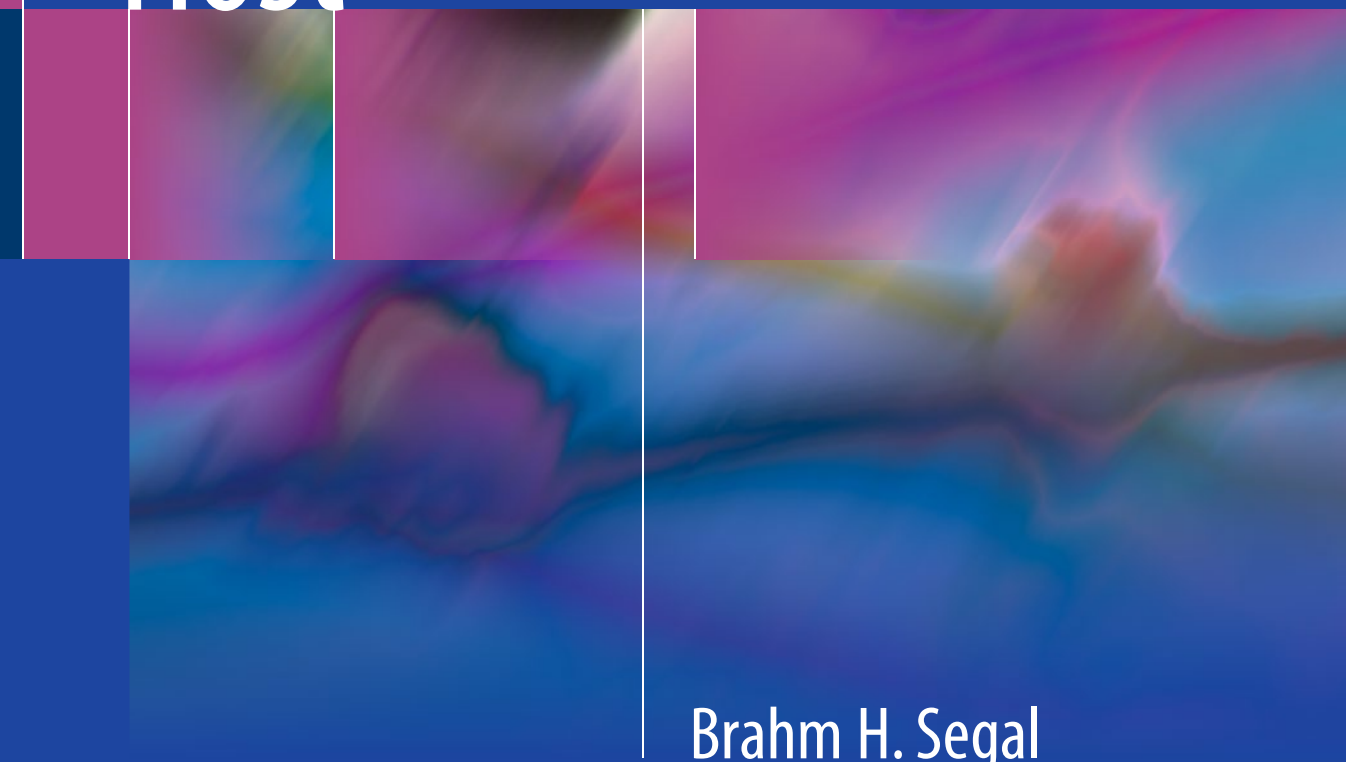
Management of Infections in the Immunocompromised Host



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To my wife, Stephanie, and to our children, Joshua and Emily.

Preface

"Now inflammation as understood in man and the higher animals is a phenomenon that almost always results from the intervention of some pathogenic microbe. So it is held that the afflux of mobile cells towards points of lesion shows the organism's reaction against foreign bodies in general and against infectious microbes in particular. On this hypothesis, disease would be a fight between the morbid agent, the microbe from outside, and the mobile cells of the organism itself. Cure would come from the victory of the cells and immunity would be the sign of their acting sufficiently to prevent the microbial onslaught" (Ilya Mechnikov, Nobel lecture, 1908). Mechnikov's conclusions were based on his seminal experiments involving the application of splinters to larvae of the starfish, Bipinnaria, that led to the discovery of phagocytosis as a critical factor in host defense. In the same lecture, Mechnikov noted that individuals have different susceptibility to infections: "It is often seen that in households where all members are exposed to the same danger, or again in schools or troops where everyone lives the same life, disease does not strike everyone indifferently."

The overriding theme of this textbook – that our immune system must sense pathogens, migrate to sites of infection, and kill pathogens or at least limit their growth to avoid disease, and that disorders of the immune system predispose to infection – is echoed in Mechnikov's prescient statements made 110 years ago. The progress made in our understanding of the immune system and development of novel immunotherapies for infectious diseases, cancer, autoimmunity, and other disorders has been extraordinary. The challenge of this textbook is to link knowledge about host defense to assist clinicians in a practical fashion in the care of patients with suspected or known immunodeficiencies and infectious diseases. In addition to practical knowledge applied at the bedside, we also aim to provide an understanding of gaps in knowledge, cutting-edge technology in immunotherapy, and future directions of research.

Because of the importance in understanding the normal immune system as a prerequisite for understanding immunodeficiencies, this textbook provides detailed overviews of phagocyte biology, complement, cytokines, and other soluble mediators of immunity, mucosal immunity, and T-cell and B-cell immunity. The next section of the textbook is focused on primary immunodeficiencies. Indeed, hundreds of primary immunodeficiencies have been described, the majority resulting from defects in single genes. From these patients, we learn that our immune system has redundant pathways for host

defense, and deficits in specific genes lead to susceptibility to specific pathogens.

The majority of immunodeficiencies are acquired rather than inherited. The major acquired immunodeficiencies include HIV infection, cancer, transplantation, and immunosuppressive therapy for autoimmune diseases. Among this large group of patients with acquired immunodeficiencies, important differences in infection risk are observed, and even within these patient groups, substantial heterogeneity exists regarding the underlying disease and intensity of immunosuppressive therapy. Severely immunocompromised patients can have substantial exposure to antibacterial, antifungal, and antiviral agents, both as prophylaxis and as treatment. In addition, these patients are frequently hospitalized and are at risk for nosocomial infections. It is therefore important to understand the growing trends in antimicrobial resistance and judicious use of antibacterial, antifungal, and antiviral agents to guide appropriate therapy. Chapters written by expert clinicians provide practical evidence-based approaches to prevention, diagnosis, and treatment of infectious complications in these patient populations.

