Forum in immunology

Phagocytosis by neutrophils

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Abstract

Phagocytosis is central to the microbicidal function of neutrophils. Pathogens are initially engulfed into a plasma membrane-derived vacuole, the phagosome, which proceeds to acquire degradative properties by a complex process termed maturation. In this chapter, we discuss the current knowledge of the molecular mechanisms underlying phagosome formation and maturation in neutrophils.

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1. Introduction

Neutrophils, which along with macrophages comprise the professional phagocytes, are endowed with a unique capacity to engulf (Fig. 1) and thereby eliminate pathogens and cell debris. Phagocytes are equipped with specialized receptors to recognize their targets; this complex machinery mediates internalization and initiates an assortment of degradative mechanisms that culminate in killing and disposal of the engulfed particles. Most of our knowledge of the molecular mechanisms of phagocytosis has been derived from studies of phagocytosis in macrophages, and much less is known about neutrophils, due to the fact that they are refractory to genetic manipulation by either transfection or microinjection. For this reason, frequent reference will be made to phagocytosis in macrophages, although neutrophils remain the principal focus of this review.

Like macrophages, neutrophils can internalize both opsonized (Fig. 1) and non-opsonized particles. The principal osonin receptors of neutrophils, Fc receptors and a subgroup of β2 integrins, bind to immunoglobulin or complement-coated particles, respectively. The main Fc receptors of human resting neutrophils are FcγRIIA (CD32) and FcγRIIb (CD16), while the high-affinity FcγRI (CD64) functions predominantly after neutrophils have been primed with interferon [1]. Complement fragment C3bi is recognized by the activated β2 integrin MAC1 (CD11b/CD18). Studies in macrophages suggest that while engulfment of the particle into a membrane-derived vacuole is the end result in both cases, the processes underlying internalization are not identical in IgG- or complement-mediated events. While complement-opsonized particles are internalized by gently “sinking” into the cell, Fcγ receptor ligation initiates the vigorous extension of pseudopods that surround and ultimately entrap the particle [2]. Once formed, the vacuole undergoes a rapid series of remodeling events that alter its composition, conferring onto it the ability to kill pathogens and dispose of debris. These remodeling events are collectively termed maturation. This chapter presents an overview of the current knowledge of the molecular processes that mediate phagosome formation and maturation in neutrophils.

2. Early signaling events in phagocytosis

As discussed above, multiple types of receptors can initiate phagocytosis and, while they are superficially similar, the molecular events underlying particle internalization are most likely to differ in each individual case. For simplicity, only the signals elicited by Fcγ receptors, which have been studied most extensively, will be reviewed briefly here. Activation of the transmembrane Fcγ receptors requires phosphorylation of tandem tyrosine residues within the context of
an immunoreceptor tyrosine activation motif or ITAM. Phosphorylation is brought about by recruitment of activated Src-family kinases to the immediate vicinity of the receptors, as a result of receptor clustering by multivalent ligands. Phosphorylation of the ITAM motifs in turn serves to generate docking sites for proteins bearing SH2 domains, particularly the tyrosine kinase Syk. Localized Syk activity has been shown to be essential for Fc-mediated phagocytosis, as neutrophils from Syk-deficient mice are unable to ingest IgG-opsonized particles [3]. Engagement of Syk is accompanied by stimulation of phosphatidylinositol 3-kinase (PI3K), which is largely responsible for converting phosphatidylinositol 4,5-bisphosphate (PI4,5P2) to phosphatidylinositol 3,4,5-trisphosphate (PI3,4,5P3). Syk-deficient mice show reduced PI3K phosphorylation, implying that activation of the tyrosine kinase is an early event that contributes indirectly to phosphoinositide metabolism. PI4,5P2 is not only converted to PI3,4,5P3, but acts also as a substrate for phospholipase C (PLC) to locally generate diacylglycerol (DAG) [4]. The latter produces focal activation of classical and novel isoforms of protein kinase C (PKC) and other C1 domain-containing enzymes in the immediate vicinity of the phagosome [5]. Despite intensive conversion to PI3,4,5P3 and DAG, the level of PI4,5P2 is maintained and even increased at the early stages of phagocytosis by localized stimulation of phosphatidylinositol 4-phosphate 5-kinase [4]. Normal activation of the phosphoinositide kinases [2] and of PLC [4] is required for phagocytosis, while the requirement for individual PKC isoforms is still the subject of debate.

