles leukemia, but progressive maturation eventually restores a normal marrow cell population. Diagnosis may require reevaluation of the bone marrow after a few days.

Myelomonocytic leukemia (M4) is characterized by combined production of neutrophils and monocytes by the bipotential stem cell resulting in myeloblasts and monoblasts that equal or exceed 30% of nonerythroid cells in the bone marrow. This is the most common nonlymphoid leukemia. Percentage of monocytes and neutrophils may change as the disease progresses. Moderately severe anemia is expected.

Monocytic leukemia (M5) occurs infrequently. Cytochemical staining may prevent misdiagnosis of monocytic leukemia as acute lymphocytic leukemia. Monoblasts are increased to 30% or greater of the nonerythroid cells of the bone marrow. Monoblasts have basophilic cytoplasm that lacks any obvious granulation; nuclei are irregularly round, producing a folded or creased appearance.

Erythroleukemia (M6) involves proliferation of immature and atypical erythroid and granulocytic cells. The erythroid component exceeds 50% with myeloblasts and monoblasts (together) equal to or greater than 30% of nonerythroid cells. A variant form called *M6Er*, consists predominantly of erythroid precursors; in this form rubriblasts exceed 30%. The M6 conditions are more common in cats than in dogs. Severe nonregenerative anemia and dysplastic changes are frequently prominent. Over time, this form of leukemia may change in appearance and progress to involve predominantly granulocytic precursors. It is often associated with FeLV infection in cats.

Megakaryoblastic leukemia (M7) has been reported in both dogs and cats. Leukocyte and platelet counts vary, and circulating megakaryoblasts may be found. These cells have a round nucleus and scant basophilic cytoplasm with a ragged irregular cell surface. Platelet morphology is often bizarre, characterized by giantism and abnormal granulation. Diagnosis of this form of leukemia requires positive cytochemical reactions to periodic acid-Schiff, alpha naphthyl acetate esterase, acetylcholinesterase, and factor VIII-related antigen. Electron microscopy may reveal characteristic alpha granules or early internal membrane demarcation systems. Some previously reported cases have been misdiagnosed as AML-M7 that were more correctly identified as myeloid hyperplasia with myelofibrosis (discussed later in the chapter).

Chronic Myeloproliferative Diseases

Chronic granulocytic (myelogenous) leukemia (CGL, CML) differs from acute granulocytic leukemia in that segmented and band neutrophils predominate. The left shift in CGL may extend back to promyelocytes, and bone marrow myeloid proliferation may involve orderly myeloid maturation with a marked increase in the myeloid : erythroid ratio (Fine and Tvedten, 1999). This ratio may fall between 4:1 to 25:1 or as high as 36:1. The bone marrow contains less than 30% myeloblasts of nonlymphohistiocytic cells (see Figure 4-9). Diagnosis of CGL is often based on finding marked, persistent leukocytosis (40,000 to 200,000/µl) and exclusion of a leukemoid reaction, discussed earlier under inflammatory leukocytosis. Anemia is mild-to-moderate and platelet counts are variable. Lymphadenopathy with lymph node aspirates that resemble marked extramedullary hematopoiesis may occur in CGL. The prognosis and response to treatment is better

than for AML, with death occurring months after diagnosis because of a blast cell crisis.

Eosinophilic leukemia is a variant form of myelogenous leukemia. This type of leukemia is rare but has been reported in the dog and documented in the cat associated with FeLV infection. It is characterized by a marked, persistent eosinophilia (often > 50,000/µl) and a shift toward immaturity. A moderate anemia may be present. It may be difficult to differentiate from hypereosinophilic conditions (e.g., hyeneosinophilic syndrome, allergies, parasitism, eosinophilic inflammatory diseases, mast cell tumors, certain lymphomas). Leukemic eosinophils will leave the bone marrow and infiltrate solid tissues, such as the lymph nodes, liver, and spleen.

Basophilic leukemia is another variant form of myelogenous leukemia that is uncommon and reported to occur mostly in dogs. This form of neoplasia can be distinguished from mast cell leukemia by subtle nuclear indentation, segmentation, or lobulation. In dogs, the cytoplasmic granules in neoplastic basophils may be coarser than in mast cells. The disorder has been associated with thrombocytosis and anemia. Prognosis is good with treatment.

Chronic myelomonocytic leukemia (CMML) is an uncommon disorder in which blast cells of both granulocytic and monocytic lines involve less than 30% of nonlymphohistiocytic bone marrow cells. Cases often display peripheral monocytosis greater than 4000/µl. Diagnosis is suspected if marked monocytosis accompanies neutrophilia, without indications of an inflammatory condition. CMML may progress over time to an AML.

Polycythemia vera (primary erythrocytosis) is a rare disease in dogs and cats involving the neoplastic production of mature, anucleated erythrocytes. Polycythemia is suggested by brick-red mucous membranes related to markedly increased hematocrits (i.e., PCV 65% to 82%). Splenomegaly if present is mild. Polyuria, polydipsia, hemorrhage, and neurologic disorders occur in 50% of canine cases. Definitive diagnosis of polycythemia vera requires ruling out other causes of erythrocytosis or absolute polycythemia (see Chapter 3). Diagnosis is based on demonstration of an absolute increased red cell mass, normal PaO_2, and a decreased serum erythropoietin concentration that is measured at specialized laboratories. Renal cysts, pyelonephritis, and tumors must be excluded, because they can also produce absolute polycythemia (i.e., inappropriate secondary erythrocytosis) as a paraneoplastic syndrome with increased serum erythropoietin concentration.