The last chapters address standard and novel approaches for enhancing host defense in immunocompromised patients. They include vaccination of patients and household members and immunoglobulin therapy. Finally, dedicated chapters on stem cell transplantation for patients with primary immunodeficiencies, adoptive cellular immunotherapy, and gene therapy will provide readers with insight into these rapidly evolving and cutting-edge therapies.

I hope that this textbook will be of value to a broad readership, from trainees to clinicians and scientists interested in the fields of infectious diseases and immunology. I want to extend my gratitude to the expert authors who contributed chapters to this textbook. Needless to say, the success of this textbook is a direct result of their knowledge and effort. I also want to thank the staff at Springer for their helpful suggestions, efficiency, and commitment to the project.

Buffalo, NY, USA Brahm H. Segal

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Phagocytes

1

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Abbreviations

APC	Antigen-presenting cell
BMCP	Basophil-MC progenitor cell
BPI	Bactericidal/permeability-increasing protein
CCL2	CC-chemokine ligand 2
cDC	Classical DC
CDP	Common DC progenitor
CLP	Common lymphoid progenitor
CMP	Common myeloid progenitor
CXCL8	CXC-chemokine ligand 8 (IL-8)
DAMP	Damage-associated molecular pattern
DC	Dendritic cell
EPC	Embryonic progenitor cell
ESL1	E-selectin ligand 1
ET	Extracellular trap
GAG	Glycosaminoglycan
GMP	Granulocyte-macrophage progenitor
H_2O_2	Hydrogen peroxide
HMGB1	High-mobility group box 1
HNP	Human neutrophil peptide
HOCl	Hypochlorous acid
HSC	Hematopoietic stem cell
ICAM-1	Intercellular adhesion molecule 1
iNOS	Inducible nitric oxide synthase
JAM-A	Junctional adhesion molecule A
JAM-C	Junctional adhesion molecule C
LMPP	Lymphoid-myeloid multipotent progenitor
LPS	Lipopolysaccharide
MDP	Macrophage-DC progenitor
MEP	Megakaryocyte-erythrocyte progenitor
MHC	Major histocompatibility complex
MPO	Myeloperoxidase
MPP	Multipotent progenitor
NET	Neutrophil extracellular trap
PAMP	Pathogen-associated molecular pattern
pDC	Plasmacytoid DC
PECAM-1	Platelet-endothelial cell adhesion molecule-1 (CD31)
PMN	Polymorphonuclear leukocyte (or neutrophil)
PRR	Pattern recognition receptor
PSGL1	P-selectin glycoprotein ligand 1
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SYK	Spleen tyrosine kinase
TCR	T cell receptor
TEM	Transendothelial cell migration
TNF	Tumor necrosis factor
VCAM-1	Vasculature intercellular adhesion molecule 1
VLA-4	Very late antigen 4

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Origin and Development of Phagocytes

Phagocytic leukocytes are important for innate and acquired immunity. These cells are also involved in the initiation and resolution of the inflammatory response, and they maintain tissue homeostasis in the steady state. There are multiple types of phagocytes, and each can contribute uniquely to the maintenance of human health and the defense against microorganisms. Phagocytes originate from self-renewing and multipotent hematopoietic stem cells in bone marrow or during embryogenesis from yolk sac and/or fetal liver stem cells [1–3]. In the traditional model of hematopoiesis, multipotent progenitor (MPP) cells differentiate into common lymphoid progenitor (CLP) and common myeloid progenitor (CMP) cells (Fig. 1.1). CLPs differentiate ultimately into B cells, T cells, and natural killer cells. The CMPs give rise to granulocyte-macrophage progenitors (GMPs), which can then differentiate ultimately into phagocytes, including granulocytic phagocytes (neutrophils, eosinophils, and mast cells), and mononuclear phagocytes (monocytes, macrophages, and dendritic cells) [1].

Recent findings indicate that MPPs can differentiate into a lymphoid-myeloid multipotent progenitor cell (rather than a direct differentiation of MPPs to CLPs as described above), which in turn gives rise to GMPs, CLPs, or early thymic precursors (Fig. 1.1) [4]. Differentiation to phagocytes from GMPs in this model is similar to that in the traditional model. It is also noteworthy that dendritic cells and monocytes/macrophages can