Downstream effectors of the protein and lipid kinases induce the polymerization of actin and the localized membrane remodeling that are essential for particle ingestion. Rac1 and Cdc42 are thought to be required for actin assembly following Fcγ receptor stimulation, while Rho plays an equivalent role in complement-activated cells [2]. Another small GTPase, Arf6, seems to play a dual role in phosphoinositide synthesis and in regulating the delivery of endomembranes to sites of phagocytosis [6]. The precise guanine nucleotide exchange factors (GEF) involved in each instance have not been fully identified, but Vav is believed to contribute to activation of Rho-family GTPases [7], as it contains a pleckstrin homology (PH) domain that has a high affinity for PI3,4,5P3. Further downstream, the Arp2/3 complex is felt to play a central role in actin assembly [8]. Arp2/3 is stimulated by Cdc42 through WASP and by Rac1 via SCAR/WAVE. Polymerization of actin, together with the activation of the PI3,4,5P3-sensitive myosin X [9] and the localized delivery of endomembranes, drives the formation of the phagosomal cup and its sealing.

3. Phagosomal maturation

Even after a phagosome is formed around a particle, its initial composition is not antimicrobial: the term “phagosome maturation” therefore refers to the process by which the nascent phagosome acquires the cellular machinery necessary for the killing and disposal of internalized microorganisms. This development is a dynamic process, thought to involve multiple fusion events with components of the endocytic pathway, as well as removal of components by vesicular fission. These sequential interactions (nicknamed the “kiss and run” hypothesis) bring about tremendous changes to the phagosomal contents and membrane, including the acquisition of microbicidal enzymes, vacuolar (V) ATPases and the NADPH oxidase complex. Although much has been
learned about the molecular determinants of this maturation process in recent years, the majority of the studies have been conducted in macrophages or in non-phagocytic cells (e.g. fibroblasts) transfected with phagocytic receptors (so-called engineered phagocytes). Somewhat less is known about phagosome maturation in neutrophils, largely because they are not as amenable to molecular manipulation.

3.1. Endocytosis and phagocytosis

Shortly after sealing, the phagosomal vacuole initiates the process of maturation. Because of the obvious parallels with endocytosis, a review of phagosome maturation cannot be accomplished without some understanding of the endocytic pathway [10]. After internalization of a receptor–ligand complex by endocytosis, the contents of an endocytic vesicle are targeted to an early endosome. At this stage, the ligand and its receptor often dissociate, with the receptor being recycled to the cell surface and the ligand continuing along the degradative arm of the endocytic pathway. An early endosome can be identified by its moderately acidic lumen (pH 6.5–6.0) and by its characteristic associated molecules, including the early endosomal antigen 1 (EEA1), transferrin receptors, and the small GTPase Rab5. Those components of the early endosome that are not recycled to the cell surface are delivered to late endosomes, where degradation may begin. Late endosomes are more acidic (pH 5.5–6.0), and because they contain intraluminal vesicles, are often termed multivesicular bodies. Late endosomes can be identified by their association with the GTPases Rab7 and Rab9, and with lysosomal-associated membrane proteins (LAMPs). Subsequently, endocytic cargo is delivered to lysosomes, which are characterized by their extreme acidity (pH ≤5.0) and elevated concentrations of proteases. Lysosomes are the ultimate destination of endocytic cargo that is intended for degradation. Because lysosomes share many markers with late endosomes (e.g. LAMPs), they can be best identified in “pulse-chase” labeling experiments, using protocols that will ensure delivery of internalized probes to a late, terminal compartment, i.e. the lysosome.

In many ways, phagosomal maturation in macrophages appears to be a recapitulation of the endocytic sequence [11]. After closure of the phagosomal cup, the newly formed phagosome undergoes sequential fusion events with early, then late endosomes, before finally fusing with lysosomes to yield a phagolysosome. The phagolysosome is an acidic compartment rich in hydrolases, where the bulk of degradation of the luminal contents is thought to occur.

Unlike macrophages, which undertake active endocytosis, mature neutrophils are remarkably passive. Conventional early and late endosomes, and lysosomes are not readily found in these cells. Instead, they contain a variety of specialized vesicles and granules that are neither subject to traffic among themselves, nor are accessible from the external medium. These are described in more detail below.

