Phagocytosis: At the Crossroads of Innate and Adaptive Immunity

Isabelle Jutras and Michel Desjardins
Détartement de pathologie et biologie cellulaire, Université de Montréal, Montréal, Quebec H3C 3J7, Canada; email: isabelle.jutras@umontreal.ca, michel.desjardins@umontreal.ca

Key Words
phagosomes, phagolysosome biogenesis, antigen cross-presentation

Abstract
Phagocytosis, the process by which cells engulf large particles, requires a substantial contribution of membranes. Recent studies have revealed that intracellular compartments, including endocytic organelles and the endoplasmic reticulum (ER), can engage in fusion events with the plasma membrane at the sites of nascent phagosomes. The finding that ER proteins are delivered to phagosomes, where degraded peptides are loaded onto major histocompatibility complex (MHC) class II molecules, has significantly enhanced our understanding of the immune functions associated with these organelles. Although it is well known that pathogens are killed in phagosomes, the contribution of ER proteins to phagosomes has provided a novel pathway for the loading of exogenous peptides onto MHC class I molecules, a process known as cross-presentation. Thus, phagocytosis has evolved from a nutritional function in unicellular organisms to play key roles in both innate and adaptive immunity in vertebrates.
INTRODUCTION

Eukaryotic cells continuously internalize constituents of their environment. The nature of the internalized constituents, either fluids or particles of different sizes, delineates the various internalization processes (Conner & Schmid 2003). Phagocytosis involves the entry of large particles, typically 1 µm or more, including particles as diverse as inert beads, apoptotic cells, and microbes. Phagocytosis is used for different purposes by a wide range of organisms from amoeba to vertebrates. The capacity to engulf large particles likely appeared as a nutritional function in unicellular organisms, exemplified by the amoeba Dictyostelium discoideum, which feed on bacteria. More complex organisms have taken advantage of the phagocytic machinery to fulfill additional functions, such as the clearance of apoptotic cells during embryonic development. The molecular framework of phagocytosis appears to have been conserved throughout evolution, hence enabling the use of genetically tractable model organisms, such as the aforementioned Dictyostelium and the nematode Caenorhabditis elegans, to decipher the mechanisms underlying phagocytosis. For example, the study of phagocytosis in C. elegans has provided a better understanding of the molecular mechanisms associated with the engulfment of apoptotic cells; these mechanisms are often conserved in mammals (Reddien & Horvitz 2004).

Although it is easy to imagine how, in theory, cells could invaginate their cell surface to form a phagosome, the early events leading to particle engulfment during phagocytosis are, in fact, extremely complex. Recognition of specific ligands on the particle by cell-surface receptors initiates the phagocytic process. Carbohydrate binding to lectin receptors, used by a variety of cell types and organisms for phagocytosis, is probably the most widespread and ancient ligand-receptor combination (McGreal et al. 2004). This binding motif on bacteria, for instance, contributes to their engulfment by both amoeba and mammalian immune cells. Via phagocytosis, amoeba derive nutritional benefits whereas mammals may avoid infection (Cardelli 2001, Underhill & Ozinsky 2002). Mammalian professional phagocytes, such as neutrophils, dendritic cells, and macrophages, display a more substantial array of phagocytic receptors, coupled to distinct signal transduction pathways, than do unicellular organisms. In mammalian phagocytes, the study of phagocytic pathways has been focused mainly on those triggered by the Fc receptors (see
underscore the importance of phagocytosis review, we focus on recent developments that phagocytosis to adaptive immunity. In this cells to activate specific lymphocytes, also link peptides, which are presented by phagocytic some lumen and the production of antigenic the degradation of pathogens in the phago- sequent killing of microbes in phagosomes immune response. Indeed, phagocytosis and the sub- importance of phagocytosis in both the innate and adaptive immune functions.

**PHAGOLYSOSOME BIOGENESIS: A TALE OF MULTIPLE MEMBRANES**

**Contribution of the Plasma Membrane to Nascent Phagosomes**

In the classical view of phagocytosis, which had prevailed since the original description of phagocytes by Metchnikoff, the PM extended around the particle and formed the phagosome membrane (for a review on the early history of phagocytosis, see Stossel 1999). Several observations have since led to a reconsideration of the sole involvement of the PM in phagocytosis. The membrane surface required to enclose the multiple particles that professional phagocytes can phagocytose may represent an area equivalent to the entire cell surface (Werb & Cohn 1972). This is well illustrated by the fact that macrophages display a gargantuan appetite and can internalize beads larger than themselves without impairing their basic functions (Cannon & Swanson 1992). Nevertheless, no substantial decrease in the cell surface occurs following phagocytosis (Werb & Cohn 1972). The phagocytic rate observed in professional phagocytes may also imply an important waste of PM, a crucial organelle in monitoring the extracellular milieu. Thus, it appeared highly improbable that the PM would serve as the only source of membrane involved in phagocytosis, because the replacement of that membrane by neosynthesis may be expected to occur only within hours (Werb & Cohn 1972). Capacitance measurements made during the internalization of latex beads in J774 macrophages showed that phagocytosis is accompanied by an increase, rather than a decrease, in the cell surface area (Holevinsky & Nelson 1998). These observations were the first to suggest that endomembranes are recruited at the cell surface for the formation of phagosomes in a process referred to as focal exocytosis (Bajno et al. 2000).

