Introduction
The circulating white blood cells include the granulocytes, the monocytes, and the lymphocytes. From a functional perspective, these cells may be divided into two systems: the phagocytes and the immunocytes. The phagocytes include the granulocytes and the monocyte/macrophage continuum. The immunocytes include the B and T lymphocytes.

The phagocytic system is the first line of defense against infectious disease and may be thought of as the nonspecific immune system. The immunocytic system responds to specific antigens by producing antibodies and lymphokines and is termed the specific immune system. The two systems are linked through the functions of the monocyte/macrophage continuum.

Clearly, the peripheral blood leukocytes play a central role in the animal’s response to disease. Understanding this role and being able to interpret abnormal peripheral leukocyte responses will provide the clinician with significant diagnostic, therapeutic, and prognostic insights. The following paragraphs detail the normal structure and function of the various circulating leukocytes and briefly address the interpretation of abnormal leukocyte morphology and number. Evaluation of the phagocytic system is emphasized, since changes in this system are the most significant to clinical evaluation.

The Granulocyte System

Normal Granulocyte Structure and Function
Granulocytes of dogs and cats include neutrophils, eosinophils, and basophils. In terms of circulating numbers, the neutrophil is by far the most prevalent. The three granulocytes are distinguished on the basis of their cytomorphology; they are also distinct functionally, and changes in circulating cell numbers of the three granulocytes are interpreted quite differently.

In peripheral blood films, neutrophils are round cells measuring 12–15µ with pale pink granular cytoplasm and nuclei with condensed chromatin forming nuclear lobes connected by thin bands of chromatin. Nuclear morphology of neutrophils is classically described as segmented.

From a functional perspective, the neutrophil is a fully armed phagocyte, which can be immediately mobilized when microorganisms, particularly bacteria, invade the animal. Circulating neutrophils are drawn from blood into the tissues at sites of inflammation where there is a high concentration of chemoattractants (chemotactic stimuli). This process of directed movement of phagocytes along a chemotactic gradient is known as chemotaxis. Chemoattractants for neutrophils include bacterial lipopolysaccharide, complement fragments, immune complexes, etc.

When neutrophils arrive at a site of inflammation, they adhere to those particles for which they have receptors on their surfaces. In particular, neutrophils have surface receptors for things such as immunoglobulins and complement fragments; when bacteria are coated by such molecules (called opsonins), adherence is facilitated.

Adherence is followed by phagosome formation. In this process, the cell membrane of the phagocyte literally flows around the adhered bacterium, resulting in internalization of the organism within a membrane-bound vacuole.

Phagosome membranes fuse with membranes of neutrophil cytoplasmic granules, thereby releasing granule content in close proximity to the internalized bacterium yet separated from the neutrophil cytoplasm. Neutrophil granules are of two types. Specific granules contain lysozyme, lactoferrin, collagenase, and plasminogen activators. Azurophilic (primary) granules are lysosomes, which contain acid hydrolases, myeloperoxidase, lysozyme, and cationic proteins. Collectively granule content of the neutrophil is responsible for both bacterial killing and digestion. Altogether, the process of bacterial identification, entrapment, killing, and digestion is the process of phagocytosis.
Eosinophils are granulocytes that contain orange (eosinophilic) cytoplasmic granules. Granules stain with eosin because of their content of basic mucopolysaccharide (including antihistamines) and major basic protein. Granule appearance in eosinophils is characteristic of the species. Dog eosinophils have variable numbers of variably sized round granules. Cytoplasm of canine eosinophils is often vacuolated. Feline eosinophils contain large numbers of uniform rod-shaped granules. Like neutrophils, both canine and feline eosinophils have segmented nuclei, but in general eosinophil nuclei stain less intensely than neutrophil nuclei.

Like neutrophils, eosinophils are phagocytes capable of ingesting, killing, and digesting bacterial organisms. However, eosinophils are less effective at bacterial phagocytosis than neutrophils, and this is not their primary function. Rather, they are involved primarily in the IgE mediated destruction of metazoan parasites, in controlling allergic reactions, and as moderators of acute inflammation (due to their antihistamine content). Whenever circulating eosinophilia is observed, systemic allergic reaction should be suspected. It is emphasized that intestinal parasitism is not a cause of systemic allergy and therefore should not be accepted as a cause of peripheral eosinophilia.