3.2. Secretory vesicles and granules

The phagosome in neutrophils acquires its antimicrobial effects through fusion with secretory vesicles and granules. These endomembrane organelles are thought to form from vesicles that bud off the trans-Golgi network during neutrophil development, and contain a remarkably heterogeneous and powerful arsenal of microbicidal peptides and proteolytic enzymes, as well as numerous membrane-bound proteins that contribute to pathogen elimination. Four categories of granules have been identified: primary (or azurophilic) granules characterized by the presence of myeloperoxidase and membrane-bound CD63; secondary granules (also known as specific granules) whose contents include lactoferrin and membrane-bound CD66b; tertiary granules, which lack CD66b but contain gelatinase; and secretory vesicles, which contain albumin and express alkaline phosphatase and CD35 on their membranes. The mechanisms of degranulation in neutrophils are the subject of a separate article in this issue [12]; in this section, we will confine our discussion to neutrophil granules as they pertain specifically to phagocytosis.

While much has been learned about the mechanisms that underlie granule fusion, the readers should appreciate that many of these studies used soluble mediators to stimulate granule exocytosis at the plasmalemma (generalized secretion), while a smaller number analyzed localized granule coalescence with the phagosomal membrane, using particular stimuli. Whether the results of these studies are directly comparable is unclear, but it is safe to assume that the two phenomena are related. Indeed, use of generalized secretion as a model for localized fusion may be a reasonable approximation, given that fusion of granules with the unsealed plasma membrane has been demonstrated to occur near forming phagosomes [13].

3.3. Signals for secretion

The abrupt nature of fusion following phagocytosis implies the acute generation of effective signals. As in other systems, calcium can trigger the fusion of granules in neutrophils. Moreover, elevated cytosolic free calcium is known to accompany particle ingestion during phagocytosis. Multiple sources contribute to increasing the cytosolic calcium: the cation is released from the endoplasmic reticulum [14], which in turn activates the influx of extracellular calcium. In addition, it is conceivable that calcium may also be released locally by the target compartment, i.e. the phagosome itself [15].

Changes in the level of free cytosolic calcium are required for granule secretion [16] and, more importantly, for granular fusion with phagosomes in neutrophils [17]. The individual secretory compartments have different calcium thresholds for secretion, with secretory vesicles having the lowest and azurophilic granules the highest threshold [18]. The mechanism responsible for this differential sensitivity to calcium is
unknown. A family of calcium-responsive proteins, the synaptotagmins, has been postulated to mediate calcium sensitivity in other cells. One isoform, synaptotagmin II, was found to be associated with specific granules and translocated to the phagosome in a calcium-dependent fashion [19]. The authors of this study speculated that synaptotagmin II serves as the calcium sensor for these granules. It will be of interest to define whether different synaptotagmin isoforms exist in the individual secretory compartments of neutrophils and to validate their involvement in fusion.

Other sites of action could contribute to the effect of calcium on phagosome maturation. The actin coat that lines the phagosomal cup is rapidly disassembled after sealing, and this step is thought to be permissive for the docking and fusion of vesicles and granules with the phagosomal membrane. Of note, the depolymerization of periphagosomal actin requires changes in intracellular calcium [20]. The cation could therefore promote secretion by granting the vesicles access to their target, the phagosomal membrane. Calmodulin is another possible effector of calcium during phagosome maturation. Studies in yeast suggested that calmodulin may be recruited in a calcium-dependent manner to vacuoles, where it can catalyze the coalescence of apposed membrane bilayers [21]. The downstream effectors of calmodulin in this process likely include calmodulin-dependent protein kinase II [22]. Lastly, the calcium-binding proteins annexin I and annexin III are known to associate with the phagosome in neutrophils, and may contribute to its maturation, though their function remains unclear [23].

While the requirement for calcium in the secretion of granules is well-established, the role of PKC isoforms in this process is less well defined. Although at least four isoforms of PKC translocate to the plasma membrane upon phagocytosis, it appears that while PKC is essential for superoxide generation, the kinase is not strictly required for degranulation [24]. On the other hand, the Src family of protein tyrosine kinases has been implicated in phagosome maturation in neutrophils. As stated earlier, Src-family kinases are instrumental in the early activation sequence triggered by receptor clustering. In addition, however, Hck has been localized to azurophilic granules and upon phagocytosis of opsonized zymosan, is activated and translocates towards the phagosome [25]. Interestingly, it has been shown that when neutrophils internalize mycobacteria, Hck fails to be activated and does not translocate to the phagosome. More importantly, under these conditions, fusion of azurophilic granules with the phagosome is ablated, while fusion with specific granules is unaffected [26]. These tantalizing observations implicate Hck in azurophilic granule fusion, but the mode of action of the kinase remains to be defined.

