Published in final edited form as:

Curr Opin Immunol. 2009 February ; 21(1): 98–104. doi:10.1016/j.coi.2009.01.001.

MHC Molecules and Microbial Antigen Processing in Phagosomes

Lakshmi Ramachandra, Daimon Simmons, and Clifford V. Harding

Department of Pathology, Case Western Reserve University, Cleveland, Ohio, USA.

Summary

Macrophages and dendritic cells are phagocytic antigen presenting cells that internalize bacteria and other particulate antigens into phagosomes. The phagosome must then balance microbicidal and proteolytic degradation functions with the generation of antigenic peptides for presentation by class I and class II MHC molecules to CD8 and CD4 T cells, respectively. Understanding the host and bacterial factors that affect phagosomal antigen processing may help facilitate new strategies to eliminate pathogens.

Introduction

Dendritic cells (DCs) and macrophages are phagocytic antigen presenting cells (APCs) at the interface between the innate and adaptive immune systems. They internalize bacteria, fungi and parasites into phagosomes, where proteolytic processing of microbial antigens (Ags) produces antigenic peptides that are subsequently presented by class I major histocompatibility complex (MHC-I) and class II MHC (MHC-II) molecules to T cells. MHC-I presentation of exogenous Ags, e.g. those derived from extracellular or intravacuolar microbes, is also referred to as MHC-I cross processing to distinguish this mechanism from conventional MHC-I processing of cytosolic Ags, although most MHC-I cross processing involves components of the cytosolic Ag processing pathway as well as phagosomes. Thus, phagosomes contribute to both innate antimicrobial host defenses and specific immune responses to microbes by MHC-II-restricted CD4+ T cells and MHC-I-restricted CD8+ T cells. This review will focus on selected recent advances in the areas of phagosomal Ag processing (including cross processing) and its regulation. Other recent reviews have covered related areas of Ag processing and phagosome function [1–3].

Phagosomes

Bacteria are internalized into phagosomes that exchange material initially with early endosomes and subsequently with late endosomes and lysosomes to form phagolysosomes in a process referred to as phagosome maturation. These vesicular interactions deliver host molecules with anti-microbial properties that include the generators of toxic reactive oxygen species, anti-microbial peptides, proteases, thiol reductases and proton pumps. Reactive oxygen species may be created by nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, as reviewed elsewhere [4]. When stimulated by inflammatory signals, phagocytic cells use NADPH oxidase 2 (NOX2) to generate reactive oxygen species in phagolysosomes. Anti-microbial peptides in phagosomes include defensins. Proteases (including cathepsins) are activated in the acidic environment of the phagosome, enhancing

Corresponding author: Clifford V. Harding (cvh3@case.edu).

Conflict of Interest:

The authors have no conflict of interest with the work cited in this paper.

catabolic functions that contribute to both killing of microbes and proteolytic processing of their Ags. Together, these components of the phagolysosome contribute both antimicrobial defenses and antigen processing functions.

While the phagosome must acquire proteolytic function to process Ags, peptides must be spared from complete proteolytic destruction for presentation by MHC molecules, and this is achieved by regulating the level of proteolysis and phagosomal acidification in different APCs at different stages of APC activation or maturation [5–7]. How does the phagosome achieve this fine balance? Although activation of macrophages has been associated with increased lysosomal protease expression [8,9], recent studies in macrophages using fluorometric techniques [10] or proteomic analysis [11] have suggested that activation of macrophages with interferon-gamma and/or lipopolysaccharide leads to reduced vacuolar protease activity that may avoid complete proteolytic destruction of peptides and favor generation of peptides for Ag presentation. Phagosome acidification is less extreme in DCs than in macrophages, which may contribute to the high efficiency of MHC-I cross processing by DCs, which are particularly important APCs for Ag presentation to naïve T cells. DCs blunt acidification of their phagosomes by Rab27a-dependent recruitment of NOX2 [12–14]. This apparently reduces Ag proteolysis to a level that allows processing but does not fully destroy antigenic peptides, favoring increased MHC-I cross processing [13]. This again demonstrates the interplay of anti-microbial and Ag processing functions in the phagolysosome, as NOX2 contributes to both.

MHC-I cross processing in phagosomes

All nucleated cells process and present cytosolic Ags on MHC-I, but only phagocytic APCs can internalize and process particulate Ags for MHC-I cross presentation. Direct cross processing occurs when phagocytic APCs process particulate Ags (e.g. microbes) that they directly internalize, while indirect cross processing occurs when phagocytic APCs process Ags present in other host cells that they internalize. This may occur after apoptosis of the Ag-donor cell [15] and the resulting cross presentation can contribute to either induction of antimicrobial [16] or anti-tumor CD8 T cell responses, or cross tolerance [17] to tissue-specific self Ags [18]. This process is critical for the induction of adaptive immunity against viral Ags [19] or other Ags that are not endogenously synthesized by the professional APCs that can license Ag-specific CD8+ T cells.

Do phagosomes export antigenic fragments into the cytoplasm for loading by the conventional MHC-I loading pathway in the endoplasmic reticulum (ER) or are they capable of mediating formation of peptide:MHC-I complexes themselves? Several models have addressed this question in different ways (Fig 1). The phagosome to cytosol model of cross processing involves transfer of Ag across the phagosomal membrane into the cytosol, where it is degraded by the proteasome and loaded onto MHC-I in the ER [20]. Alternatively, Ag may be cross processed through a vacuolar mechanism [21] that involves trafficking of MHC-I molecules to phagosomes, where they bind antigenic peptides generated by vacuolar antigen proteolysis. For example, in DCs ovalbumin bound to beads is processed by vacuolar cathepsin S for presentation by MHC-I [22]. Other studies have supported a third cross processing mechanism, the ER-phagocytosis model, wherein the ER fuses with the phagosome, introducing molecules that may transport Ag to the cytoplasm and peptides back into the phagosome for loading of MHC-I. It is likely that all of these mechanisms contribute to cross processing to a degree that may vary with different types of Ag and APCs of different type or state of activation.