Basophils are named for their basophilic (blue to purple) cytoplasmic granules, which acquire their stain affinity from their content of sulfated (acid) mucopolysaccharides (histamine, heparin, serotonin). In reality, in canine and feline basophils these granules can be quite sparse, making these cells difficult to identify. Basophils are generally slightly larger than neutrophils, the nucleus is less hyperchromic, and the background cytoplasm is more basophilic.

Functionally, basophils are not phagocytes. For years, their role has remained somewhat obscure. Current theories are that basophils (and their tissue counterparts the mast cells) are initiators of the acute inflammatory response, and in this function they are opposed by the moderating effects of the eosinophil (see above). Additionally, basophils release their histamine when stimulated by reaginic antibodies (Ig E) and therefore are important as facilitators of systemic allergic reactions. When circulating basophilia is seen, systemic allergy should always be suspected.

**Normal Granulocyte Production, Circulation, and Utilization**

The granulocytes—neutrophils, eosinophils and basophils—are all produced in the bone marrow. Five distinct granulocyte precursor cells—the myeloblast, promyelocyte, myelocyte, metamyelocyte, and band cell—can be identified in the bone marrow. In addition, mature granulocytes are also present in large numbers. The granulocytic cells of the marrow can be divided into three distinct cell pools. The mitotic pool comprises the myeloblasts, promyelocytes, and myelocytes. These are the only granulocytic precursor cells capable of cell division. The metamyelocytes and band cells comprise the maturing pool. It takes about 5–7 days for a myeloblast to pass through the mitotic pool and maturing pool, with the resultant formation of a mature granulocyte. Mature granulocytes are not released immediately into the blood but instead are stored in the marrow and comprise the storage pool. Mature granulocytes remain in the marrow an average of 3 to 5 days before being released into the blood. In dogs approximately a 5-day supply of granulocytes is stored in the marrow.

Granulocytes released from the marrow into the peripheral blood enter first the freely circulating pool and eventually the marginated pool. The number of cells in each of the two peripheral blood pools is approximately equal at any point in time under normal conditions. Cells leave the freely circulating pool and enter the marginating pool at random. Cells in the marginating pool eventually leave the peripheral blood and enter the last of the body’s granulocyte pools, the tissue pool. Altogether, granulocytes remain in the peripheral blood for relatively short periods of time, usually in the range of 9 to 12 hours. It is important to note that when blood is collected it is the freely circulating pool that is sampled.

**Altered Granulocyte Responses**

Numerous conditions can alter the delicate balance of granulocyte production, circulation, and utilization described above. A number of these conditions will be described in detail. Comments will be directed toward the neutrophils, since this is the most prevalent and diagnostically significant granulocyte of the peripheral blood of dogs and cats.

**Physiologic Leukocytosis**

Whenever an animal becomes excited, endogenous epinephrine is released and blood flow is increased. Other causes of increased blood flow include tachycardia, convulsions, and exercise. Increased blood flow washes marginating cells back into the freely circulating pool, where they can be sampled when blood is taken. The net effect is a leukocytosis. Since under normal circumstances there are as many marginating cells as there are freely circulating
cells, white blood cell counts could be theoretically doubled. Physiologic leukocytosis is predominantly a
neutrophilia and occurs immediately at the time of excitement. The elevation persists only as long as the excitement
persists.

Physiologic leukopenia is the converse of physiologic leukocytosis and occurs whenever blood flow is slowed. For
example, blood samples collected from dogs under general anesthesia are generally leukopenic. In these instances
there is increased margination of circulating neutrophils.

Steroid Induced Leukocytosis
Corticosteroids cause several alterations in neutrophil pools. The principal changes are a prolonged circulating time,
a shift from the marginating to the circulating pool, and increased release of neutrophils from the marrow storage
pool. The net effect is a leukocytosis that is predominantly a neutrophilia. In addition corticosteroids induce a
monocytosis, an eosinopenia, and lymphopenia in dogs. The eosinopenia and lymphopenia may result from either a
lytic effect of steroids on these two cell lines or from redistribution of freely circulating cells as to the marginalizing
pools. The steroid effect is not seen immediately but takes approximately four hours to develop. The response
persists for approximately 24 hours.