In summary, comparatively little is known about the events that trigger membrane fusion during maturation of phagosomes in neutrophils. Calcium and protein kinases most certainly play a key role, but their targets and the possible existence of additional signals should be the focus of future work.

3.4. Specificity of secretion

While secretory vesicles and granules are clearly capable of fusing throughout the plasma membrane when cells are activated by soluble agonists, during engulfment of particles, fusion is restricted to the membrane of the nascent and formed phagosome. Clearly, some mechanism(s) must exist to ensure the vectorial nature and specificity of these fusion events. Obvious candidates for this task are the SNARE family of proteins. It is thought that a SNARE (soluble N-ethylmaleimide-sensitive-fusion-protein attachment protein receptor) on a donor membrane interacts with a cognate SNARE on a target membrane to form a stable complex consisting of four helices, contributed by the two SNARE proteins. This tetrahedral complex serves to bring the two membranes together and promotes their fusion.

To date, comparatively little is known about the role of defined SNAREs in phagosome maturation. However, the exocytosis of granules by neutrophils activated by soluble stimulants has been studied. In neutrophils activated with phorbol-12-myristate-13-acetate, the SNARE protein SNAP-23 translocated from specific and tertiary granules to the cell surface [27], where it was found to interact with syntaxin 6, another SNARE. Antibodies to SNAP-23 prevented exocytosis of specific granules but not azurophilic granules; in contrast, antibodies to syntaxin 6 prevented exocytosis of both specific and azurophilic granules. The authors of this report proposed that syntaxin 6 on the neutrophil plasma membrane acts as a target for the secretion of both specific and azurophilic granules, while SNAP-23 is involved only in the secretion of specific granules. A similar study by the same investigators suggested that syntaxin 4 on the plasma membrane interacts with VAMP-2 on the surface of specific and tertiary granules, leading to their exocytosis [28]. Clearly, much remains to be learned about the role of SNAREs in neutrophil physiology.

Although SNAREs are important mediators of membrane fusion events, the degree of specificity of their interactions remains controversial. Other determinants of specificity and directionality would be required in the event that SNAREs do not dictate the entire process. The Rab family of GTPases is likely to fulfill this role. By working in concert with SNAREs, Rab proteins confer an extra layer of specificity to the docking of donor to target membranes. Of the Rab family of GTPases, Rab5 is by far the best studied. Like other members of the family, Rab5 is a monomeric GTPase whose ability to cycle between GTP- and GDP-bound states allows it to function as a molecular switch [29]. In macrophages, Rab5 has been detected to reside transiently in early phagosomes, becoming apparent shortly after scission from the surface membrane. More importantly, Rab5 was recently shown to regulate phagosome maturation in neutrophils: inhibition of Rab5a (one of three isoforms of Rab5) using antisense oligonucleotides impaired LAMP-1 and lactoferrin acquisition by the maturing phagosome [30]. The precise mode of action of Rab5 is incompletely understood.
GTPase is thought to interact with a large number of proteins, termed Rab5 effectors, which include Rabaptин-5, Rabenosyn-5, EEA1, and the class III PI3K, the mammalian homologue of yeast Vps34, that generates phosphatidylinositol 3-phosphate (PI3P). How these and possibly other Rab5 effectors function together to facilitate membrane docking is not known for certain. Recruitment of Vps34 is felt to be an early step, leading to the rapid formation of PI3P. The generation of the phosphoinositide by the Rab5 effector complex might serve to recruit other proteins. Indeed, some Rab5 effectors, like EEA1 and Rabenosyn-5, have so-called FYVE domains, cysteine-rich sequences that bind specifically to PI3P. In addition, PI3P is also a ligand for certain proteins bearing Phox homology (PX) domains. Interestingly, EEA1 contains two spatially distinct Rab5 binding sites, and may thus serve to tether two membranes containing Rab5.

In addition to bringing membranous compartments together, Rab proteins may also regulate the direction of the fusion processes. Phagosomes are known to move bidirectionally along microtubules, and inhibitors of microtubules prevent phagosomes from acquiring markers of the late endocytic pathway [31]. Rab5 has been shown to interact with dynemin, a minus-end directed microtubule-based motor protein [32]. Similarly, Rab7, which is found on late endosomes and lysosomes, has been shown to interact with a protein called RILP (Rab7-interacting lysosomal protein), RILP recruits the dynein–dynactin complex, which mediates centripetal movement of organelles along microtubules [33]. To what extent these motor proteins are involved in the maturation of phagosomes in neutrophils is not yet known.