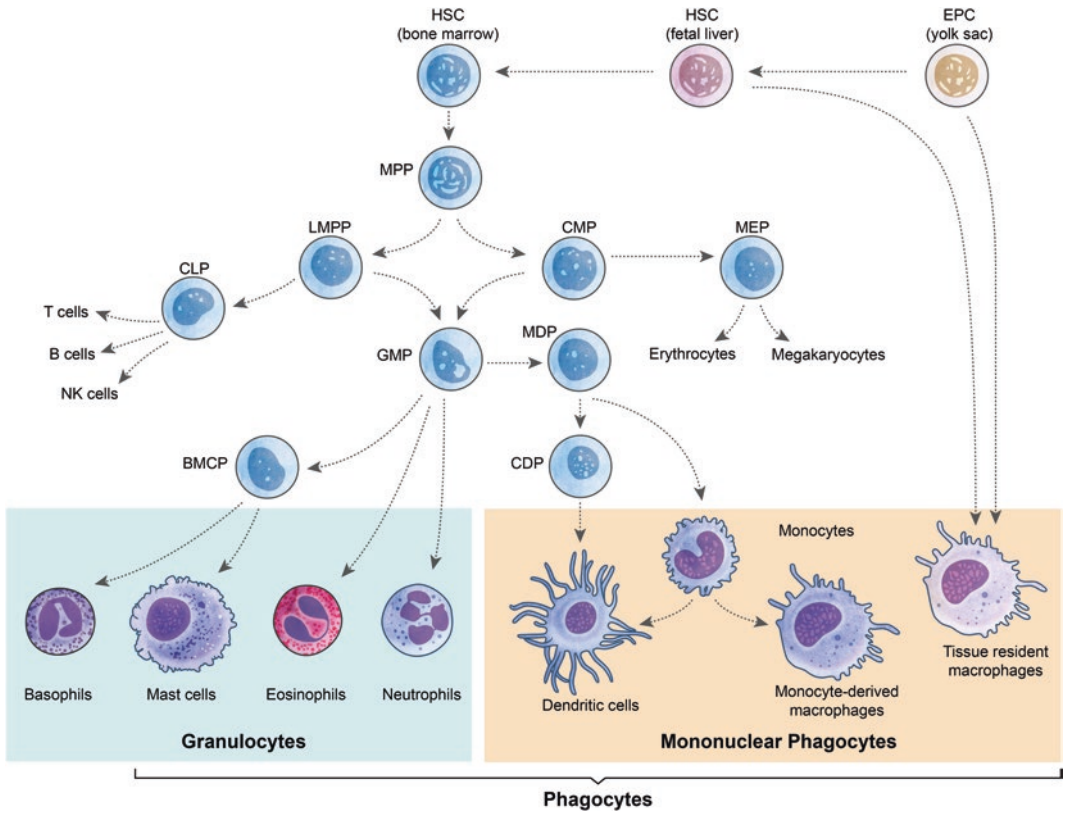

Fig. 1.1 Hematopoiesis and production of phagocytes. Leukocytes originate from embryonic progenitor cells in the fetal yolk sac, multipotent hematopoietic stem cells in bone marrow, and/or fetal liver stem cells. BMCP basophil/mast cell progenitor, CDP common dendritic cell, CLP common lymphoid progenitor, CMP common myeloid progenitor, DC dendritic cell, EPC embryonic progenitor cell, GMP granulocyte-macrophage progenitor, HSC hematopoietic stem cell, LMPP lymphoid-primed multipotent progenitor, MDP macrophage-dendritic cell progenitor, MEP megakaryocyte-erythrocyte progenitor, MPP multipotent progenitor

be derived from reprogramming of CLPs by specific cytokines [1, 5]. Therefore, the current model of hematopoiesis is not absolute and will need revision and updating as new discoveries are made.

Not all phagocytic leukocytes are produced during hematopoiesis in bone marrow. Based on studies in mice, a significant proportion of tissue-resident macrophages are now known to originate from stem cells during embryogenesis (Fig. 1.1) [2, 3]. These tissue macrophages develop from embryonic progenitor cells in the yolk sac or fetal liver and then self-renew and are thus maintained independent of blood monocytes [2, 6]. This is a major deviation from the traditional model of bone marrow hematopoiesis and the process of myeloid cell differentiation, from which all mononuclear phagocytes were thought to be derived [7]. A detailed review of hematopoiesis and phagocyte development is beyond the scope of this chapter, and we refer the reader to relevant articles on the topic [1–4, 8–15]. Instead, we highlight a few features of phagocyte development that are important for our understanding of the function of each cell type.

Mononuclear Phagocytes

Monocytes, macrophages, and dendritic cells (DCs) comprise cells of the mononuclear phagocyte system [16]. These cells are important for innate and adaptive immunity, and they play a key role as antigen-presenting cells and in maintaining immune system homeostasis. In humans, monocytes comprise ~10% of all leukocytes in blood (considerable variability between individuals exists), and production (as determined by turnover) is on the order of 7×10^6 cells/h/kg body weight [17–19]. There are ~3 times more monocytes in the marginal pool than in circulation in blood (~2×10^5 cells/ml) [17, 18]. The half-life for monocytes in human blood is ~1–2 days, although there is considerable variance among individuals [17, 19]. Hematopoiesis maintains steady-state production of monocytes that originate initially from a CMP and, then more proximally, from a recently described progenitor cell known as a macrophage-dendritic cell progenitor (MDP) [20, 21]. MDPs can differentiate to monocytes or to classical or plasmacytoid DCs via an intermediate known as a common DC progenitor cell (CDP) (Fig. 1.1) [22]. Human monocytes are characterized by cytochemistry, nuclear morphology, and surface expression of selected receptors. For example, monocyte subsets can have high, intermediate, and low surface expression of CD14. Those with comparatively high levels of CD14 on the cell surface represent the vast majority of monocytes in healthy humans and are known as classical monocytes [22]. More recent studies have shown that monocytes can be segregated further into distinct subsets based on high or low surface expression of CD16 [23], or those with high or low expression of CX3CR1, the fractalkine receptor [24]. Fractalkine (CX3CL1) has a number of functions, including stimulation of adhesion of leukocytes to activated endothelial cells. Depending on the stimulus or condition, a subset of monocytes can differentiate further to monocyte-derived dendritic cells or monocyte-derived macrophages in tissues (Fig. 1.1) [3]. For example, during severe inflammation or inflammation-related injuries, macrophages are replenished by blood-derived monocytes [3]. In mice, monocytes with high expression of CX3CR1 differentiate into long-term persisting tissue-resident phagocytes, whereas those with comparatively low CX3CR1 expression are inflammatory monocytes that serve as precursors for antigen-presenting cells [24]. Importantly, monocytes are innate immune effector cells that phagocytose (ingest) and kill a wide range of microbes, such as bacteria and fungi.

Tissue-resident mononuclear phagocytes are diverse and include macrophages (e.g., microglia, osteoclasts, Kupffer cells, Langerhans cells, and monocyte-derived macrophages) and DCs (classical DCs, plasmacytoid DCs, and monocyte-derived DCs) [3, 9, 25]. Like monocytes, these cells are defined by morphology, phenotype (cell surface markers), and function. Macrophages maintain steady-state tissue homeostasis by phagocytosing and removing dead cells and debris. During infection, they ingest and kill microbes and produce many different chemokines and cytokines that contribute to the acute

inflammatory response. Tissue-resident macrophages are well-known for their ability to function as antigen-presenting cells and thus serve as a bridge between innate and acquired immunity. Historically, activated macrophages have been categorized as classic (M1) and alternative (M2), so named to reflect the prototypical Th1 and Th2 mouse strains from which they were isolated [26, 27]. In accordance with this nomenclature, M1 macrophages are those activated by interferon gamma and Toll-like receptor ligands (e.g., lipopolysaccharide) or tumor necrosis factor (TNF-α), whereas M2 macrophages are activated by IL-4, IL-10, IL-13, or IL-33 [26–29]. M1 macrophages are proinflammatory and produce reactive nitrogen or reactive oxygen intermediates and cytokines such as IL-1, IL-6, and TNF-α [28, 29]. By comparison, M2 macrophages have been characterized by production of polyamines and IL-10 and IL-12, regulate wound healing, and in general suppress immune responses [28, 29]. Inasmuch as macrophage activation is complex and varied among mammals, and there is inconsistent use of defining features for macrophage activation, the M1-M2 macrophage nomenclature has been brought into question recently, and new guidelines have been proposed [30]. Although it is widely acknowledged that a primary purpose of macrophages is to kill ingested microbes, these phagocytes are readily parasitized by a number of bacterial pathogens [31]. For example, *Brucella* spp., *Chlamydia pneumoniae*, *Coxiella burnetii*, *Francisella tularensis*, *Legionella pneumophila*, *Listeria monocytogenes*, *Mycobacterium tuberculosis*, and *Salmonella enterica* can replicate in macrophages [31]. This interesting topic has been reviewed recently by Price and Vance, and they suggest several factors contribute to the ability of bacteria to survive and replicate within macrophages [31]. These factors include intracellular access by phagocytosis, extended host cell lifespan, and nutrient availability [31]. It is also noteworthy that macrophages have limited bactericidal activity compared with neutrophils, which are infrequently parasitized by bacteria. Tissue-resident macrophages are maintained in steady state by self-renewal, and it is only under immune system duress, as with acute inflammatory processes, that monocytes are recruited to tissues to replenish tissue macrophages.