Granulocyte Responses in Inflammation

1) Peracute Inflammation

Peracute inflammatory responses are rarely seen by the small animal practitioner, primarily because the owner does
not recognize the disease until it has been present long enough to progress beyond the peracute stage. Nevertheless,
whenever severe tissue damage occurs, a transient phase of peracute inflammation results. The peracute
inflammatory response is characterized by movement of inflammatory cells out of the peripheral blood marginating
pool and into the damaged tissue in response to localized chemotactic stimuli. Concomitantly, cells move out of the
freely circulating pool and into the marginating pool. Both responses serve to diminish the peripheral leukocyte
count, inducing leukopenia that is primarily a neutropenia.

2) Acute Inflammation

If peracute inflammation persists, it eventually progresses to the acute stage. In acute inflammation, blood cells
continue to enter involved tissue sites in response to chemotaxins. At the same time that cells are moving out of the
blood and into the tissues, increased numbers of neutrophils are being released from the marrow storage pool into
the circulation. In addition, the continued mobilization of granulocytes out of circulation into the tissue causes the
release of granulocytopoietin (colony stimulating factors). CSFs cause expansion of the mitotic pool and maturing
pool of the bone marrow. Eventually expanded marrow production and release into circulation exceed tissue
demand, and a build-up of circulating white cells (leukocytosis) is observed. Because of expanded marrow
production, immature white blood cells are usually also released into circulation during this phase of inflammation.
This peripheral blood response, a leukocytosis with a neutrophilia and increased numbers of immature granulocytes
(a left shift), is termed a regenerative left shift and is generally regarded as a proper early response to an
inflammatory reaction in dogs, cats, horses, and young ruminants.

3) Overwhelming Inflammation

Occasionally, tissue injury sites may be so severe that marrow production and release of neutrophils cannot keep
pace with tissue demand. In other words, neutrophils are moving out of the blood and into the tissues faster than
they are moving from the bone marrow into the blood. The net effect in the peripheral blood is leukopenia
characterized by neutropenia, usually with an attendant left shift. Such a response is termed a degenerative left shift,
and in dogs, cats, horses, and calves indicates overwhelming inflammation and a guarded prognosis.

Because adult ruminants have a small bone marrow storage pool of mature neutrophils, leukopenia with neutropenia
and a left shift is the expected typical acute inflammatory response in adult cattle, sheep, and goats. In these animals,
a degenerative left shift therefore does not have the same negative implications that it does in other species. In
ruminants, the neutrophil count should rebound to reference range levels or higher in about four days after the onset
of inflammation. Failure of neutrophil counts to recover in this time frame leads to a guarded prognosis.
4) Chronic Inflammation

Two forms of chronic inflammatory responses can be recognized. The first is generally seen in association with serious but circumscribed and/or walled off suppurative lesions. In these instances (e.g., pyometra, pyothorax, pancreatic abscess, prostatic abscess, etc.), there is often ongoing massive release of CSFs from the sites of inflammation. This results in marked marrow granulocytic hyperplasia, which in turn leads to severe leukocytosis (>75,000/µl). In most cases there will also be a left shift, which can be marked with the presence of metamyelocytes and even myelocytes seen on the blood film. Toxicity may or may not be observed. Monocytosis and hyperglobulinemia are commonly present. This reaction is termed a leukemoid response and must be differentiated from a true myelogenous leukemia.

The second form of chronic inflammation is seen when a new steady state has been reached between marrow production and release of granulocytes and tissue demand. In these instances, the granulocyte marrow compartments are markedly expanded, but the rate at which white blood cells enter the circulation is identical to the rate at which they leave the blood and enter the tissues. In this circumstance total white cell count is within the reference range or only slightly elevated, and the only significant change in the leukogram is a monocytosis.

Morphologic Granulocyte Abnormalities
The preceding paragraphs have outlined quantitative changes that occur in the various granulocyte pools in disease. In addition, specific morphologic abnormalities can also be described.

Toxicity of circulating granulocytes in dogs is most commonly characterized by cytoplasmic basophilia and vacuolation. Peripheral blood toxicity is a reflection of bone marrow toxicity and indicates a cytoplasmic maturation arrest in developing granulocytes. Basophilia is due to the retention of cytoplasmic RNA and failure of developing granulocytes to form specific granules. Because toxic change represents a developmental arrest, it is most commonly seen in circulating band cells. Toxicity is a relatively constant feature of severe bacterial infections and is seen whenever systemic toxemia occurs.