**Innate immunity:** the initial defensive response against encountered microbes. This immune response involves the recognition of microbes by germ line-encoded receptors and relies mainly on phagocytic cells

**Adaptive immunity:** immune response that occurs as an adaptation to the microbes encountered during the organism’s lifetime and that confers protection against these microbes. This response involves the recognition of peptide antigens by specific T cells and the production of antibodies

**PM:** plasma membrane
Recycling endosomes: intracellular compartments, composed of vesicles and tubular structures, where proteins internalized by endocytosis are sorted and transported back to the plasma membrane or to other organelles.

Syt VII: synaptotagmin VII

Phagosomes: organelles resulting from extensive fusion events between phagosomes and lysosomes; these organelles are the sites where phagocytosed material is degraded.

Focal Exocytosis of Endosomes and Lysosomes

The observation that the cell surface area initially rises prior to the engulfment of particles raises several questions. What is the source of the intracellular membranes contributing to this increase? Where does exocytosis occur at the PM? How is exocytosis synchronized with phagocytosis? Although still incomplete, some answers to these questions have started to emerge. Focal exocytosis of VAMP-3-containing vesicles, presumably originating from recycling endosomes, was shown to occur in the vicinity of nascent phagosomes, and insertion of these membranes was suggested to account for the growth of pseudopods (Bajno et al. 2000). The small GTP-binding protein ARF6, which is activated during Fc receptor–mediated phagocytosis, appears to regulate the recruitment of these vesicles at phagocytic sites (Niedergang et al. 2003). The exocytosis of late endosomes at sites of phagocytosis was also recently demonstrated (Braun et al. 2004). This process appears to be regulated by VAMP-7, as pseudopod extension was inhibited in the absence of VAMP-7 (Braun et al. 2004).

Lysosomes are also capable of fusing with the PM, a process particularly important in pathogen phagocytosis. The secretory properties of specialized lysosomes, such as azurophil granules in neutrophils, are well known (Henson et al. 1992). Fusion of azurophil granules occurs with nascent unsealed phagosomes (Tapper & Grinstein 1997), leading to the release of azurophil granule enzymes in the extracellular medium. The release of these enzymes, which is likely to depend on the type of receptors triggered by phagocytosis, can be inhibited by certain pathogens, including mycobacteria (Cougoule et al. 2002). More recently, neutrophils have also been shown to secrete chromatin, which, together with the degradative content of the granules, forms an extracellular trap capable of retaining and killing bacteria (Brinkmann et al. 2004). The exocytosis of conventional lysosomes during phagocytosis was first demonstrated with the parasite Trypanosoma cruzi (Tardieux et al. 1992). Host-cell lysosomes were observed to aggregate at sites of parasite attachment and appeared to fuse with nascent phagosomes (Tardieux et al. 1992). This process was later shown to depend on the elevation of intracellular free Ca^{2+} concentrations, which are triggered upon the attachment of the parasite to the host cell (Andews 2002). Syt VII, a member of the synaptotagmin family of Ca^{2+}-binding proteins, regulates lysosome exocytosis and is required for cell invasion by T. cruzi (Caler et al. 2001). Importantly, Ca^{2+}-regulated exocytosis appears to be a general property of lysosomes and does not occur exclusively during parasite invasion. Lysosome exocytosis has been suggested, for instance, to play a key role in the repair of PM injuries, a process also regulated by Syt VII (Reddy et al. 2001). Consistent with these findings, fibroblasts from Syt VII-deficient mice are less susceptible to T. cruzi infection and are defective in membrane repair (Chakrabarti et al. 2003). Altogether, these studies clearly indicate that, in addition to the PM, membranes of various origins along the endosomal/lysosomal pathway may contribute directly to the formation of nascent phagosomes (Figure 1).

Phagosome Maturation

Although generally referred to as phagosomes, the compartments formed by phagocytosis can be widely heterogeneous depending on the type of particles internalized and the type of receptors used at the cell surface. Nevertheless, regardless of their content, phagosomes are extremely dynamic organelles that are continuously modified upon their formation. The conditions prevailing in the phagosome lumen, as well as their overall protein composition, change significantly from when phagosomes are formed at the cell surface to when phagolysosomes, the mature compartment where killing and degradation of pathogens occur, are generated. The
Multiple origins of the phagosome membrane. Phagocytosis of large particles involves the extension of plasma membrane pseudopods around the particle. However, the plasma membrane alone is insufficient to engulf the particle; phagocytosis is a process that requires the contribution of intracellular membranes. Several intracellular compartments—including the endoplasmic reticulum (ER), recycling endosomes (VAMP-3-containing vesicles), late endosomes (VAMP-7-containing vesicles), and lysosomes—may donate membranes to nascent phagosomes. Fusion of ER and plasma membranes involves the ER-resident SNARE protein ERS24, the mammalian homolog of yeast Sec22. Sec22 is capable of pairing with the plasma membrane t-SNARE Sso1/Sec9c to mediate fusion, a mechanism that may also take place in mammalian cells. Focal exocytosis of VAMP-3 vesicles is regulated by the small GTP-binding protein ARF6. The secretion of lysosomes is triggered by an increase in intracellular free Ca\textsuperscript{2+} concentration and is regulated by the Ca\textsuperscript{2+}-binding protein Syt VII.
multiple ways by which phagosome composition can be modulated include direct exchange of molecules with the cytoplasmic pool and transient interactions with various cell organelles. Early kinetic studies of phagosome maturation have indicated that cell surface markers rapidly disappear from newly formed phagosomes, and that maturing phagosomes sequentially display markers of early endosomes, late endosomes, and lysosomes (Desjardins et al. 1994, Pitt et al. 1992).