DCs have the capacity to phagocytose microbes and produce high levels of cytokines (depending on the type of DC), but their primary function is largely as antigen-presenting cells that activate naive T cells [8, 9, 32]. There are three or four subsets of DCs, depending on whether Langerhans cells are classified as macrophages or DCs. Langerhans cells were traditionally classified as DCs, but recent gene expression data and their origin from fetal liver precursor cells are more in line with characteristics of tissue macrophages [33, 34]. Regardless, Langerhans cells are abundant resident phagocytes in human skin and serve as sentinels of the immune system [35]. Classical DCs (cDCs; originally identified by Steinman and Cohn [36]) present antigen to T cells in the context of major histocompatibility complex (MHC) I and MHC II [37] molecules. These cells are present in many types of tissues and organs, and ultimately migrate (if necessary) to areas that promote interaction with T cells, such as the spleen or lymph nodes [37]. The lifespan of cDCs is relatively short (~1 week), and they are replenished by hematopoiesis from blood-borne CDPs [9]. Plasmacytoid DCs (pDCs) also originate from a CDP, but unlike cDCs, pDCs have a long lifespan and are involved in the response to viral infections [9]. Monocyte-derived or inflammatory DCs, such as TNF and iNOS-producing DCs, originate from monocytes during the inflammatory response [9, 38]. A more detailed discussion of DC subsets is outside the scope of this chapter, but there are several recent articles on this topic [35, 39].

Granulocytes

Polymorphonuclear leukocytes (PMNs or neutrophils) are the most numerous circulating leukocytes in humans and are the most prominent cellular defense against bacterial and fungal infections. Indeed, 60% of the cells in bone marrow are granulocytes or granulocyte precursors,

and ~60% of white cells in human blood are neutrophils [40]. Neutrophils and eosinophils are identified readily by cytochemistry and phenotype (e.g., nuclear morphology). Under steady-state conditions, neutrophils develop in bone marrow for ~14 days (5–6 days excluding mitotic precursors), circulate in blood for a day, and then enter tissues, where they remain for another 1–2 days before undergoing apoptosis [40, 41]. Compared to mononuclear phagocytes, especially macrophages, the lifespan of mature neutrophils is short. They are terminally differentiated end cells. However, this short lifespan is offset by the tremendous number of cells produced during hematopoiesis. Neutrophil turnover in humans is approximately 10^{11} cells per day in an average healthy adult [41, 42]. Such turnover is remarkable, and a mechanism dependent on the mononuclear phagocyte system is in place to remove dead and dying neutrophils from tissues, thereby maintaining immune system homeostasis.

The myeloblast is an early neutrophil precursor cell and is followed in sequence by the promyelocyte, myelocyte, metamyelocyte, band cell, and mature neutrophil [40]. As neutrophils mature in bone marrow, they develop protein machinery and specialized organelles known as granules that are necessary for microbicidal activity. Azurophilic or primary granules (peroxidase-positive granules) appear first during granulopoiesis and contain numerous antimicrobial peptides and proteins, including myeloperoxidase, alpha defensins, elastase, cathepsin G, proteinase 3, and azurocidin [43, 44]. Azurophilic granules are synthesized largely during the promyelocyte stage of cell development. Specific granules, gelatinase granules, and secretory vesicles, which are peroxidase-negative, appear after azurophilic granules during neutrophil development in bone marrow [40]. The membranes of the specific and gelatinase granules and those of the secretory vesicles contain receptors and other membrane-bound proteins important for virtually all neutrophil functions. For example, at least 90% of neutrophil gp91*phox*/p22*phox* heterodimer (flavocytochrome b_{558}), which forms the nidus of the superoxide-generating NADPH oxidase in neutrophils, is located in the membranes of these granules [45, 46]. These organelles serve as storage compartments for the molecules required for neutrophil microbicidal activity, which is discussed below. Although mature neutrophils are fully equipped with the molecules required for PMN microbicidal activity, they retain some biosynthetic capacity [45]. Importantly, neutrophil production can be rapidly increased as needed, as, for example, during severe systemic bacterial or fungal infections. This process is known as emergency granulopoiesis [14, 47].

Basophils, eosinophils, and mast cells are granulocytes that participate in innate and acquired immunity. They are key cells in the response to allergens and function as antigen-presenting cells [29, 48, 49]. Basophils are not typically considered as phagocytes and will not be discussed further [48]. Eosinophils and mast cells can phagocytose microbes, but phagocytic capacity is either significantly less than that of other phagocytes or incompletely characterized and the role in vivo not fully understood [29, 49, 50]. The ability of eosinophils to kill bacteria has also been linked to extracellular release of cytotoxic molecules [51, 52]. These leukocytes, like basophils, are known historically for their role in the host defense against parasites, especially helminths [49, 53]. Mast cell precursors develop in bone marrow and then migrate to tissues, where they differentiate and mature (Fig. 1.1) [29, 54]. Mast cells are long-lived cells that have been reported to phagocytose and kill multiple bacterial species [55]. However, the in vivo significance of this direct bactericidal activity remains unknown, and these cells are more characterized for their ability to coordinate immune and allergic responses. For simplicity, much of the discussion of phagocyte function is based on studies with mononuclear phagocytes and neutrophils. For those interested in a more detailed review of basophils, eosinophils, and mast cells, we recommend specific articles on these cell types [29, 48–50, 53, 56, 57].

