The phagocytes: neutrophils and monocytes

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The production and deployment of phagocytes are central functions of the hematopoietic system. In the 1950s, radioisotopic studies demonstrated the high production rate and short lifespan of neutrophils and allowed researchers to follow the monocytes as they moved from the marrow through the blood to become tissue macrophages, histiocytes, and dendritic cells. Subsequently, the discovery of the colony-stimulating factors greatly improved understanding the regulation of phagocyte production. The discovery of the microbiidal myeloperoxidase-H2O2-halide system and the importance of NADPH oxidase to the generation of H2O2 also stimulated intense interest in phagocyte disorders. More recent research has focused on membrane receptors and the dynamics of the responses of phagocytes to external factors including immunoglobulins, complement proteins, cytokines, chemokines, integrins, and selectins. Phagocytes express toll-like receptors that aid in the clearance of a wide range of microbial pathogens and their products. Phagocytes are also important sources of pro- and anti-inflammatory cytokines, thus participating in host defenses through a variety of mechanisms. Over the last 50 years, many genetic and molecular disorders of phagocytes have been identified, leading to improved diagnosis and treatment of conditions which predispose patients to the risk of recurrent fevers and infectious diseases. (Blood. 2008;112:935-945)

Introduction

The importance of “lauable pus” in the response to injury and infection was recognized in ancient times. In the book The Healing Hand: Man and Wound in the Ancient World, Majno wrote:

Pus is therefore a noble substance: it is made of brave cells that never sneak back into the blood vessels to escape; they all die in the line of duty. Note also the double meaning of suppuration: it indicates that there is an infection, but also that the body is fighting it well. The outcome of the battle can be predicted, to some extent, from the aspect of the pus, as was observed even in ancient times. The whitish, creamy kind (and therefore rich in polys) is “preferable,” because it indicates that an infection is being fought effectively. Hence its ancient Latin name of pus bonum et laudable, “good and laudable pus.” Thin or malodorous pus suggests a poor defense of especially vicious bacteria.1

Much of what we know about the cellular components of the inflammatory response was gradually discovered in the 19th and early 20th centuries.2 Landmark reports include the following: (1) In 1841, William Addison compared colorless corpuscles in the blood with those of inflamed tissues and proposed that leukocytes get to the tissues by diapedesis.3 (2) In 1873, Cohnheim reported the margination of leukocytes along vessel walls and leukocyte protrusion and extravasation through the vessel wall (ie, transmigration) to reach the extravascular tissues.4 (3) In 1880, Ehrlich developed the staining techniques that facilitated identification of developing phagocytes in the bone marrow, blood, and tissues.5 Using Ehrlich’s stains, leukocyte counting and microscopic observations of blood cells became common, leading to clear definitions of normal counts, leukemia, leukopenia, neutropenia, and agranulocytosis.6 (4) In 1884, Metchnikov microscopically observed the phagocytic process, first in ameboid cells of the marine sponge and later in higher species. He is credited with the origin of the terms phagocyte and phagocytosis.7 (5) In 1904, Arneth introduced the counting of the lobes of neutrophils as an index for the maturity of neutrophils and coined the term shift to the left to describe an abnormal number of immature neutrophils on a blood smear.8 (6) In 1911, Schilling developed the current format of the leukocyte differential count. He coined the term, regenerative shift, to designate the outpouring of neutrophils in response to infection. The term, degenerative shift, was used to describe a failure of this response.9 (7) In 1942, the first edition of Wintrobe’s text, Clinical Hematology, was published and contained clear descriptions and excellent illustrations of normal and abnormal leukocytes. The text described neutrophil chemotaxis, phagocytosis, the proteolytic enzymes of the neutrophil granules, and “toxic granules.” Wintrobe also described physiological leukocytosis and the changes in leukocyte, neutrophil, and monocyte counts with age. He gave excellent descriptions of leukemia, agranulocytosis, infectious mononucleosis, and leukopenia with splenomegaly.10 (8) In 1948, Beeson described the release of “endogenous pyrogen” from leukocytes exposed in vitro to particles. This pioneering study was instrumental in our understanding the association of fever, leukocytosis, and neutrophilia. Furthermore, findings from his studies provided the fundamental basis for future studies on the physiological effects of endogenous cytokines.11

The fifth edition of Wintrobe’s Clinical Hematology, published soon after the beginning of Blood, had extensive chapters on phagocytes. The text described their abundant glycogen in the cytoplasm of neutrophils and aerobic glycolysis with oxygen consumption, glucose utilization, and lactic acid. It also described leukocyte alkaline phosphatase and its variation in chronic myeloproliferative diseases.
By 1960, hematology sections in general medical texts, such as Harrison's Principles of Internal Medicine, contained specific sections on leukocyte disorders, particularly leukemia, agranulocytosis, and the diseases causing pancytopenia. This brief review outlines some of the pivotal discoveries in phagocyte biology during the past 50 years. These discoveries have significantly increased our understanding of host defense and immunity, leading to important advances in the practice of medicine.