By monitoring the transfer of BSA-gold particles from endocytic organelles to nascent phagosomes at the electron microscope level, our group showed that phagosomes rapidly fuse with early endosomes and subsequently with late endosomes and lysosomes (Desjardins et al. 1997). This process is likely to influence the acquisition of proteolytic and microbicidal activities. Interestingly, the exchange of BSA-gold particles from endosomes to phagosomes was shown to be size-selective, suggesting that fusing membranes form a pore between the compartments in order to prevent the complete mixing of endosomal and phagosomal contents (Desjardins et al. 1997). Phagosome maturation could thus involve multiple but transient membrane fusion/fission events with endosomes, a process referred to as kiss and run fusion (Desjardins 1995). Fusion/fission events during phagolysosome biogenesis are controlled by a subset of Rab GTPases, including Rab5 and Rab7, which are thought to regulate the transient nature of the fusion events with early endosomes and late endosomes, respectively (Duclos et al. 2000). The expression of Rab5 molecules blocked in their active GTP-bound form impairs the transient nature of these fusion events and results in the formation of giant phagosomes that are unable to kill the intracellular parasite Leishmania donovani (Duclos et al. 2000). An effector of Rab7 termed RILP (Cantalupo et al. 2001) has been shown to bind dynein-dynactin, a microtubule-associated motor complex (Harrison et al. 2003). As maturing phagosomes are known to move along microtubules from the cell surface to a perinuclear region (Blocker et al. 1997), RILP may bind Rab7 on phagosomes and hence function in the attachment of phagosomes to microtubules (Harrison et al. 2003).

Phagosome maturation is also accompanied by the acquisition of sets of proteins and lipids that contribute to the segregation of certain phagosome constituents in membrane microdomains. Lipid raft-associated proteins, including flotillin-1 and subunits of the proton pump ATPase, appear to be absent from nascent phagosomes and to be recruited from endocytic compartments during phagosome maturation (Dermine et al. 2001). Lipid microdomains on phagosomes may serve as platforms for the assembly and nucleation of actin (Defacque et al. 2002). Because actin nucleation on phagosomes has been shown to facilitate fusion between phagosomes and late endosomes but has no significant involvement in fusion with early endosomes (Defacque et al. 2000, Kjeken et al. 2004), lipid rafts may play key roles in phagosome maturation and the acquisition of a phagosome’s microbicidal properties. Phagosomal lipid rafts have also been proposed to be the privileged platforms for the recruitment of cytosolic NADPH oxidase factors and for their assembly in an active NADPH oxidase complex (Vilhardt & van Deurs 2004, Shao et al. 2003); NADPH oxidase is a crucial enzyme for the microbicidal function of phagosomes and innate immune defense against infections.

How changes in the composition of phagosome proteins and lipids are modulated and coordinated in time represents a complex issue. Although substantial progress has been made, via a proteomics approach, in characterizing the protein composition of phagosome populations (Garin et al. 2001), the dynamics of association and dissociation of proteins on maturing phagosomes are largely unknown. To make things even more complex, a recent study indicated that rather than being regulated at the cell level, some features of phagosome maturation, such as the pattern of phospholipid association, may vary...
between individual phagosomes in a given cell (Henry et al. 2004).

**Toll-Like Receptors and Phagosome Maturation**

Phagocytic cells express a large number of receptors that bind specifically to various microbial molecules, allowing the innate immune system to respond to different types of infection in a process referred to as microbial sensing (Janeway & Medzhitov 2002, Gordon 2002). Among these receptors, recent studies have focused on the family of TLRs, which may suffice to recognize all types of microbes (Beutler 2004). In mammals, 13 TLRs have been identified; these directly sense microbial molecules (Beutler 2004). For instance, bacterial peptidoglycan and lipopolysaccharide are recognized by TLR2 and TLR4, respectively. The observed recruitment of TLR2 to the phagosome membrane suggests that TLRs may sense the microbial content of phagosomes to elicit an appropriate inflammatory response (Underhill et al. 1999). The effect of TLR activation on phagocytosis has been recently investigated. Pretreatment of macrophages with various TLR ligands, especially with the ligand of TLR9, apparently increased the number of *Escherichia coli* taken up by macrophages (Doyle et al. 2004). In agreement with this finding, one study showed that phagocytosis of various bacteria was impaired in macrophages lacking both TLR2 and TLR4 signaling (Blander & Medzhitov 2004). This study also highlighted an unexpected function for TLRs, as TLR activity was shown to stimulate fusion between endocytic organelles and phagosomes containing bacteria (Blander & Medzhitov 2004). This study found that the maturation of phagosomes containing apoptotic cells was not affected by TLR signaling (Blander & Medzhitov 2004); however, a concomitant study suggests that TLR4 has an inhibitory effect on the fusion of endocytic organelles with phagosomes containing apoptotic cells in macrophages (Shiratsuchi et al. 2004). Clearly, more studies are needed to decipher the role that TLRs might play in membrane fusion and phagosome maturation.