Recruitment, Chemotaxis, and Priming

The rapid recruitment of phagocytes to damaged tissue is critical for an effective inflammatory response. Circulating phagocytes must quickly

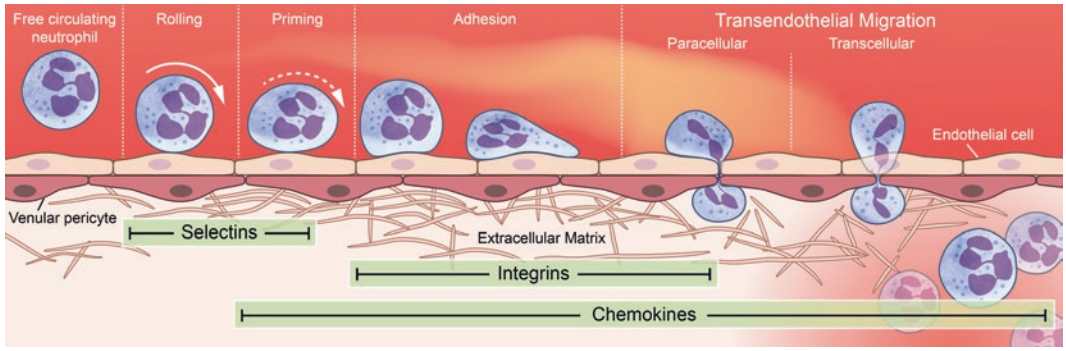

Fig. 1.2 Neutrophil chemotaxis and transmigration. Migration of neutrophils from blood to infected or injured tissues is characterized by four distinct stages: rolling, priming, adhesion, and transendothelial cell migration. Chemokines and other chemoattractants promote neutrophil rolling and priming, which in turn leads to integrin-dependent interactions. Selectins and integrins present on the endothelium and neutrophils promote neutrophil adhesion and transendothelial migration

recognize danger signals emanating from distressed host cells, efficiently breach the postcapillary venule wall, and immigrate to the site of tissue damage to successfully resolve host injury. Phagocyte extravasation, namely, the migration from circulation into extravascular tissue, follows a process referred to as the leukocyte adhesion cascade that can be divided into four primary events: rolling, priming, adhesion, and transendothelial cell migration (TEM) (Fig. 1.2) [58, 59]. These events are largely coordinated by a hierarchical chemokine gradient and through direct interactions with activated host cells that act in concert to sequentially recruit specific phagocyte subsets to the site of host insult [60–62]. Following extravasation, phagocytes are transformed from patrolling sentinel cells in circulation to fully activated effector cells that play critical roles in orchestrating subsequent immune responses, destroying pathogens, and removing unwanted debris.

Rolling

Circulating phagocytes appear to roll along the wall of postcapillary venules as they near the site of host tissue distress. This rolling motion is primarily mediated by the transient on and off binding of cell surface molecules called selectins under the shear-force conditions encountered in postcapillary venules [63]. There are three members of the selectin family, with the nomenclature of these molecules indicating the cell type in which they were first identified (E for endothelia, L for leukocytes, and P for platelets). Selectins bind to glycosylated proteins on the surface of adjacent host cells. Although P-selectin glycoprotein ligand 1 (PSGL1) was first identified as a ligand for P-selectin, this molecule is now known to be a primary ligand for all three selectins. E-selectin on endothelial cells also binds to E-selectin ligand 1 (ESL1) and glycosylated CD44 on the surface of phagocytes. Surface expression of selectins is varied among cell types and is dependent on the activation state of the cell. These expression attributes facilitate efficient targeting of phagocytes to specific sites of host tissue inflammation. The constitutive expression of L-selectin by circulating phagocytes largely mediates rolling at high velocities. In contrast, E-selectin is only expressed by activated endothelial cells early during the inflammatory response and acts to decrease the velocity of phagocytes as they near injured tissue. As the velocity of rolling phagocytes decreases, signals localized near the site of compromised host tissue enhance the activation state of these cells in a process referred to as priming.

Priming

Phagocytes in circulation detect relatively low concentrations of host- and/or microbe-derived signaling molecules, such as chemokines, cytokines, and bacterial *N*-formylated peptides, as they near the site of damaged tissue or infection. These molecules can "prime" phagocytes for enhanced function, and this phenomenon has been investigated extensively in neutrophils [64]. Priming of neutrophils was described originally as the ability of a primary agonist (at substimulatory concentrations) to enhance or influence production of superoxide in response to a second stimulus [65, 66]. It is now known that priming enhances multiple neutrophil functions, including adhesion, phagocytosis, superoxide production, and degranulation [64, 67]. In general, neutrophil priming promotes the timely recruitment of these leukocytes to damaged and infected tissues and enhances capacity to destroy infectious microbes.

Many molecules that promote phagocyte priming contain molecular moieties that are normally absent or concealed in the healthy host but are exposed during infection and injury. For example, infectious agents generate structurally conserved molecules that display pathogen-associated molecular patterns (PAMPs) [68]. These molecules include lipopolysaccharide (LPS) or muramyl dipeptide specific to bacteria, double-stranded RNA that comprises the genome of certain viruses, or β-glucan located on the surface of fungi. Alternatively, sterile injury induces the release of damage-associated molecular patterns (DAMPs) that are normally confined within the cytosol of host cells [69]. High-mobility group box 1 (HMGB1) and cytosolic heat-shock proteins are examples of DAMPs released by necrotic cells [69]. PAMPs and DAMPs are recognized by a number of cell surface and cytosolic receptors that are collectively referred to as pattern recognition receptors (PRRs) [69]. These molecules include the Toll-like receptors, scavenger receptors [70], and C-type lectin receptors (e.g., the mannose receptor and Dectin-1) located on the cell surface or within endolysosomes, as well as the NOD-like receptors and RIG-like receptors that are only found within the cytosol and act to recognize infection by intracellular pathogens. Phagocytes generally express a large number of PRRs, and they play a major role in recognizing host injury, immune surveillance, and directing subsequent immune responses. Other resident tissue cells, such as endothelial cells and keratinocytes, express PRRs to a lesser degree and can also alert the immune system to tissue insult [71].

In addition to priming of recruited phagocytes, engagement of PRRs with corresponding ligands activates pathways that increase local cytokine concentrations. These molecules contribute to the ongoing inflammatory process, which includes continued recruitment of phagocytes and other leukocytes toward the site of tissue damage. In humans, CXC-chemokine ligand 8 (CXCL8 or IL-8) is a major cytokine that influences neutrophil recruitment and activation [60], while CC-chemokine ligand 2 (CCL2) plays an important role during monocyte recruitment to inflamed tissue [72]. Cytokines released by stimulated host cells bind to glycosaminoglycans (GAGs) such as heparin sulfate that are located on the surface of endothelial cells and attached to the extracellular matrix [59, 61]. As phagocytes travel through circulation, they encounter increasing concentrations of chemokines that are presented on the surface of vascular endothelium as they approach distressed tissue. These chemokines bind to cognate G-coupled-protein receptors on the surface of rolling phagocytes and induce very rapid cellular changes that result in arrest and firm adhesion to the postcapillary venule wall near the site of host injury.