Neutrophils (polymorphonuclear leukocytes)

Production and kinetics

Radioisotopic tracers were first introduced to study the origin and fate of hematopoietic cells in the early 1950s. Results from this line of investigation and the growing understanding of hematopoietic stem cells by Osgood, Lajtha, Fliedner, McCullough, and others provided important foundations for many major advances in hematology and oncology in the 1960s and the years that followed. At the beginning of this era, studies of bone marrow and blood counts after radiation-induced injury or administration of early chemotherapeutic agents, such as nitrogen mustard, suggested that neutrophil precursors have a very high proliferative rate and that mature neutrophils have a short lifespan. Craddock et al used 32P to investigate replicating marrow cells in dogs and defined the mature nondividing compartment of neutrophils in the normal marrow, as well as the effects of leukopheresis, endotoxin, and inflammation on developing and mature neutrophils in the marrow.13 Cronkite and Fliedner quantified the high proliferative rate of neutrophil precursors using tritiated thymidine.14 In this same time period, Athens et al used 32P-diisopropylfluorophosphate (32P DFP), which binds irreversibly to the serine proteases of the neutrophil granules, to investigate and quantify the production and fate of blood neutrophils in dogs and in humans.15 These studies confirmed early reports that neutrophils have a short half-life and high turnover rate in peripheral blood and that the circulating and marginal pools of blood neutrophils are in a dynamic equilibrium16 (Figure 1).

Quantitation of marrow production of neutrophils and monocytes took another step forward when Finch et al, using radioactive iron to determine the erythropoietic mass,17 and his trainees, Dancey, Dubelbeiss, and coworkers quantified the absolute and relative size and turnover of myeloid cell compartments in the marrow and described the “effectiveness” of production based on the ratio of marrow production versus peripheral cell turnover (Dancey and Dubelbeiss18 and Dubelbeiss et al19). Price et al then extended these studies and showed that patients with chronic neutropenia have “ineffective production” with cell loss along the developmental pathway.20 This work presaged the concept of accelerated apoptosis as a common mechanism for chronic neutropenia and myelodysplasia. Although kinetics and turnover of radiolabeled blood cells are now rarely done, they were instructive in the 1990s in the development of clinical applications and understanding of the effects of the hematopoietic growth factors, granulocyte colony-stimulating factor (G-CSF), and granulocyte-macrophage colony-stimulating factor (GM-CSF).21 Nuclear medicine studies with labeled cells are also now frequently used to detect occult infections and abscesses.

Hematopoietic growth factors and the regulation of neutrophil production

Prior to the 1960s, there were many efforts to define leukopoietins, the myeloid equivalent of erythropoietin, but essentially all of these efforts were unsuccessful. In the mid 1960s, Bradley and Metcalf in Australia and Pluzink and Sachs in Israel independently developed in vitro culture techniques for hematopoietic cells and discovered the colony-stimulating factors (Figure 2).25 It was soon learned that these factors were endogenously produced and released during neutropenia and infection and after endotoxin administration. Subsequently, rapid advances in molecular biology during the late 1970s allowed cloning of the genes for these growth factors and their receptors. These studies led to fundamental insights into the mechanisms responsible for physiological regulation of neutrophil production. Targeted genetic disruption (“knock-out”) experiments in mice clearly showed that G-CSF and its receptor are essential for the maintenance of normal levels of blood neutrophils,26,27 similar to findings in dogs made neutropenic by immunologic neutralization of their endogenous G-CSF. Moreover, G-CSF was demonstrated to play a critical role in the leukocytosis caused by bacterial infection.28

Therapeutically, the development of the colony-stimulating factors as therapeutic agents has had a major impact on the practice of hematology and oncology. Both G-CSF and GM-CSF have a multitude of pharmacological effects, including increasing the proliferative activity of progenitor cells, shortening the time for
neutrophil production and maturation in the marrow, accelerating the release of maturing cells from the marrow to the blood, augmenting the production of neutrophil granule proteins, and stimulating the release of proteases and perhaps other constituents from the cells to their surroundings. Somewhat serendipitously, it was learned that these factors, as part of their effect to expand the hematopoietic tissue mass and the production and deployment of phagocytes, stimulate the release of progenitor cells from the marrow to the blood. The harvest of these cells (ie, CD34+ hematopoietic stem cells) by leukapheresis has dramatically changed the practice of hematopoietic stem cell transplantation.