**FUNCTION OF PHAGOSOMES IN ANTIGEN PRESENTATION**

The killing of pathogens in the phagosome lumen after phagocytosis is a simple defense mechanism that is derived from the fact that phagosomes specialize in the degradation of complex molecules for recycling or nutritional purposes. This feature is sufficient for organisms such as amoebae to survive in their challenging environment and feed on bacteria. The degradative properties of phagosomes were exploited, through evolution, to improve the ability of complex organisms to monitor their environment and build more efficient defense mechanisms. In jawed vertebrates, peptides generated through the degradation of exogenous proteins can be loaded in the phagosome lumen onto MHC molecules and presented at the cell surface to initiate an adaptive immune response. This response involves recognition of MHC-associated peptides by TCR molecules, which have coevolved with the MHC (Du Pasquier 2004). Phagosomes in jawed vertebrates thus represent a compartment that has evolved to integrate functions of the innate and adaptive immune system.

**Antigen Presentation by MHC Class II Molecules**

The proteins present on engulfed particles encounter an array of degrading proteases in phagosomes. Yet, this destructive environment generates peptides that are capable of binding to MHC class II molecules. Interestingly, evidence indicates that hydrolases are not acquired by phagosomes through a simple “one-step” fusion event with lysosomes; as implied in the term “phagolysosomes,” such a process would transfer the bulk of hydrolases all at once. Instead, as indicated by the protein profile of phagosomes...
obtained by two-dimensional gel electrophoresis, the subsets of hydrolases acquired by phagosomes vary throughout the maturation process whereby newly formed phagosomes become phagolysosomes (Garin et al. 2001). The kinetics of protease acquisition may also vary between phagocytes; for instance, it is faster in macrophages than in dendritic cells (Lennon-Duménil et al. 2002). These conditions may limit the proteolysis of proteins in dendritic cells and favor the generation of peptide antigens of appropriate length for loading onto MHC class II molecules.

In the ER, newly synthesized MHC class II α and β chains associate with the Ii, which occupies the peptide-binding groove of the MHC class II molecules and targets them to the endocytic pathway (Watts 2004). There, Ii undergoes a series of proteolytic cleavages involving intermediates known as p22 and p10 and culminating in a fragment referred to as the CLIP. The processing of Ii can be initiated by AEP, which generates p22 and p10. However, other unidentified proteases can also mediate Ii degradation (Manoury et al. 2003). Importantly, humans and mice may exhibit differences in these proteases; for instance, AEP does not generate p10 in humans (Manoury et al. 2003). Although several cysteine proteases are capable of converting p10 into CLIP, this step is mainly dependent on cathepsin S in antigen-presenting cells (Watts et al. 2004). The protease responsible for processing p10 into CLIP may also vary between species. In murine thymic epithelial cells, cathepsin L appears to be the main p10-cleaving protease (Nakagawa et al. 1998), whereas in humans p10 may be processed only by cathepsin S (Bania et al. 2003).

CLIP remains bound to the peptide-binding site of the MHC class II molecules until it is replaced by a peptide antigen, in a process catalyzed by the DM protein. DM favors the dissociation of weakly bound peptides, such as CLIP, and the binding of high-affinity antigens (Watts 2004).

Antigen loading on MHC class II molecules takes place in various compartments, from early endosomes to lysosomes, of the endocytic pathway (Hiltbold & Roche 2002). Phagosomes containing latex beads have been shown to acquire MHC class II molecules both from a recycling pool on the PM and from a newly synthesized pool (Ramachandra & Harding 2000). The capacity of pathogens to prevent phagosome maturation is likely to affect antigen processing and presentation. For instance, in phagosomes containing Mycobacterium tuberculosis, the formation of bacterial antigen-MHC class II complexes is decreased when live bacteria, rather than heat-killed bacteria, are phagocytosed (Ramachandra et al. 2001). Likewise, phagocytosed Legionella, which reside in an ER-derived vacuole, may be expected to avoid the MHC class II presentation pathway. On the contrary, however, Legionella peptide antigens access compartments competent for peptide loading on MHC class II molecules (Neild & Roy 2003). The pathway by which these ER-derived antigens are trafficked to endocytic compartments remains to be defined.

Newly formed antigen-MHC class II complexes are delivered to the cell surface for presentation to CD4⁺ T cells. Recent results using GFP-tagged MHC molecules in dendritic cells suggest that peptide-MHC complexes are transported to the cell surface on lysosome-derived tubules, which extend toward the interface between dendritic cells and T cells (Boes et al. 2002, Chow et al. 2002). These lysosomal tubular structures, or vesicles derived from them, were also observed to fuse with the PM (Chow et al. 2002, Kleijmeer et al. 2001). Although mostly examined in dendritic cells, the trafficking of peptide-MHC class II complexes is likely to follow a similar pathway in other types of antigen-presenting cells.