Finally, although the secretion of granules by neutrophils requires calcium, it is interesting to point out that the focal, polarized nature of granule fusion at sites of phagosome is not dictated by calcium. This localized delivery can be disrupted by colchicine, but not cytochalasin, suggesting a role for microtubules in the preferential delivery of granules to the phagosome [13].

4. The neutrophil NADPH oxidase

Upon activation, neutrophils are highly effective at generating reactive oxygen species (ROS) by a process known as the respiratory burst. In stimulated neutrophils, ROS are generated almost exclusively by an NADPH oxidase that belongs to the family of NOX proteins. The leukocyte specific NADPH oxidase is a multisubunit entity with membrane-bound and soluble components that assemble into a heteromeric complex when the cells are stimulated. While a fraction of the intrinsic components of the oxidase are present at the plasma membrane at the time of phagocytosis, most are delivered through fusion with intracellular storage sites. Secondary granules are thought to be the main source of phagosomal NADPH oxidase [34].

Assembly can occur at the plasmalemma, when soluble agonists are used, or at the phagosomal membrane during ingestion of particles. The assembled oxidase facilitates the transfer of one electron from cytosolic NADPH to molecular oxygen captured from the medium. The product of this reaction, the superoxide anion, can then be converted to other reactive oxygen metabolites, including hydrogen peroxide and hypochlorous acid. Together these ROS serve as highly effective antimicrobial agents. The clinical importance of the NADPH oxidase and its products is best exemplified in patients with chronic granulomatous disease (CGD). These patients, who have defective oxidase function, are susceptible to recurrent bacterial and fungal infections.

5. Role of pH in phagosome maturation: macrophages vs. neutrophils

In macrophages, one of the key features of the maturing phagosome is its steadily increasing acidity. Late phagosomes (phagolysosomes) can reach a luminal pH ≤ 5.0. This acidification is the key to optimal activity of proteases and other lysosomal hydrolases involved in pathogen killing. However, in addition to its direct inhibitory effect on the growth and viability of microbes, the acidic phagosomal pH is likely also important for phagosome maturation. Thus, treatment of macrophages with weak bases like NH₄Cl inhibits phagosome–lysosome fusion. The mechanism of this inhibition is not known, but the finding suggests that rather than simply being a consequence of maturation, acidification itself may be required for the phagosome to develop fully. This notion is consistent with observations made in the endocytic pathway, where inhibition of the vacuolar (V)-ATPase decreased the rate of delivery of cargo from multivesicular endosomes to lysosomes [35].

As in endosomes, the acidification of phagosomes in macrophages is established primarily by the action of the V-ATPase, a multisubunit transmembrane protein that pumps protons into the lumen of the phagosome. V-ATPases are barely detectable in the plasma membrane of quiescent cells and are therefore not initially present in the nascent phagosome. However, they are promptly inserted by fusion events with endomembrane organelles. In neutrophils, secretory vesicles, primary and tertiary granules, but not secondary granules were found to contain V-ATPases [36].

Remarkably, despite the fact that acidic granules fuse with the phagosomes of neutrophils, thereby inserting V-ATPases, the lumen of phagosomes in these cells is not very acidic. In fact, a transient initial alkalinization has been described, which subsides after a period of minutes. It is unclear whether the pH then remains near neutrality or becomes slightly acidic (for review see [37]). This unexpected behavior has been attributed primarily to the recruitment and activation of the NADPH oxidase, which is much more pronounced in neutrophils than in macrophages. The oxidase alters phagosomal pH by several independent mechanisms. First and foremost is the net consumption of luminal H⁺ during the dismutation of superoxide to hydrogen peroxide (Fig. 21). Secondly, ROS generated by the oxidase diminish the efficiency of granule fusion with the phagosomal mem-

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brane, resulting in a reduced insertion of V-ATPases and consequently lower rates of H⁺ pumping [38]. Because the pH itself plays a role in the maturation process, the inability of phagosomes to develop an early acidification will impact on its subsequent fusion with granules bearing additional V-ATPases, compounding the effect on pH. Lastly, the oxidative effect of the ROS also increases the passive or “leak” permeability to H⁺ (equivalents), preventing their accumulation in the lumen [38].