Although basophilia and vacuolation are the most frequent features of toxicity, other changes may also be observed. Bizarre giant band cells and metamyelocytes are occasionally seen. Giantism is believed to result from a reduction in the number of cell divisions in the marrow. Döhle bodies are also occasionally seen in toxic canine neutrophils. Döhle bodies are basophilic cytoplasmic bodies, which have been identified as precipitates of endoplasmic reticulum ultrastructurally.

The Monocyte/Macrophage System

Normal Monocyte Structure and Function
Normal blood monocytes are large cells (15–20 µ) with lacy, irregularly shaped nuclei and abundant basophilic granular cytoplasm. When blood films are made from samples collected into EDTA anticoagulant, cytoplasm often contains vacuoles. Cytoplasmic blebs or pseudopods are also seen in monocytes from EDTA blood.

Circulating monocytes are the precursors of tissue macrophages. Once differentiated, they perform a diversity of functions.

The best known function of tissue macrophages is phagocytosis. Macrophages are more efficient phagocytes than neutrophils and are more capable of digesting particulate debris. Consequently, macrophages are the cleanup cell whenever there is tissue necrosis.

Tissue macrophages are also important to the immune response in several ways. Antigens phagocytized by macrophages subsequently are presented to lymphocytes in such a way as to be more immunogenic. Interactive lymphocytes then initiate cell-mediated and humoral immunity. In addition, macrophages carry surface receptors for both immunoglobulins and complement. Organisms or antigens coated with either antibody or complement may therefore be more readily phagocytized by macrophages.
A third major function of macrophages is regulation of iron metabolism. Whenever red blood cells are destroyed in the body, hemoglobin is degraded and the iron contained in hemoglobin is stored as hemosiderin within macrophages. All tissue macrophages except alveolar macrophages can release iron back into circulation. The iron then can be mobilized to the bone marrow and reutilized in red blood cell production.

Finally, a pivotal role for macrophages in regulation of both the inflammatory response and cell growth and differentiation has recently been described. Macrophages are now recognized as secretory cells as well as phagocytic cells. Among the products secreted are interleukins, prostaglandins, complement fragments, tumor necrosis factor, leukokinitins, etc.—all mediators of inflammation. Additionally, macrophages are the principal source of a variety of colony stimulating factors (CSFs), which are essential for production and development of bone marrow elements.

Normal Monocyte Production, Circulation, and Utilization
Like the granulocytes, monocytes are produced in the bone marrow, circulate transiently in the blood (circulating half-life of approximately 10–36 hours), and enter the tissues, where they differentiate into macrophages and perform their functions. Unlike the granulocytes, however, monocyte precursors do not accumulate in the marrow; in fact, marrow monocyte precursors are rarely seen. In effect, as suggested earlier, circulating monocytes are the precursors of the monocyte/macrophage system. Whenever there is a demand for phagocytosis and a stimulus for macrophage production, a peripheral monocytosis therefore is seen. Circulating monocytosis is a common feature of chronic inflammatory conditions but can also occur acutely. Whenever mild to moderate peripheral monocytosis is observed, an inflammatory leukogram is said to be present. If monocytosis is marked, or if monocytosis is the only evidence of inflammation, then evaluation of a buffy coat preparation is recommended (see below).

Altered Monocyte Morphology
Both infectious agents and noninfectious but opsonized particles can occasionally be found in circulating monocytes. When present they are usually of diagnostic significance. Such phagocytosed particles are best seen in buffy coat preparations where monocytes are concentrated.

One noninfectious condition where phagocytosis can be seen in circulating monocytes is immune-mediated hemolytic anemia. In this disease, circulating monocytes occasionally contain phagocytosed red cells. Phagocytosis of circulating RBCs will only occur if red cells are in some way altered or damaged, so finding red cells in circulating monocytes on peripheral blood films is significant. If buffy coat preparations are made to search for erythrophagocytosis, it is important that the buffy coats are made from fresh blood and that the buffy coat smears are made immediately after the blood has been centrifuged. If monocytes are allowed to stand in contact with RBCs for any length of time, some in vitro nonpathogenic erythrophagocytosis is expected.

Infected diseases in which phagocytosed etiologic agents can be seen in circulating monocytes are primarily fungal, protozoal, or rickettsial. We have seen monocytes containing leishmania, histoplasma, toxoplasma, and ehrlichia.