**Neutrophil granules and granule-associated proteins**

The presence of granules in neutrophils, monocytes, and eosinophils was recognized by Metchnikov and Ehrlich, and the proteins associated with neutrophils were first defined through biochemical and histochemical studies beginning early in the 20th century. In 1952, Chediak, a Cuban physician, described patients with an autosomal recessive disease with several distinctive characteristics, including abnormal leukocyte granules. This disease subsequently became known as the Chediak-Higashi-Steinbrinck syndrome. In pioneering work first reported in the early 1960s, Cohn and Hirsch, building on earlier studies of de Duve et al., isolated and characterized granules from rabbit neutrophils and described the fusion of the neutrophil granule with ingested particles to form a “digestive vacuole” or “phagosome.” Hirschhorn and Weissmann then extended these studies to human neutrophils, thus providing a dynamic description of the process of phagocytosis described earlier by Metchnikov.

Advances in electron microscopy in this same era allowed dissection of the phagocytic process, and phase contrast microscopy allowed visualization of the killing of microbes. Extension of this work has defined organisms that are relatively readily killed by neutrophils and monocytes, such as streptococci and yeast, and microorganisms that are relatively protected and survive in phagocytes, such as *Mycobacterium* and *Salmonella*.

Purification, quantification, and understanding of the role of each of the phagocyte granule proteins have proved a complex task. From the work of Bainton et al., it was learned that granule proteins are produced in sequence, with the earliest proteins produced in myeloid progenitors and packaged in primary granules. Subsequently, phagocytes produce proteins that are packaged in secondary and tertiary granules, under control of genes that in turn are regulated by distinct transcription factors.

Neutrophil granules serve as reservoirs for digestive and hydrolytic enzymes prior to delivery into the phagosome. Pioneering studies by Spitznagel, Elsbach and Weiss, and Ganz et al. indicated that azurophilic granule contents possess microbicidal activity and may play an important role in the tissue destruction observed during inflammatory reactions.

Probably because of their overlapping functions, natural deficiencies or “knockouts” of the granule proteins are not necessarily associated with enhanced susceptibility to infection. The condition termed specific granule deficiency, a disorder in which all the secondary granules of the neutrophil are absent secondary to the functional loss of the transcription factor C/EBP γ or Gf-1, is associated with recurrent bacterial infections caused by *Staphylococcus aureus* and *Pseudomonas spp*. In contrast, congenital deficiency of myeloperoxidase from the primary granules of neutrophils is generally not associated with an increased risk of serious infections. More recently, researchers found that mutations in the gene for another primary granule protein, neutrophil elastase or ELA-2, are the cause for most cases of cyclic and severe congenital neutropenia.

**Microbicidal mechanisms of neutrophils**

The killing of microbes is a critical physiological function of phagocytes. How this occurs was perhaps the most interesting and important observation related to these cells of the last half century. Before the 1950s, the general aspects of the process of phagocytosis—from the rich glycogen supply of the neutrophil cytoplasm to the enzymatic contents of the neutrophil granules—were already recognized. In the early 1950s, Valentine and Beck described glycolysis by leukocytes and Sbarra and Karnovsky described the burst of glycolysis that occurs associated with phagocytosis. Klebanoff then discovered that myeloperoxidase, known from work in the 1920s to be released from neutrophils during phagocytosis, was involved in generating hydrogen peroxide and proposed that it is potentially the critical antimicrobial substance generated within neutrophils. Further experimentation clarified that another oxidase, NADPH oxidase, also played an important role in the generation of hydrogen peroxide and other reactive oxygen species. This observation led Klebanoff to pursue further experiments demonstrating that hydrogen peroxide interacted with myeloperoxidase and a halide, predominantly chloride, to generate the potent antibacterial substance, hypochlorous acid, within the phagosome (Figure 3). Although phagocytes have other microbicidal mechanisms, including antimicrobial peptides (eg, defensins) and broadly acting proteases, phagocytosis with generation of reactive oxygen species and hypochlorous acid is still regarded as the critical killing mechanism for most invading pathogens.