Adhesion

The arrest and firm adhesion of circulating phagocytes in response to chemokines expressed at the site of distressed host tissue is mediated by the activation and binding of integrins. Integrins are a class of heterodimeric cell surface proteins consisting of α and β subunits. In mammals, 18 α sub-

units and 8 β subunits have been identified that give rise to at least 24 different types of integrins [73]. The binding of activated integrins expressed by phagocytes to immunoglobulin superfamily members on the surface of activated endothelial cells is imperative for firm adhesion of phagocytes to the vascular endothelium. Integrins and ligands that are important during this process include integrins $\alpha_M\beta_2$ (CD11b/CD18 or Mac-1) and $\alpha_L\beta_2$ (CD11a/CD18, also known as leukocyte function-associated antigen 1 or LFA-1), which bind to intercellular adhesion molecule 1 (ICAM-1) on endothelial cells, and $\alpha_4\beta_1$ (also known as very late antigen 4 or VLA-4) which binds vascular intercellular adhesion molecule 1 (VCAM-1) [73, 74]. In general, the integrins of unprimed circulating phagocytes are not in an active state. In a process termed inside-out signaling, the recognition of chemokines by G protein-coupled receptors on rolling phagocytes rapidly increases the avidity of integrins, resulting in almost immediate cell arrest via adhesion to adjacent activated endothelial cells [75].

The avidity of integrin-mediated adhesion is dependent upon the affinity of individual integrin molecules for their ligands and by the distribution of integrins on the cell surface [76]. Inactive integrins are diffusely spread on the cell membrane and hold a bent conformation with the binding region tightly pressed against the membrane surface, resulting in a low affinity for corresponding ligands. Inside-out signaling through activated G protein-coupled chemokine receptors quickly opens this bent conformation, exposing the integrin-binding domain to allow high-affinity interactions with ligands. Inside-out signaling also induces integrin clustering, further increasing the overall avidity of these molecules for ligands on the surface of endothelial cells.

When clustered integrins bind to corresponding ligands, changes are induced in the cytoplasmic domain of these molecules that activate intercellular tyrosine kinase-dependent signaling pathways in a process referred to as outside-in signaling [77]. Activation of these pathways leads to rearrangement of the phagocyte actin cytoskeleton that flattens the cell against the vessel wall, increasing surface area contact with the vascular endothelium and enabling sustained adherence under sheer-flow conditions. In addition, outside-in signaling further primes phagocytes by mobilizing factors that are important for antimicrobial activity and TEM into the extravascular space.

Transendothelial Cell Migration

Primed phagocytes that are firmly adhered to activated vasculature endothelium must exit the capillary lumen to reach compromised host tissue and perform effector immune functions. As with previous steps in the leukocyte adhesion cascade, this process is largely directed by increasing concentrations of different chemotactic factors in conjunction with signals derived from direct interactions with activated host cells [78]. Collectively these cues orchestrate the migration of phagocytes between or even through activated endothelial cells lining the postcapillary venule wall, across the underlying endothelial basement membrane, and through the extravascular space to the site of distressed tissue.

Once phagocytes rolling through circulation have become firmly adhered to activated endothelium, they will often crawl in an amoeba-like fashion along the capillary lumen wall in search of suitable extravasation sites. Neutrophil and monocyte crawling requires interactions between integrin $\alpha_M\beta_2$ (Mac-1) with ICAM-1 on the surface of activated endothelium [59]. Forward cell displacement during crawling is dependent upon the reorganization of the phagocyte actin cytoskeleton and the polarization of intracellular signaling proteins, surface receptors, and adhesion molecules across the cell. On the leading edge of crawling phagocytes, new bonds are formed with adhesive molecules on the surface of activated vascular endothelium, while bonds at the trailing end are simultaneously broken. As phagocytes crawl, they extend pseudopods that probe the vessel wall for chemotactic factors and signals from underlying activated endothelial cells that indicate optimum sites for TEM.

The majority of phagocytes crossing the endothelial layer pass through the junctions between

vascular endothelial cells in a process termed paracellular TEM [61]. A number of adhesion molecules expressed by both phagocytes and endothelial cells are important for paracellular TEM. These include platelet-endothelial cell adhesion molecule-1 (PECAM-1 or CD31), junctional adhesion molecules A and C (JAM-A and JAM-C), and CD99 [79]. VE-cadherin expressed by endothelial cells plays an important role in maintaining tight junctions between adjacent cells, and the expression of this molecule deters paracellular TEM [80]. Phagocytes can also pass directly through endothelial cells in a process referred to as transcellular TEM. In some instances recruited phagocytes prefer the transcellular path, such as TEM across brain vasculature endothelium that maintain very tight junctions between adjacent cells [61].

Migrating phagocytes that have crossed the vascular endothelium encounter the next major barrier during extravasation—the underlying basement membrane that ensheathes postcapillary venules. The basement membrane is composed of elongated mural cells termed venular pericytes imbedded in a complex layer of extracellular matrix proteins that include collagen IV, various laminins, and glycoproteins such as nidogens and perlecan [78]. Immigrating phagocytes preferentially breach the basement membrane between venular pericytes at areas where the density of extracellular matrix proteins is low. The expression of integrin ligands such as ICAM-1 by venular pericytes allows migrating phagocytes to use these cells as an adhesive substrate for traversing the basement membrane toward these extravasation hot spots [61]. Perivascular macrophages residing in the extravascular region adjacent to the basement membrane are thought to guide migrating phagocytes toward areas of optimal extravasation via the expression of chemokines such as CXCL1 and CXCL2 [78].

Once migrating phagocytes have breached the basement membrane, they crawl through the interstitial space toward increasing concentrations of so-called end-stage chemotactic factors [78, 80]. These molecules, which include formylated peptides and complement protein C5a, take precedence over other chemotactic factors and play a dominant role guiding phagocytes through the interstitial space directly to the site of host tissue injury.

Phagocytosis and Microbicidal Activity

The ability of phagocytes to ingest and subsequently kill invading microbial pathogens is paramount to maintenance of host health. Phagocytosis is functionally defined as the intracellular uptake of particles greater than 0.5 μm in diameter and is primarily executed by neutrophils and mononuclear phagocytes (monocytes, macrophages, and dendritic cells). Phagocytes have an enormous capacity for ingestion, and surface area can increase up to 300% for neutrophils and 600% for macrophages [81]. The process of phagocytosis is highly complex and can be conceptually divided into two different phases: recognition and binding and internalization.