The Immunocyte System
Although the immunocyte system is extremely important to body defenses, from a hematologic perspective, circulating lymphocytes provide far less diagnostic information than cells of the phagocytic system. In the following paragraphs we review the lymphocyte as a circulating cell; detailed consideration of the immunocytic system in health and disease we leave to the immunologists.

Normal Lymphocyte Structure and Function
Normal circulating unstimulated lymphocytes are relatively small leukocytes (9–11 µ), with large, round nuclei and a scant rim of faintly basophilic cytoplasm. Nuclei stain intensely and chromatin clumps may be seen, but nucleoli are not apparent. Normal circulating lymphocytes include both B cells (responsible for humoral immunity) and T cells (responsible for cellular immunity); the two cell types cannot be differentiated in standard hematologic preparations. In most species, approximately 70% of normal circulating lymphocytes are T cells and the majority of the rest are B cells. These proportions often change in disease.
Normal and Abnormal Lymphocyte Kinetics

Normal circulation of lymphocytes is markedly different than circulation of phagocytes. Peripheral lymphocyte responses in disease often reflect disturbances in these normal circulatory patterns.

All circulating lymphocytes are believed to be produced originally in bone marrow, but those of the T cell line have probably undergone a maturation or conditioning process in the thymus. Circulating lymphocytes are long-lived cells which, unlike the phagocytes, continuously leave the blood only to reenter at a later time. Circulating lymphocytes are sometimes termed memory cells because they have been conditioned to recognize specific antigens as they circulate. When they come in contact with these specific antigens during circulation, they are then able to settle out in the peripheral lymphoid tissues and initiate the specific immune response. Once the response has been initiated, the inciting lymphocytes, as well as those produced in the nodal response, may be returned to the circulation. The normal circulating pattern for lymphocytes is therefore from blood to peripheral lymphoid tissues (lymph nodes) to lymph and finally back to blood.

Abnormal lymphocyte responses in disease include lymphocytosis and lymphopenia. Lymphocytosis is seen commonly in cats as a physiologic response to excitement; lymphocytes marginated on vessel walls are washed back into circulation by increased blood flow. Lymphocytosis can also be seen as an indication of an expanded lymphocyte compartment as a result of chronic antigenic stimulation and less commonly as a result of lymphoid neoplasia. Lymphopenia is seen whenever the normal circulatory patterns of lymphocytes are interrupted. The most common cause of lymphopenia is an increased level of circulating glucocorticoids, which is believed to cause margination of lymphocytes on vessel walls. This kind of “stress” lymphopenia accounts for the vast majority of lymphopenias seen in veterinary practice. Lymphopenia also is seen in chylothorax, where lymphocytes are dumped from the lymph into the thoracic cavity rather than being returned to the peripheral blood. Lymphosarcoma can also be a cause of lymphopenia. In this case, the circulatory pattern of the lymphocytes is mechanically interrupted in the lymph nodes, where massive proliferation of malignant cells simply blocks the migration of normal lymphocytes from the node back into lymph and eventually back to blood.

Abnormal Lymphocyte Morphology

Two forms of abnormal lymphocyte morphology are typically described: reactive lymphocytes and atypical lymphocytes.

In truth, reactive lymphocytes are not abnormal at all, but simply reflect normal function of the immunocytic system. When unstimulated lymphocytes come in contact with antigens to which they are sensitized, they undergo a process known as blast transformation. The products of blast transformation are reactive lymphocytes. Reactive lymphocytes are larger than normal, with abundant deep blue cytoplasm and vesiculate, often eccentric, nuclei. When present in large numbers, reactive lymphocytes indicate systemic antigenic stimulation.

Atypical lymphocytes are lymphocytes that exhibit any of a variety of morphologic aberrations. For example, lymphocytes with cleaved nuclei are classified as atypical. Nucleoli in circulating unstimulated lymphocytes are a feature of atypia. Other atypical lymphocytes have azurophilic cytoplasmic granules.

The significance of circulating atypical lymphocytes remains controversial. Historically, atypical lymphocytes were regarded as suggestive of viral infection. We now regard atypical lymphocytes simply as a nonspecific indicator of disease, as they have been seen in increased numbers in both infectious and noninfectious conditions.