ER-Mediated Phagocytosis and Antigen Cross-Presentation

A large body of evidence indicates that, in addition to the clear involvement of the
MHC class II pathway in the immune response against phagocytosed pathogens, antigens from pathogens, including mycobacteria, *Salmonella*, *Brucella*, and *Leishmania*, can elicit an MHC class I–dependent response promoting the proliferation of CD8+ cytotoxic T cells (**Figure 2**) (Ackerman & Cresswell 2004, Kaufmann & Schaible 2005). Yet the common view has long been that exogenous proteins, internalized by endocytosis or phagocytosis, are presented by the MHC class II pathway. In contrast, the MHC class I presentation pathway was considered restricted to endogenously synthesized proteins, including self proteins and those resulting from viral infections. The presentation of exogenous proteins on MHC class I molecules was thus referred to as cross-presentation, but the molecular basis of the mechanisms that might explain cross-presentation remained elusive. Clearly, the proliferation of CD8+ T cells is usually triggered by proteins that are from endogenous sources and processed in the cytoplasm by proteasomes to generate peptides that are loaded onto MHC class I molecules in the ER. Insights into the mechanisms enabling the cross-presentation of exogenous antigens came from observations, made by the Harding group, that phagocytosis of bacteria, with no known mechanism for cytosolic penetration, resulted in the presentation of bacterial antigens by MHC class I molecules in a process insensitive to Brefeldin A treatment (Pfeifer et al. 1993). These results indicated that because this drug disrupts the biosynthetic apparatus and blocks the classical class I processing pathway, a novel vacuolar pathway, competent for the processing of exogenous peptides and not linked to transport steps through the Golgi apparatus, also existed (Pfeifer et al. 1993).

Further insights into the molecular mechanisms linked to cross-presentation came from proteomics analysis of latex bead-containing phagosomes indicating that several ER proteins known to play a role in MHC class I–mediated antigen presentation, but no Golgi-resident protein, were present on phagosomes (Garin et al. 2001). On the basis of these observations, it was proposed that ER membranes could be recruited to phagosomes at some point during phagosome formation or maturation into phagolysosomes (Garin et al. 2001). Analysis of phagosome formation by

**Figure 2**

Antigen processing and loading on MHC molecules in phagosomes. Phagocytosed particles are degraded by proteases acquired during phagolysosome biogenesis. The degradation of foreign proteins generates peptide antigens that are loaded onto MHC class II molecules. Through ER-mediated phagocytosis and the delivery of ER proteins to phagosomes, cross-presentation of exogenous proteins on MHC class I molecules can be initiated in these organelles. Antigen cross-presentation involves the retro-translocation of exogenous peptides from the phagosome lumen to the cytosol, either by the Sec61 translocon or by the putative channel formed by the recently identified Derlin-1. Retro-translocated peptides are polyubiquitinatated, processed by the proteasome, transported back to the phagosome lumen through TAP, and loaded onto MHC class I molecules. An alternative pathway for cross-presentation involves the transport of processed peptides from the cytosol to the ER lumen, where they can be loaded onto MHC class I molecules. Some of the chaperones involved in retro-translocation (calnexin and GRP78/Bip) and in MHC class I antigen loading (calreticulin and Erp57) are shown.
WHAT INTRACELLULAR PATHOGENS TELL US ABOUT CROSS-PRESENTATION

Antigen presentation by MHC class I and class II molecules has generally been considered to involve two segregated pathways and to take place in distinct organelles. Namely, endogenous proteins processed in the cytoplasm are loaded onto MHC class I molecules in the ER, whereas exogenous proteins are processed and loaded onto MHC class II molecules in endocytic compartments. However, this model is difficult to reconcile with our comprehension of the immune response to pathogens. Intracellular pathogens have evolved ways to survive in their host by subverting host-membrane trafficking to establish replicative niches in compartments lacking the properties of phagolysosomes. For instance, *Salmonella* and *Mycobacteria* species reside in immature phagosomes lacking the typical markers of lysosomes (Holden 2002, Vergne et al. 2004), whereas *Legionella* and *Brucella* bypass the endocytic pathway and reside in ER-like compartments (Roy & Tilney 2002, Celli & Gorvel 2004). Hence, the segregated presentation pathways imply that peptides derived from the former species are presented on class II molecules, whereas those derived from the latter are presented on class I molecules. In fact, all of these pathogens can elicit responses from both the MHC class I and II presentation pathways. Recent findings in the trafficking of intracellular organelles during phagocytosis indicate that all types of phagosomes interact, at least to some level, with the endocytic and biosynthetic pathways to build mixed compartments in which, in principle, both class I and class II processing can take place. Thus, the concept of cross-presentation may have arisen from a misunderstanding of the complex trafficking events occurring in antigen-presenting cells.