Phagocytosis

Phagocyte recognition of invading microbial pathogens is mediated by receptors present on the outer surface of the host cell membrane. There are two primary types of receptors that are used to recognize microorganisms: (1) PRRs, which directly recognize microbial-derived structures, and (2) opsonic receptors, which recognize host proteins that are deposited on the microbial surface. Ligation of PRRs initiates a complex series of signal transduction cascades that modulate phagocyte effector functions such as enhanced phagocytosis, killing, and regulation of inflammation via cytokine production. Ligation of PRRs is generally insufficient to promote phagocytosis directly, but there are exceptions (e.g., Dectin-1 is a PRR that binds fungal β-glucans and promotes ingestion of bound fungi) [82–84]. Phagocytosis is most efficient in the presence of opsonins—soluble host molecules that promote uptake—of which specific IgG and complement are the major constituents and phagocyte recognition of these molecules

directly mediates uptake (Fig. 1.3). IgG bound to the microbial surface activates the classical complement pathway and leads to deposition of complement C3 and derivatives. In addition, C3 can be deposited on the microbial surface following activation of the alternative pathway or the mannose-binding lectin pathway. Neutrophils and mononuclear phagocytes express distinct receptors for IgG (FcγRI, FcγRII, and FcγRIII) [85] and opsonic complement molecules C3b and C3bi (CR1, CR3, and CR4). Receptors that contribute to phagocytosis have varied affinities for target ligands. For example, FcγRI is a high-affinity receptor, whereas FcγRII and FcγRIII are constitutively expressed low- to moderate-affinity receptors. Integrins such as $\alpha_M\beta_2$ (CR3, CD11b/CD18, Mac-1), by contrast, dynamically equilibrate between conformational states on the cell surface—a closed conformation with low affinity and an open conformation with high affinity [86]. CR3 ligand affinity can increase following cell activation by inflammatory mediators such as tumor necrosis factor-α, LPS, and platelet-activating factor [87]. Efficient particle binding is enhanced by the engagement (simultaneous or sequential) of multiple receptors (of similar or differing types) on the phagocyte surface. In addition, elaboration of cellular extensions such as membrane ruffles [88] and macrophage filopodia [89] facilitate target binding by an actin-dependent mechanism [90], and membrane protrusions can be enhanced by stimulation of PRRs [88, 91].

Engagement of phagocyte receptors initiates a complex series of molecular signals that contribute to internalization of microbes or some other target object (e.g., debris) and is followed by complete activation of antimicrobial systems. There is inherent diversity in signaling between phagocyte receptors, and the variability in signals

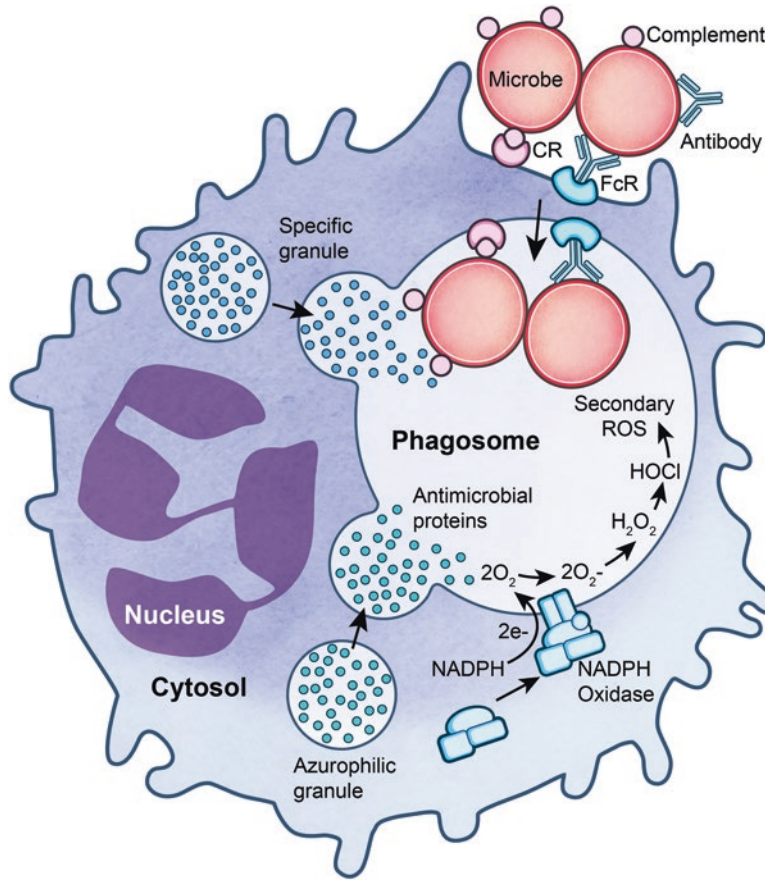

Fig. 1.3 Neutrophil phagocytosis and microbicidal processes. Binding and ingestion of microbes (phagocytosis) are mediated optimally by host opsonins such as serum complement and antibody. Phagocytosis triggers fusion of cytoplasmic granules with the newly formed phagosome, thereby enriching the phagocytic vacuole with antimicrobial agents. Granule-phagosome fusion is followed by assembly and activation of NADPH oxidase. The NADPH oxidase produces superoxide ($O_2\bullet^-$), which in turn leads to the production of hypochlorous acid (HOCl) and other reactive oxygen species (ROS)

transduced extends to cell type-specific differences elicited by the same receptor. Although a detailed compendium on phagocyte receptor signal transduction is beyond the scope of this section (for reviews on the topic, see [92, 93]), we highlight features of FcR-mediated signaling elicited by a prototypical phagocytosis receptor. The complex signals govern cellular processes such as membrane reorganization and cytoskeletal remodeling that are required for phagocytosis. The cytosolic domain of the Fc receptor contains a region known as the immunoreceptor tyrosine-based activation motif (ITAM), which serves as a substrate for phosphorylation by tyrosine kinases of the Src family such as Lyn and Hck [94]. The signaling cascade is amplified by spleen tyrosine kinase (Syk), a cytosolic kinase essential for phagocytosis [95], and is followed by recruitment of adapter proteins and activation of lipid-modifying enzymes such as phosphatidylinositol 3-kinase and small GTPases [96]. Actin polymerization is requisite for phagocytosis and is facilitated by the Arp2/3 nucleator complex, a seven-protein complex that nucleates branched actin filaments. Actin polymerization, in conjunction with progressive FcR binding, provides the cytoskeletal framework to advance the phagocyte plasma membrane over the particle. Actin is concentrated at the tips of the advancing membrane cup during particle internalization, and depolymerization of actin occurs at the base of the cup. In addition, several classes of myosin, including myosin X, have been implicated in execution of FcR-mediated phagocytosis [97]. Although the final stage of particle internalization requires sealing of the opposing membrane leaflets to complete formation of the nascent vacuole, little is known about the mechanism of closure.