6. Electrophysiology of the maturing phagosome

Activation of the NADPH oxidase is an electrogenic event. During the respiratory burst, the oxidase mediates the vectorial translocation of electrons across the bilayer, to catalyze the reduction of extracellular or luminal oxygen. Simultaneously, protons are released into the cytoplasm during the oxidation of NADPH to NADP⁺. The combined effects of this charge separation render the cytosol positive with respect to the extracellular or luminal side of the membrane. The magnitude of this depolarization has been estimated to exceed 100 mV [39].

A recent detailed analysis of the current–voltage relationship of the oxidase demonstrated that, as anticipated, the activity of the enzyme diminishes as it struggles against an increasing electrochemical gradient [39]. It follows that continued, robust activity of the oxidase, which is essential for its microbicidal action, can only be sustained if a charge dissipation pathway develops in parallel. The most important contributor to this pathway appears to be a unique, highly selective H⁺ (equivalent) channel (see [40] for a review). Three features of this channel make it ideally suitable for its dissipative role in phagocytes: firstly, it is markedly voltage sensitive, becoming activated as the cell depolarizes. The current–voltage relationship displays a profound outward rectification, ensuring that H⁺ (equivalents) will leave, rather than enter the cytosol, relieving the developing acidification. Secondly, the channel is activated by cytosolic acidification, favoring the export of cytosolic H⁺ during the respiratory burst. Finally, the activation of the channel seems to be tightly linked to the stimulation of the oxidase, ensuring
coupling of their functions [40]. Indeed, it has been argued that the conductive activity is inherent to the transmembrane subunits of the oxidase (Fig. 2II), i.e. that the oxidase is also the channel [41]. Evidence both in favor of and against this hypothesis has been reported and is summarized and debated in a recent article [42].

6.1. The oxidase and degranulation

As discussed above, the NADPH oxidase has marked effects on the development of the luminal acidification that is thought to be, in turn, required for optimal maturation of the phagosome. Accordingly, patients with CGD exhibit abnormal neutrophil degranulation, and inhibition of the oxidase in normal cells has marked effects on phagosomal maturation [43]. While these effects can be most readily explained by the well-established alterations in phagosomal pH induced by the oxidase, an alternative explanation was recently put forward. Based on measurements of alkali cation fluxes and contents, Reeves et al. [44] proposed that K\(^+\), rather than H\(^+\), may be the primary counterion that neutralizes the electrogenic displacement of electrons by the oxidase. According to these authors, the electrically driven accumulation of K\(^+\) in the lumen of the phagosome generates a hypertonic milieu of high ionic strength. This unique solution is then envisaged to facilitate the dissolution of the matrix that holds the contents of secretory granules together. Failure to activate the oxidase, e.g. in individuals with CGD, would deprive the phagosome of the ability to disperse the granular contents, causing the observed secretory deficiency. Albeit attractive, this model requires independent confirmation, since it is unclear how a hypertonic solution can be generated in the water-permeable and distensible phagosomal membrane. Moreover, the purported high concentration of K\(^+\) generated in the phagosomal lumen by the oxidase was not demonstrated directly. Lastly, the conductive ionophore valinomycin had paradoxical effects in the report of Reeves et al. [44], which are not readily compatible with their hypothesis. Clearly, a more direct quantitation of the relative contribution of H\(^+\) and K\(^+\) to the electrical current and to the secretory events associated with maturation is required.

7. Concluding remarks

It is becoming increasingly apparent that phagosome formation and maturation are not identical in all cases. Obvious differences are noted between neutrophils and macrophages, which necessarily impact on the ability of phagosomes to eliminate pathogens or apoptotic bodies. Moreover, not all ligand and receptor pairs trigger comparable modes of phagosome formation, and the rate and extent of maturation also seem to differ among them. As a result, the mode of entry of pathogens is most likely to influence their fate, which may additionally turn out to be very different in neutrophils and macrophages. This realization makes it imperative to define the distinct molecular events that underlie phagocytosis mediated by individual receptors and the differential contribution of the NADPH in specific cell types. In addition, another challenge will be to explain the parallels and the differences between the “classical” maturation of phagosomes in macrophages, which parallels the endocytic sequence, and that in neutrophils, in which the unique and very specialized secretory organelles are not active components of the endocytic machinery. Clearly, much remains to be learned.