Cytotoxic T cells: a class of T lymphocytes that kills infected target cells displaying pathogens’ peptide fragments bound to MHC class I molecules

various biochemical and morphological methods led to the surprising observation that during phagocytosis, the ER is recruited to the cell surface, where it appears to fuse underneath phagocytic cups to provide some of the membrane required for the formation of phagosomes, a process referred to as ER-mediated phagocytosis (Gagnon et al. 2002). Although several types of organelles were shown to be able to contribute membrane to forming phagosomes (see above and Figure 1), the direct fusion of the ER with the PM at the cell surface was unexpected, as no molecular basis could explain the fusion of these two compartments. Interestingly, the Gerisch group showed that the ER was likely to play a direct role in the formation of phagosomes, because a double knockout for the ER proteins calnexin and calreticulin in *Dictyostelium* abolished phagocytosis in this cell (Müller-Taubenberger et al. 2001).

Membrane fusion requires the formation of a SNARE complex, which is composed of SNAREs located on the vesicle and the target membranes, termed respectively the v-SNARE and the t-SNARE. The appropriate pairing of v-SNAREs and t-SNAREs ensures the specificity of these fusion events. Using an in vitro assay that measures the fusion of reconstituted liposomes, Rothman’s group tested different combinations of yeast SNAREs for their capacity to sustain fusion (McNew et al. 2000). This study revealed that liposomes containing the PM t-SNARE Sso1/Sec9c could fuse with liposomes bearing the ER-resident SNARE Sec22—a finding that was, at the time, difficult to reconcile with the SNARE hypothesis as ER-PM were not expected to be fusion partners. The significance of this finding was recently investigated in the context of phagocytosis using ERS24, the mammalian homolog of Sec22. It was shown that ERS24 is recruited to nascent phagosomes and that its inhibition impairs the phagocytosis of particles larger than 0.8 µm (Becker et al. 2005).

The Phagosome is a Competent Organelle for Cross-Presentation

Arguments in favor of the proposal that the ER contributes a significant part of the phagosome membrane came from morphological and biochemical studies showing that calnexin was present in its proper orientation in the membrane of isolated phagosomes, whereas calreticulin, a luminal protein, was protected from hydrolysis by proteases, as expected from a direct fusion event between the ER and the phagosomes (Gagnon et al. 2002).
Calnexin was also observed on membranes surrounding latex particles in dendritic cells (Guermonprez et al. 2003). In addition to calnexin, most of the molecules known to participate in the processing and presentation of peptides on MHC class I molecules were shown to be present on phagosomes and to contribute to defining a compartment competent for the processing of exogenous proteins for MHC class I presentation (Ackerman et al. 2003, Guermonprez et al. 2003, Houde et al. 2003).

The ability of phagosomes to degrade microorganisms and generate derived peptides is well established. Moreover, the sequential acquisition of hydrolases by phagosomes (Garin et al. 2001) may favor regulated patterns of peptide degradation suitable for presentation (Lennon-Duménil et al. 2002). Recently, cathepsin S-deficient phagosomes were shown to generate poorly presented class I peptides (Shen et al. 2004). However, the overall efficiency of intraphagosomal degradation to generate peptides for direct loading onto MHC class I molecules remains to be established. The 8- to 10-amino acid peptides preferentially loaded on MHC class I molecules are best produced by proteasomes; this implies that peptides partially degraded in the phagosome lumen would be better suited for presentation if they underwent further processing by proteasomes. This step would require the translocation of peptides from the phagosome lumen to the cytoplasm. This process has been shown to occur and be part of a “phagosome-to-cytosol” pathway that feeds exogenous peptides to the “classical” MHC class I pathway (Kovacsovics-Bankowski & Rock 1995). Swanson and colleagues tested various carriers as a source of exogenous proteins by phagocytosis and found that translocation of ovalbumin to the cytoplasm was more efficient when delivered by polystyrene particles or biodegradable particles rather than by sheep red blood cells (Oh et al. 1997).

Although these translocated peptides could be targeted effectively to the classical MHC class I pathway, recent findings suggest that phagosomes from macrophages possess a machinery of their own for antigen processing (Figure 2). Proteomics analyses have revealed that proteins involved in each step of the process required for MHC class I antigen processing and presentation, including chaperones, the ubiquitination machinery, proteasome subunits, and elements of the loading complex, were present on purified latex bead-containing phagosomes (Houde et al. 2003). In functional assays, peptides from fluorescently labeled ovalbumin that were loaded onto latex beads were shown to be efficiently translocated to the cytoplasm, where they were polyubiquitinated and observed to associate with proteasomes on the phagosome membrane (Houde et al. 2003). It is still unclear which molecule is responsible for the translocation of peptides from the phagosome lumen to the cytoplasm. Because CTA1 can also be translocated to the cytoplasm after phagocytosis, it was proposed that Sec61, the ER translocon also detected on phagosomes, might be involved in this process (Houde et al. 2003). Indeed, Sec61 was shown previously to be involved in the retro-translocation of CTA1 from the ER lumen to the cytoplasm (Schmitz et al. 2000). Recently, a novel membrane protein, Derlin1, was shown to mediate retro-translocation from the ER lumen to the cytosol (Lilley & Ploegh 2004, Ye et al. 2004). Whether Derlin 1, which also localizes to phagosomes (P. Cresswell, personal communication), can also function on this organelle remains to be established. Using in vitro assays, two groups have also demonstrated that functional TAP transporters on dendritic cell phagosomes are able to translocate added peptides to the phagosome lumen (Ackerman et al. 2003, Guermonprez et al. 2003). Intraphagosomal loading of peptides on MHC class I molecules was also observed (Ackerman et al. 2003, Guermonprez et al. 2003, Houde et al. 2003). Finally, it was shown that following phagocytosis, the MHC class I ovalbumin–derived peptide SIINFEKL could trigger the proliferation of
CD8+ T cells by a mechanism that was only partially inhibited by Brefeldin A (Houde et al. 2003).