Maturation of Phagosomes

The newly formed phagosome lacks quintessential antimicrobial properties, and its lumen resembles the extracellular environment. To assemble microbicidal machinery and acquire antimicrobial properties, the nascent phagosome undergoes a dynamic process of maturation. In macrophages, this process starts immediately after the phagosome is sealed and is dependent on the endocytic pathway. In mononuclear phagocytes, nascent phagosomes fuse with early endosomes, followed by fusion with late endosomes and lysosomes, to yield a hybrid vacuole called phagolysosome. For simplicity, the terms phagosome and phagolysosome will be used interchangeably. Sequential early and late endosome-phagosome fusion events progressively acidify the lumen of the macrophage phagosome largely by incorporating vacuolar ATPase complexes (V-ATPases) [98]. This is followed by fusion of phagosomes with lysosomes, which, in turn, enrich the vacuole lumen with lysosomal proteases and other hydrolytic enzymes. The pH of the mature macrophage phagosome is ~5–6 [99–101], which is optimal for lysosomal protease activity [102]. In contrast to macrophages, the pH of the DC phagosome is near neutral, if not slightly alkaline (pH 7.0–7.6). This attribute of DCs is due to the comparatively limited phagosomal V-ATPase activity and sustained intraphagosomal production of superoxide by NADPH oxidase, a process that consumes protons [100]. More recent studies suggest NADPH oxidase alters the redox capacity of DC phagosomes and thereby controls proteolysis (of antigens) mediated by cysteine proteases [103]. In addition, DC lysosomes have reduced levels of proteases and associated proteolytic activity compared with those of macrophages [104]. From a functional standpoint, DC antigen processing and presentation are optimal in phagosomes that maintain near neutral/slightly basic pH and have limited lysosomal protease activity [100, 104, 105]. It is noteworthy that not all studies agree about the pH of the DC phagosome or the mechanism by which proteolysis is regulated [100, 103]. This may be a reflection of differences in DC subsets.

In contrast to mononuclear phagocytes [106], in which phagosome maturation involves the endocytic pathway to a significant extent, maturation of neutrophil phagosomes is based largely on fusion with specialized granules. Neutrophils can undergo granule exocytosis (also called

degranulation), whereby the granules mobilize to and fuse with the plasma membrane, and release their contents into the extracellular space. Alternatively, granules fuse with forming phagosomes, and the lumen and membrane are enriched with granule proteins. In the resting state, human neutrophils in circulation have limited capacity to interface with the external environment, as there are relatively few proinflammatory receptors present on the cell surface. However, this attribute changes rapidly upon exposure to very low levels of host or microbe-derived proinflammatory molecules, such as chemokines or PAMPs. The cytoplasmic granules and secretory vesicles fuse with membranes in a hierarchy that is stimulus and calcium threshold dependent: secretory vesicles are mobilized first, followed by tertiary granules (gelatinase granules), secondary granules (specific or beta granules), and ultimately primary granules (azurophilic or alpha granules) [43]. Consistent with their ability to mobilize readily, secretory vesicles enrich the cell surface with receptors and other molecules needed for chemotaxis, transmigration, and microbicidal activity. The primary and secondary granules were known traditionally as peroxidase-positive and peroxidase-negative granules, respectively, nomenclature that reflects the presence and absence of myeloperoxidase (MPO). As indicated above, these granules contain antimicrobial peptides and enzymes required for oxygen-dependent and oxygen-independent killing of microbes by neutrophils. For a more comprehensive review of neutrophil granule synthesis, content, and mobilization, we refer the reader to excellent articles on the topic [43, 44, 107].

Compared with macrophages, there are fewer V-ATPase channels in neutrophil phagosome membranes early after ingestion, and the activity of these molecules is inhibited by reactive oxygen species [108, 109]. Notably, the pH of neutrophil phagosomes is near neutral (pH ~7.2) [99]. Although not all findings concur about the initial pH of neutrophil phagosomes, neutral pH in the phagocytic vacuole is needed for optimal bactericidal activity, and proton channel activity is required to offset charge differential caused by production of superoxide by NADPH oxidase (see below) [99, 109–112]. Moreover, charge compensation in neutrophils is essential for optimal NADPH oxidase activity, and protons are needed for subsequent formation of hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl), each of which is important for oxygen-dependent killing of microbes [110]. In the end, multiple factors contribute to regulation of phagosome pH and function, including vesicle fusion events, production of superoxide, redox potential, recruitment of V-ATPases, and proton channel activity, each of which appears specific to phagocyte function and type [113].

Production of Reactive Oxygen Species

Professional phagocytes use oxygen-dependent and oxygen-independent processes to eliminate ingested microorganisms. Phagocytes have two main oxygen-dependent antimicrobial systems: NADPH oxidase and inducible nitric oxide synthase (iNOS). NADPH oxidase is a multicomponent enzyme complex that produces superoxide in activated cells. In unactivated cells, NADPH oxidase components are segregated in membrane and cytosolic compartments. Flavocytochrome b_{558} is a heterodimeric transmembrane protein comprised of gp91*phox* (NOX2) and p22*phox* subunits; it contains the electron transport machinery for the enzyme complex and forms the nidus of the assembling oxidase at the plasma or phagosome membrane. p47*phox*, p67*phox*, p40*phox*, and the small GTPase Rac (Rac1 or Rac2) are oxidase components located in the cytosol of resting cells. Upon phagocyte activation, p47*phox*, p67*phox*, and p40*phox* translocate to the plasma or phagosome membrane en bloc and interact directly with flavocytochrome b_{558}. Rac translocates to the membrane independent of the other cytosolic components and, in turn, associates with components (p67*phox* and flavocytochrome b_{558}) of the assembling enzyme complex [113]. NADPH oxidase catalyzes the transfer of electrons from cytosolic NADPH to molecular oxygen, thereby producing $O_2^{\bullet -}$. Although $O_2^{\bullet -}$ is weakly microbicidal, it is rapidly converted to other, more effec-