It had been known since 1932 that a marked increase in neutrophil oxygen consumption, termed the respiratory burst, occurred during phagocytosis. Stimulated neutrophils oxidize NADPH through a reaction yielding hydrogen peroxide. The clinical significance of these findings was recognized by Baehner and Nathan, who observed that neutrophils from chronic granulomatous disease (CGD) patients failed to generate the products of the respiratory burst.
The neutrophil metabolic burst and chronic granulomatous disease

Following the discovery of the importance of NADPH oxidase, mentioned above, investigators elucidated its components and the effects of mutations in the NADPH complex over a period of several decades. Segal et al. and Segal and Jones identified a cytochrome within phagocytic vacuoles that was missing or functioned abnormally in cell homogenetics from CGD patients. It was referred to as cytochrome b558 and was later found to be composed of a heavy and light chain. The gene for X-linked CGD at p2.1 was identified by the first use of positional cloning by the Orkin laboratory (Royer-Pokora et al.), and in complementary studies Parkos et al. purified the peptide constituents of cytochrome b, which led to the identification of the gene product as the heavy chain of cytochrome b558 that was lacking in X-linked CGD. Further studies by Roos documented a number of different mutations responsible for X-linked CGD. The disease caused by mutations in NADPH oxidase, CGD, was first described in 1957 by Berendes et al. and Landing and Shirkey. In 1967, Quie et al. reported that a microbicidal defect in the neutrophils of affected children caused recurrent bacterial and fungal infections, associated with early mortality. It is now recognized that CGD can be caused by genetic alterations in one of the components of NADPH oxidase, including gp91phox, p47phox, p67phox, and p22phox. Nunoi et al. and Volpp et al. identified 2 forms of autosomal CGD in which either a 47-kDa or a 67-kDa protein was genetically altered. Subsequently, defects in the light chain of cytochrome b558 known as p22phox were found to account for some CGD cases. The lack of function of any of the 4 components of the NADPH oxidase leads to a failure to generate hydrogen peroxide in response to bacterial or fungal infection with catalase-positive organisms. Mouse knockout models by Goebel and Dinauer and Jackson et al. of CGD have provided further insight on pathogen virulence factors (Figure 4). Unraveling the details of the molecular biology, biochemistry, and potential therapies for this disorder have been a major focus of phagocyte research for more than 40 years. Such studies have shed light on the role of prophylactic therapies with gamma-interferon and itraconazole as well as the use of curative therapies with stem cell transplantation and gene therapy.

The neutrophil surface and its receptors

The surface of the neutrophil is complex, with myriads of folds, crevices, and sites for interaction of the neutrophil with its surroundings. Receptors for interaction with opsonins (eg, receptors Fc R-I, -II, and -III, and fragments of the third component of complement) are expressed on both neutrophils and monocytes that are critical surface receptors for facilitating phagocytic movement.
and ingestion through pathways affecting cytoskeletal reorganization.77,78 The complement-mediated reactions that generate chemotactic factors in plasma also produce by-products that coat microbes and opsonize them such as C3b as shown by Alper and Rosen79 and Muller-Eberhardt80 to be critical in preventing infection in patients with C3 deficiency. Antibody participation in opsonization including IgM and in some circumstances IgG was elegantly demonstrated by Brown et al to activate complement components, which resulted in the deposition of C3 on the surface microbes to initiate complement-dependent opsonization of encapsulated virulent pathogens.81

The birth of understanding of immunodeficiency came with the report of the first case of X-linked agammaglobulinemia in 1952 by Bruton et al.82 The primary abnormality in this disorder is the B-cell failure to produce gamma-globulins, but the functional abnormality rests with the failure of the phagocytic process to halt bacterial invasion of tissues and to clear bacterial pathogens from the blood.83 Abnormalities in the complement system (ie, deficiency in components C3 and C5) that were discovered later led to defective opsonization and chemotaxis of neutrophils, respectively, because of deficiencies of these proteins.84

Neutrophils and monocytes also express cell-adhesion molecules, such as selectins and integrins, which, if mutated as seen in leukocyte adhesion deficiency 2 and leukocyte adhesion deficiency 1, respectively, affect the trafficking of neutrophils by impeding their rolling and subsequent adhesion to the capillary vascular wall in the process of diapedesis and eventual migration into tissue.85-87 As first described by Hayward et al.88 and Crowley et al.,89 patients with mutations impairing the expression of CD18, which affects the function of the leukocyte integrin CD11/CD18, are usually recognized by having neutrophilia with the inability to produce “laudable pus.”90 It is now well recognized that there are several variants of leukocyte adhesion deficiency, each illustrating the critical importance of neutrophil adherence for normal host defenses.91