Essential thrombocythemia (i.e., primary thrombocythemia) is a rare chronic myeloproliferative disease characterized by proliferation of megakaryocytes and unregulated platelet production reported in the dog and cat. It occurs unrelated to physiologic or responsive thrombocytosis. Clinical signs include splenomegaly and platelet function abnormalities, such as spontaneous bleeding and thromboembolism (see Chapter 5). Platelet counts are persistently above 600,000/µl and often greater than 1 million/µl, which predispose to microthrombosis and microvascular ischemia. Neutrophilia or basophilia may also be seen. A bioassay for thrombopoietin performed at a specialized laboratory can confirm the diagnosis.

Myeloid metaplasia/myelofibrosis (MMM) has been termed *agnogenic myeloid metaplasia, idiopathic myelofibrosis*, or *chronic megakaryocytic-granulocytic myelosis*. This uncommon condition results in intramedullary and extramedullary hematopoiesis that is accompanied by a reactive or secondary marrow fibrosis late in the course of the disease. The hematopoietic precursors most involved are granulocytic and megakaryocytic forms, which infiltrate the spleen and liver. Some cases may be mistaken for AML of megakaryocytic origin. The peripheral blood often has concurrent immature granulocytes and erythroid cells, termed a *leukoerythroblastic reaction*. Erythrocytes may display poikilocytosis with tear-drop formation. Bone marrow aspiration is often difficult, related to the presence of myelofibrosis; therefore core biopsy is recommended to confirm the diagnosis. Survival varies from months to years, depending on the response to treatment for the nonregenerative anemia.

Mast Cell Leukemia and Mastocytemia

Mast cell leukemia may originate in the bone marrow of dogs and is rare. *Mastocytemia* or systemic mastocytosis may occur secondary to a solid mast cell tumor (see Chapter 16) or may suggest severe inflammatory disorders, particularly parvoviral enteritis (Stockham, Basel, and Schmidt, 1986). In general, the larger the number of mast cells in blood, the more likely that systemic mast cell neoplasia is present, especially in the absence of enteritis. Total number of mast cells per blood

smear in dogs with enteritis and mastocytemia usually ranged from 2 to 9, but 30 to 90 mast cells per smear could be found. It is rare to find circulating metastatic mast cells from the mast cell tumors of the skin in dogs and cats. Circulating mast cells are more common in cats affected with the visceral form of mast cell neoplasia producing diffuse, moderate-to-marked splenomegaly. Erythrophagocytosis by the circulating mast cells is not unusual, and this may contribute to anemia. Typically, disseminated disease involves the spleen, liver, distant lymph nodes, or bone marrow. Cytology or surgical biopsy document disseminated mast cell neoplasia best.

Mast cells are not expected in blood smears from healthy dogs (Bookbinder, Butt, and Harvey, 1992). Normal canine marrow has 0 to 1 mast cells/1000 nucleated cells, and greater than 10 mast cells/1000 nucleated cells in bone marrow smears was considered increased and supportive of hemolymphatic involvement in disseminated mast cell neoplasia by O'Keefe and colleagues (1987). Mast cell leukemia can be distinguished from basophilic leukemia based on morphologic and cytochemical criteria.

Malignant Histiocytosis

Malignant histiocytosis or disseminated histiocytic sarcoma is an aggressive multisystem disease with a disseminated neoplasm of myeloid dendritic cells (Affolter and Moore, 2002). Older animals are at greater risk and certain breeds such as golden and flat-coated retrievers, rottweilers, and Bernese Mountain dogs have increase incidence. Primary sites affected include spleen, lung, and bone marrow; secondary sites are liver, lymph nodes, subcutis, and kidney. Anemia, thrombocytopenia, and hyperbilirubinemia are the most common laboratory abnormalities. Cytology demonstrates malignant histiocytes to be large, frequently markedly pleomorphic round or stellate cells with abundant (sometimes vacuolated) basophilic cytoplasm. Nuclei are oval to reniform with lacy chromatin and prominent multiple nucleoli (see Color Plate 6C). Multinucleate cells are common and mitotic figures are often frequent.

Erythrophagocytosis and leukophagocytosis are common but not consistently present. Another condition with erythrophagocytosis and multiple cytopenia is hemophagocytic syndrome of well-differentiated macrophages that may be associated with infectious or inflammatory disease. A more anaplastic appearance of malignant cells and the lack of a history of concurrent infection may distinguish malignant histiocytosis from this condition. Differentiation of malignant histiocytosis from other neoplasms is confirmed by demonstration of positive histiocytic cytochemical and immunohistochemical markers with negative lymphoid or epithelial markers. Similar-appearing cells may actually be T-cell or B-cell lymphoma when immunophenotyping or gene rearrangement studies are performed.

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