Taken together, these results indicate that the contribution of the ER to phagosome formation and/or maturation is not only meant to spare the use of the PM but also brings additional functional properties to the phagosome. The advantage of using the ER as a source of membrane for phagosome formation has been discussed in previous reviews (Aderem 2002, Desjardins 2003). Recent evidence further extends the possible use of the ER as a source of membrane for the formation of macropinosomes, where it might also contribute to the ability of this compartment to process soluble exogenous material for cross-presentation (Ackerman et al. 2003). Moreover, a pathway that brings exogenous molecules to the ER via endocytosis has also been described (Ackerman & Cresswell 2004). This pathway might also be used to allow contact between the ER-localized TLR9 and its endocytosed ligand consisting of bacterial unmethylated CpG DNA (Latz et al. 2004). ER-mediated phagocytosis would indeed favor the natural encounter of bacterial DNA with TLR9 after the killing and degradation of pathogens in phagosomes.

**CONCLUSION**

The contribution of both endocytic organelles and the ER to phagosome formation and maturation obviously allows for the building of a cellular compartment where killing, degradation, processing, and presentation of exogenous peptides can take place in an integrated way. Mammalian cell phagosomes have clearly inherited part of their innate immune function from the “digestive” properties of their amoeba counterparts. However, the functions related to antigen presentation have appeared only recently during evolution with jawed vertebrates, in which the essential elements of the adaptive immune system have co-evolved in the immunoglobulin-TCR-MHC unit (Du Pasquier 2004). Because several of the molecules involved in MHC class I antigen presentation encoded in the MHC are expressed in the ER—e.g., TAP—this raises the question of whether the contribution of the ER to phagosome formation is restricted to vertebrates.

Interestingly, the few pieces of evidence available at the moment indicate that this is not the case. As mentioned before, ER-mediated phagocytosis is a likely process in *Dictyostelium* because phagosomes formed in these cells appear to be surrounded by membrane-containing ER molecules, as shown by confocal microscopy (Müller-Taubenberger et al. 2001). Moreover, a double knockout of calnexin and calreticulin inhibits phagocytosis (Müller-Taubenberger et al. 2001). These results are supported by a recent study showing that the uptake of *Legionella* in *Dictyostelium* is accompanied by the recruitment of GFP-tagged calnexin and calreticulin to phagocytic cups (Fajardo et al. 2004). This contrasts with current models in mammals suggesting that ER is recruited to phagosomes at later points during infection (Roy & Tilney 2002). Furthermore, preliminary results using a proteomics approach indicate that latex bead–containing phagosomes purified from *Dictyostelium* display a significant number of ER proteins, whereas such phagosomes are mostly devoid of contaminants from other intracellular organelles (T. Soldati & M. Desjardins, unpublished results). Similar results were obtained from phagosomes isolated from Schneider S2 cells derived from *Drosophila* (J. Boulais & M. Desjardins, unpublished results). Although preliminary, these results suggest that the ER contributed to the formation and/or maturation of phagosomes from their earliest evolutionary origins. This argues for the interesting concept that because phagosome formation involved the ER in species with no adaptive immunity, novel properties beneficial to the immune system appeared on phagosomes concomitant with the appearance of the MHC locus and the expression of some of its gene products in the ER. This enabled
the evolution or refinement of phagosomes from a lytic compartment capable of playing a role in innate immunity by killing microorganisms into a well-integrated organelle linking innate and adaptive immunity. Not surprisingly, understanding of the fine molecular mechanisms supporting a central role for phagosomes in immunity awaits further study.

**SUMMARY POINTS**

1. Phagocytosis, the internalization of large particles by cells, has evolved from a nutritional process in amoeba to an immune process in complex organisms.
2. The phagosome membrane is composed of membranes of multiple cellular origins, including the plasma membrane, endocytic organelles, and the ER.
3. The protein composition of phagosomes undergoes modifications through transient fusion/fission events with compartments of both the endocytic and biosynthetic pathways.
4. Phagosome fusion with late endosomes and lysosomes leads to the formation of phagolysosomes.
5. Phagolysosomes contain proteolytic and microbicidal activities, which are essential features of the innate immune system.
6. Phagosomes are the site of peptide antigen processing and loading onto MHC class II molecules.
7. The fusion of ER membranes with phagosomes allows the delivery of the machinery required for the processing and loading of peptides onto MHC class I molecules.
8. Phagosomes are competent organelles for antigen cross-presentation.
9. The contribution of ER to phagosome formation may be a conserved process from amoeba to vertebrates.

**LITERATURE CITED**


This was the first demonstration that direct fusion events between the endoplasmic reticulum and the plasma membrane occur at the site of nascent phagosomes.