Neutrophils bear a family of receptors that facilitate the migration of phagocytes after they leave the vascular compartment. This critical response can be triggered in a multiplicity of ways, and the development of the Boyden chamber was strategically important for dissecting the specific roles of individual chemotactic factors, as demonstrated by Ward and Becker.92 In addition to the complement receptors (ie, receptors for C5a and C3b) and C3bi, neutrophils have several other chemotactic receptors. These include receptors for bacterially derived or synthesized N-formyl peptides, platelet activating factor (PAF), leukotriene B-4 (LTB-4), and a variety of other chemokines and ligands for Toll-like receptors.93-95

The importance of the chemokine receptors is illustrated by findings in patients with the myelokathexis syndrome, also referred to as WHIM (warts, hypogammaglobulinemia, infections, and myelokathexis) syndrome, first described in the 1960s by Zuelzer.96 Patients who have this disorder have severe leukopenia and neutropenia, with accumulation of neutrophils in the bone marrow, and often have many surface warts. This syndrome is now attributable to a defect in the chemokine receptor CXCR-4.97 Observations in these patients have suggested an important role for the ligand, stromal-derived factor 1 (SDF-1), in regulating neutrophil migration as well as the trafficking of lymphocytes and hematopoietic progenitor cells.98

Neutrophils also bear surface receptors for the colony-stimulating factors granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) from early in development to the mature circulating neutrophil.99 This was a surprising finding, in light of the fact that the CSFs were discovered as growth factors and that receptors for the parallel growth factor, erythropoietin, are not expressed on mature erythrocytes. Experimentally, G-CSF and GM-CSF enhance the responsiveness of mature neutrophils to other stimuli, priming them for an enhanced metabolic burst.100 In addition, these growth factors have an antiapoptotic effect on mature neutrophils, prolonging their survival.101 Thus at a tissue site of an infection, where cytokines and growth factors are generated, the inflammatory milieu promotes the survival and function of arriving phagocytes and promotes further recruitment of phagocytes from the bone marrow and blood.

**Phagocyte membrane dynamics and stimulus-response coupling**

The neutrophil is responsive to chemotactic factors and ingested particles, and undergoes metabolic and morphologic changes. Ligand binding to neutrophil surface induces hyperpolarization and calcium fluxes increase, and cyclic AMP rises transiently.102-104 Zigmund observed chemotactic factors polarize and orient attached neutrophils for locomotion.105 Hirsch found that neutrophils in response to a chemotactic source acquire a characteristic asymmetric shape.106 In the front of the cell is a pseudopodium that advances before the body of the cell containing the nucleus and the cytoplasmic granules. In the posterior of the moving cell is a knoblike tail. The formation of the pseudopodium is essential for neutrophil locomotion. Contributions of Stossel in describing cytoskeletal chemistry provided insight into the basis of neutrophil locomotion, particle ingestion, and digestion.77 The hyaline pseudopodia of the neutrophil contains filament networks composed of actin filaments and other regulatory proteins. The actin in the pseudopodia exists as a gel and is concentrated at the cell periphery along with myosin, which upon being engaged permits phagocytosis to occur. A rise in calcium concentration dissolves the actin gel by activating the protein gelsolin, which shortens actin filaments and allows for sol formation permitting neutrophil movement and directionality.

When a neutrophil comes in contact with a particle, the pseudopodium flows round the particle. Its extensions fuse and thereby it compasses the particle within the phagosome. Silverstein observed the phagocyte membrane adheres firmly to particles it ingests and surrounds the particle in a zipperlike fashion.107

Leukocyte chemoattractants induce a series of metabolic changes including activation of trimeric G-proteins followed by enhancing intracellular calcium levels, lipid remodeling, and protein kinase activations. These events culminate in fusion of granule membranes with phagosomes or with the plasma membrane.108 Leukocyte chemoattractants stimulate signaling pathways that evolve are Rho GTPases, including Rac-dependent NADPH oxidase activation, Rac- and Rho-dependent phospholipase D activity, and Rac- and CDC-42–regulated p21-activated protein kinases.109 The studies by Bokoch109 have illuminated the role of the GTPases especially the necessary requirement for Rac-dependent and NADPH oxidase activation. The importance of Rac-2 function in human neutrophil chemotaxis and NADPH oxidase activation has been highlighted by the discovery of a toddler with a naturally occurring dominant-negative Rac-2 mutation. This individual suffered from severe, recurrent infection, a markedly reduced
neutrophil migration, and NADPH oxidase–dependent activation.10,11