This paper questions the idea that phagosomes containing a single type of particle mature in a uniform manner and shows differences in phosphoinositide labeling between individual phagosomes.


Together with Ackerman et al. (2003) and Guermonprez et al. (2003), this paper reveals that phagosomes are self-sufficient organelles for antigen cross-presentation. Phagosome-associated proteins undergo retro-translocation to the cytosol, ubiquitination, proteasome-dependent degradation, and loading onto MHC class I molecules.


www.annualreviews.org • Phagocytosis
Oh YK, Harding CV, Swanson JA. 1997. The efficiency of antigen delivery from macrophage phagosomes into cytoplasm for MHC class I-restricted antigen presentation. *Vaccine* 15:511–18
Contents

Frontispiece
David D. Sabatini ................................................................. xiv

In Awe of Subcellular Complexity: 50 Years of Trespassing Boundaries
Within the Cell
David D. Sabatini ............................................................... 1

Mechanisms of Apoptosis Through Structural Biology
Nieng Yan and Yigong Shi .................................................. 35

Regulation of Protein Activities by Phosphoinositide Phosphates
Verena Niggli ................................................................. 57

Principles of Lysosomal Membrane Digestion: Stimulation of
Sphingolipid Degradation by Sphingolipid Activator Proteins and
Anionic Lysosomal Lipids
Thomas Kolter and Konrad Sandhoff .................................. 81

Cajal Bodies: A Long History of Discovery
Mario Cioce and Angus I. Lamond .................................... 105

Assembly of Variant Histones into Chromatin
Steven Henikoff and Kami Ahmad .................................... 133

Planar Cell Polarization: An Emerging Model Points in the
Right Direction
Thomas J. Klein and Marek Mlodzik ................................ 155

Molecular Mechanisms of Steroid Hormone Signaling in Plants
Grégory Vert, Jennifer L. Nemhauser, Niko Geldner, Fangxin Hong,
and Joanne Chory ............................................................ 177

Anisotropic Expansion of the Plant Cell Wall
Tobias I. Baskin ............................................................... 203

RNA Transport and Local Control of Translation
Stefan Kindler, Huidong Wang, Dietmar Richter, and Henri Tiedge ............ 223
Rho GTPases: Biochemistry and Biology
Aron B. Jaffe and Alan Hall ................................................................. 247

Spatial Control of Cell Expansion by the Plant Cytoskeleton
Laurie G. Smith and David G. Oppenheimer ......................................... 271

RNA Silencing Systems and Their Relevance to Plant Development
Frederick Meins, Jr., Azeddine Si-Ammour, and Todd Blevins .................. 297

Quorum Sensing: Cell-to-Cell Communication in Bacteria
Christopher M. Waters and Bonnie L. Bassler ...................................... 319

Pushing the Envelope: Structure, Function, and Dynamics of the Nuclear Periphery
Martin W. Hetzer, Tobias C. Walther, and Iain W. Mattaj ......................... 347

Integrin Structure, Allostery, and Bidirectional Signaling
M.A. Arnaout, B. Mahalingam, and J.-P. Xiong .................................... 381

Centrosomes in Cellular Regulation
Stephen Duxsey, Dannel McCollum, and William Theurkauf .................... 411

Endoplasmic Reticulum–Associated Degradation
Karin Römisch ................................ ......................................................... 435

The Lymphatic Vasculature: Recent Progress and Paradigms
Guillermo Oliver and Kari Alitalo .......................................................... 457

Regulation of Root Apical Meristem Development
Keni Jiang and Lewis J. Feldman ............................................................ 485

Phagocytosis: At the Crossroads of Innate and Adaptive Immunity
Isabelle Jutras and Michel Desjardins ................................................... 511

Protein Translocation by the Sec61/SecY Channel
Andrew R. Osborne, Tom A. Rapoport, and Bert van den Berg ................ 529

Retinotectal Mapping: New Insights from Molecular Genetics
Greg Lemke and Michael Reber ............................................................ 551

In Vivo Imaging of Lymphocyte Trafficking
Cornelia Halin, J. Rodrigo Mora, Cenk Sumen, and Ulrich H. von Andrian .... 581

Stem Cell Niche: Structure and Function
Lingheng Li and Ting Xie .................................................................. 605

Docosahexaenoic Acid, Fatty Acid–Interacting Proteins, and Neuronal Function: Breastmilk and Fish Are Good for You
Joseph R. Marszalek and Harvey F. Lodish ............................................ 633

Specificity and Versatility in TGF-β Signaling Through Smads
Xin-Hua Feng and Rik Derynck ............................................................... 659
The Great Escape: When Cancer Cells Hijack the Genes for Chemotaxis and Motility

John Condeelis, Robert H. Singer, and Jeffrey E. Segall ........................................ 695

INDEXES

Subject Index .................................................................................................................. 719
Cumulative Index of Contributing Authors, Volumes 17–21 .................................. 759
Cumulative Index of Chapter Titles, Volumes 17–21 .............................................. 762

ERRATA

An online log of corrections to Annual Review of Cell and Developmental Biology chapters may be found at http://cellbio.annualreviews.org/errata.shtml