Studies by Greenberg and Grinstein in macrophages have contributed to the understanding of the mechanism of phagocytosis initiated by Fc receptor engagement.12 This includes cytoskeletal alterations and membrane trafficking. The Fc receptors require phosphorylation of the receptors themselves or associated immunoreceptor tyrosine-based activating motif (ITAM)–containing subunits, by members of the Src family. Phosphorylated receptor subunit ITAMs serve as docking sites for Syk processes in phagocytosis mediated by the engagement of the Fc receptor. Pseudopodia extension in turn requires a wave of lipid remodeling in which phosphatidylinositol 3 kinase (PI3K) is generated at the phagosomes. Cessation of PI3 kinase is abrupt and in part is due to the recruitment of lipid phosphatase (SHIP) to the phagocytic cup.113,114 The synthesis of phosphatidyl 1-4,5, bisphosphate is also accelerated during phagocytosis. This lipid serves as a substrate of PI3K. It is also the target of phospholipase C, which generates diacylglycerol during phagocytosis. The latter mediator can activate protein kinase C, which can be recruited to the phagosome and participate in particle uptake. Other kinases implicated in phagocytosis include MEK-1, which may be selectively involved in Fc γR-mediated phagocytosis in neutrophils but not in macrophages.115 Both phospholipase A2 and phospholipase D are also activated and are thought to participate in the phagocytic process. The former product may participate in degranulation during phagocytosis as well as amplify the production of leukotrienes that amplify the phagocytic signal.

Co-opting phagocytic machinery by invasive pathogens

The initial response to most bacterial and fungal pathogens is phagocytosis through distinct receptors. Listeria is able to co-opt a receptor kinase to invade the host cells.116 The Listeria infection can be fatal in immunocompromised patients, neonates, and pregnant women. Southwick and Purich observed that Listeria can co-opt the receptor tyrosine kinase to invade host cells. In turn, Listeria can usurp the cytoskeletal system of the host cell and survive and thrive within the host.117 Actin assembly is essential for the cell-to-cell spread of Listeria, which allows it to move through the cytoplasm of host cells and to be transferred from one host cell to another, thereby invading the host immune system.

Other pathogens synthesize antiphagocytic factors. For an example, Yersinia secreties YepH, a tyrosine phosphatase that dephosphorylates a focal adhesion protein Cas.118 Other pathogens such as Microbacterium tuberculosis can suppress calcium signaling thereby inhibiting phagolysosome fusion and allowing for survival in the phagosome.119

Monocytes

The production and life cycle for mononuclear phagocytes is more complex than for neutrophils. Neutrophils follow a relatively simple pathway from the marrow to the blood and the tissues. Although they share some similar physiological capacities as neutrophils, marrow and blood monocytes retain a proliferative capacity and can differentiate into resident phagocytic cells, broadly termed macrophages and histiocytes in the spleen, liver, and lungs and other tissues. During chronic inflammatory conditions including sarcoidosis and tuberculosis, these cells can fuse to form giant cells. Historically, this system of tissue-based mononuclear phagocytes was called the reticuloendothelial system.120

Life cycle and progeny

The complex life cycle of monocytes led Virchow and other prominent 19th century pathologists to believe that macrophages were derived from mesenchymal tissue, rather than blood cells. Only in the modern era, using radioisotopic labeling of blood and marrow cells, has it been possible to establish that circulating monocytes are the precursors for macrophages in all tissues. The studies of Lewis and Lewis,121 Cohn and Benson,122 van Furth and Cohn,123 and Nichols et al124 were landmark papers regarding monocyte development and differentiation. Later work revealed that monocytes are not homogeneous but actually represent at least 2 distinct subsets of mononuclear phagocytes.125-127 Recently, it has been conclusively demonstrated that monocytes also serve as the precursors of dendritic cells, which play an important role in host defense as potent antigen-presenting cells during T-lymphocyte activation.128-131

Mononuclear phagocytes of the blood and tissues survive far longer than neutrophils. This feature of phagocytes is clinically very important. It protects patients from an overwhelming risk of fatal infections when neutrophil production is transiently interrupted, as occurs with cancer chemotherapy, idiosyncratic reactions to many drugs, and hematopoietic stem cell transplantation.

More comparisons of monocytes and neutrophils

Over the past 50 years, researchers have continuously focused on defining the similarities and differences between neutrophils and monocytes. Mononuclear phagocytes share many properties with neutrophils, but they also have distinctive morphologic and functional properties, depending upon their state of differentiation. For example, the granule-associated proteins are similar to those of neutrophils, but there are some distinctive differences.132 Monocytes have a preserved capacity to augment production of granule proteins through new protein synthesis, a feature that is lost in mature neutrophils. There are also significant differences in their chemotactic responses and metabolic burst activity during phagocytosis.132 At a site of acute inflammation, monocytes accumulate more slowly, but persist longer. Their metabolic burst is less extreme, but their capacity to kill many microbes is more diverse compared with that of neutrophils. Monocytes have Fc receptors and express the IgG receptor Fc,RI (CD64) constitutively in contrast to neutrophils, which express this receptor only in response to inflammatory stimuli.132

An important difference between neutrophils and monocytes is also in their capacity to produce new proteins, including a variety of cytokines associated with enhancement of the inflammatory response. Of historical interest in this regard, the "endogenous pyrogen," discovered by Beeson and thought for many years to be primarily a product of neutrophils, is now known to be predominantly produced by mononuclear phagocytes, because of their far greater capacity for cytokine production.133,134

Physiological functions

The human immune system has been divided traditionally into innate immunity and acquired (adaptive) immunity. Monocytes and their differentiated progeny play important regulatory and effector roles in both arms of the human immune system.135-137 Mononuclear phagocytes have at least 3 major functions: presentation of antigens as discussed above, phagocytosis, and immunomodulation. Mononuclear phagocytes ingest material for 2 purposes: to eliminate waste and debris and to kill invading pathogens. Mononuclear phagocytes dispose of effete and aged red cells and remove
red cell inclusions in the spleen. They also clean up debris at sites of infection or tissue damage.\textsuperscript{138,139}

Activated monocytes and macrophages also release IL-1, IL-6, TNF, and INF-\(\alpha/\beta\)—cytokines that are involved in the regulation of hematopoiesis.\textsuperscript{140} Monocytes are also subject to immune modulation through the role of chemokines. The chemokines MIP-1\(\alpha\), MIP-1\(\beta\), and RANTES produced by CD8\(^+\) T cells inhibit human immunodeficiency virus infection by monocyte trophic-1 strains.\textsuperscript{141}

Macrophages as shown by Nathan and colleagues can activate nitric oxide synthase, which leads to the synthesis of nitric oxide (MacMicking et al\textsuperscript{142}). In turn, sustained production of nitric oxide endows macrophages with cytostatic or cytotoxic activity against viruses, bacteria, fungi, protozoa, helminthes, and tumor cells. Recent studies by Tall and colleagues suggest that the macrophage contributes to the pathogenesis of both atherosclerosis and insulin resistance (Liang et al\textsuperscript{143}). Insulin-resistant macrophages in the arterial wall undergo increased apoptosis, which may lead to larger lipid-rich cores, increased inflammation, and more plaque formation.

Monocytes, macrophages, and dendritic cells express a large number of cell surface proteins that play crucial functional roles in phagocyte biology. Microbial pattern-recognition receptors are an essential component of innate immunity, in which they recognize and detect pathogen-associated molecular patterns, resulting in activation of monocytes, macrophages, and dendritic cells (and neutrophils) as part of the host response to eradicate invading pathogens.

An important class of pattern-recognition receptors is the recently described mammalian Toll-like receptor (TLR) family, which recognizes a wide range of microbial pathogens and pathogen-related products (Figure 5).\textsuperscript{144-147} TLRs are expressed to a far higher degree by monocytes than neutrophils. Upon binding of specific ligands, TLRs signal via a pathway involving the adaptor protein MyD88, or via a MyD88-independent pathway involving TRIF, to activate NF-\(\kappa\)B and stimulate proinflammatory cytokine production from monocytes and macrophages.\textsuperscript{148,149} Other cell-based receptors may cooperate with specific TLRs to enhance pathogen recognition. For example, CD14 binds LPS and interacts with TLR4 to facilitate recognition and enhance eradication of Gram-negative bacilli from the circulation and tissue sites.\textsuperscript{150}

**Congenital defects in macrophage function**

Recent progress in the elucidation of the genetic and molecular basis of congenital immunodeficiency disorders has defined critical factors regulating monocyte/macrophage-mediated innate immune responses. Functional mutations in the interleukin-12 (IL-12)/interleukin-23/interferon-\(\gamma\) (IFN\(\gamma\)) axis are associated with nontuberculous mycobacterial infections due to the inability of monocytes and macrophages to kill these organisms of relatively low pathogenicity following phagocytosis.\textsuperscript{151-153} X-linked susceptibility to mycobacterial disease has recently been attributed to functional mutations in NEMO, the \(\gamma\)-subunit of I\(\kappa\)B (inhibitor of
NF-κB) kinase (IKK) complex, that regulates the activation of the NF-κB signaling pathway.\textsuperscript{154} Deficiency of IL-1 receptor–associated kinase 4 (IKAR4), a component of the TLR/IL-1–mediated MyD88-dependent signaling pathway described above, has been shown to predispose affected individuals to invasive pneumococcal disease.\textsuperscript{155,156}

Finally, several groups have recently announced the discovery of the mutations that appear to be responsible for the pathogenesis of hyper-IgE syndrome (formerly Job syndrome)—a severe multisystemic disease associated with recurrent and severe staphylococcal infections of the lungs and skin.\textsuperscript{157,158} These findings highlight the fundamental and essential role of the cytokine-induced JAK-STAT (Janus kinase–signal transducer and activator of transcription) pathway in phagocyte-mediated host defense against microbial pathogens.

Final comments
This brief historical review only touches on the many significant events in advancing our understanding of phagocytes over the past 50 years. Only a few of the thousands of landmark papers are mentioned, and the authors regret having to omit numerous other worthy and important citations. Nevertheless, on this 50th anniversary of \textit{Blood}, it is valuable to reflect on the major progress that has been made in understanding phagocyte biology. These advances provide an excellent foundation for further research to find improved therapeutic strategies and options for our patients.

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David C. Dale

My interest in hematology began in the early 1960s when Drs David Nathan and Frank Gardner were inspiring second-year teachers in the hematology course at the Harvard Medical School. I then had Drs William Castle and Jim Jandl as advisors and teachers in a medicine clerkship at the Boston City Hospital, and they stimulated my interest in hematology and research. I began studying host defense mechanisms, neutrophils, and the problem of neutropenia under the mentorship of Dr Sheldon Wolff in the Laboratory of Investigation of the NIAID at the NIH in the late 1960s. Shelly, as we affectionately called him, got me interested in cyclic neutropenia in humans and gray collie dogs, the study of the colony-stimulating factors, the formation and fate of neutrophils, and the search for treatments to improve the well-being of patients with neutropenia. My enjoyment of the study of phagocytes has come through caring for patients who are susceptible to infections and through friendships with fellow researchers in this interesting field. My coauthors, Drs Laurence Boxer and Conrad Liles, are special friends with whom I have enjoyed working for many years.

W. Laurence Boxer

Beginning with my tour of duty in the United States Army I became interested in the pathophysiolog of neutropenic disorders. When I entered my fellowship in the laboratory of Tom Stossel at the Children’s Hospital Medical Center in Boston, I was afforded the opportunity to build a better mousetrap to identify antibodies directed against neutrophils. While a fellow, I also had the opportunity to unravel some disorders of phagocyte function, which became another passion of mine throughout my career. The subsequent study of neutrophil abnormalities obtained from patients with recurrent bacterial infections paved the way over the years to a better understanding of the components of oxidative and nonoxidative microbial killing mediated by neutrophils. The discovery and eventual clinical application of granulocyte colony-stimulating factor for the treatment of patients with severe chronic neutropenia have provided the opportunity to engage in translational research and explore the mechanisms underlying the basis for the production abnormalities associated with severe chronic neutropenia disorders. All in all, it has been highly gratifying to engage in research that has improved the understanding of the disorders of the phagocyte and in many instances improved the lives of many patients.

W. Conrad Liles

My interest in phagocytes began during my fellowship in Infectious Diseases at the University of Washington under the mentorship of Seymour Klebanoff, MD, PhD. His meticulous and insightful studies of the mechanisms of killing of microbes by phagocytes were an inspiration to me and all of his trainees. During my fellowship, I became interested in the role and regulation of apoptosis and began to study the importance of Fas/Fas ligand system in innate immunity. Over the years, my research interests have expanded to include broader studies of inflammation, sepsis, congenital immunodeficiency disorders, and immunomodulatory therapies. In 2006, I moved from the University of Washington to become Director of the Division of Infectious Diseases and Vice Chair of the Department of Medicine at the University of Toronto, where I also serve as the Canada Research Chair in Inflammation and Infectious Diseases and am a member of the McLaughlin-Rotman Center for Global Health.
The phagocytes: neutrophils and monocytes

David C. Dale, Laurence Boxer and W. Conrad Liles