The ER phagocytosis model was initially suggested by proteomic analysis of latex bead phagosomes from mouse macrophages that revealed the presence of many ER-derived

proteins (including the MHC-I loading machinery) in phagosomes [23]. This led to the hypothesis that the ER mediates phagocytosis, allowing incorporation of ER proteins into the developing phagosome [24]. Syntaxin 18, an ER-localized SNARE protein has been shown to be involved in ER-mediated phagocytosis in mouse macrophages [25]. The contribution of the ER to phagosome formation, however, has been controversial, and other investigators were unable to detect a significant contribution of the ER to forming or maturing phagosomes in either macrophages or DCs [26]. Consistent with the ER-phagocytosis model, several groups have shown that phagosomes can mediate cross processing in DCs [27,28]. This model is similar to the phagosome-to-cytosol model in that Ag is translocated to the cytosol for proteasome-mediated processing, but differs in that peptides are transported back into the phagosome (instead of the ER) for formation of peptide:MHC-I complexes. Ackerman et al suggested that only early phagosomes contribute to cross presentation in DCs [29], and additional studies have also suggested that cross processing may occur in a time-dependent manner, preferentially early after phagocytosis [30]. This suggests that cross processing occurs during the brief period when phagosomal acidification is inhibited. Since mechanisms that promote alkalization of phagosomes are transient [4], the phagosomes eventually undergo acidification, causing activation of lysosomal proteases for the generation of peptides from the particulate Ag that can bind MHC-II (Fig. 2). Phagocytosed extracellular Ags have also been demonstrated to access the cytosol via components required for ER-associated degradation [31]. These observations suggested that cross processing results from an adaptation of the retrotranslocation machinery involved in the degradation of misfolded ER proteins and further supported the observation that phagosomes acquire ER membrane. Autophagy, discussed below, may hypothetically contribute to phagosomal cross processing by delivering ER components to the phagosome.

Many factors regulate MHC-I cross processing in phagosomes. Interferon-gamma activates macrophages and promotes expression of the MHC-I loading complex within phagosomes containing latex beads [11]. In addition, it induces expression of the LMP2 and LMP7 subunits of the immunoproteasome that enhance production of peptides that fit the MHC-I binding groove. As noted above, interferon-gamma may also decrease the proteolytic activity of the phagosome, which may serve to limit destructive processing, allowing peptides to survive phagosomal processing and enhancing cross presentation of phagosomal Ags. In addition, the Fc-gamma receptor can enhance cross presentation by both promoting phagocytosis of opsonized antigens and by regulating processing functions via ITAM signaling [32,33]. As discussed above, NOX2 blunts phagosomal acidification and enhances MHC-I cross processing in murine DCs [13] and human DCs [14]. In the latter study, cross processing of model tumor Ags was decreased by NOX2 inhibitors or NOX2 deficiency (in cells from patients with chronic granulomatous disease). Thus, many mechanisms that regulate phagosome composition, acidification or proteolytic function affect MHC-I cross processing mechanisms.

Some bacterial pathogens such as *Listeria* and *Shigella* can escape the phagosome, and Ags produced by these bacteria directly enter the conventional MHC-I loading pathway. *L. monocytogenes* escapes the phagosome through the action of Listeriolysin O (LLO), which is activated in the lysosome. A recent report suggests that gamma-interferon inducible lysosomal thiol reductase (GILT), a host protein that is important for MHC-II processing [34], is also needed to activate LLO and allow phagosomal escape [35]. In contrast, intravacuolar pathogens such as *Salmonella typhimurium* and *Mycobacterium tuberculosis* survive within phagosomes. Thus, their Ags are not directly expressed in the cytosol, and cross processing of phagosomal Ags is necessary to initiate MHC-I-restricted CD8⁺ T cell responses against these intravacuolar microbes. Phagocytosed *E. coli* and *Salmonella typhimurium* are cross-processed by a vacuolar mechanism in macrophages [36] and by a

phagosome-to-cytosol mechanism in DCs [37]. *Salmonella* generate MHC-I-restricted CD8 responses by a vacuolar cross processing pathway in activated macrophages but not B cells [38] or epithelial cells [39]. Interestingly, the *yej* gene product of *Salmonella* inhibits cross processing of Ag expressed by the bacteria [40], although the mechanism for this inhibition has not been determined. Human macrophages cross process mycobacterial Ags through a vacuolar pathway [41]. Human DCs may cross process mycobacterial Ags through a vacuolar pathway [42] or by transferring mycobacterial Ag into the cytoplasm, especially in the case of Ags secreted by the bacteria [43,44]. Although *M. tuberculosis* has long been considered to be a vacuolar intracellular pathogen, a recent report suggests that virulent *M. tuberculosis* may escape phagosomes of infected macrophages after 96 hours [45], which hypothetically would allow processing of *M. tuberculosis* Ags by the conventional MHC-I Ag processing pathway.

MHC-II phagosomal processing

The role of phagosomes in MHC-II Ag processing is well established. Particulate Ags such as latex bead conjugated Ags and *M. tuberculosis* are internalized by macrophages into phagosomes and digested by proteases. The resulting peptide fragments associate with MHC-II molecules present within the phagosome [46–48]. In *M. tuberculosis*-infected DCs, MHC-II and CD1b molecules also colocalize to phagosomes [49], but rapid DC maturation results in decreased MHC-II Ag processing after an early period, while CD1b molecules are still able efficiently present lipid Ags. The loss of MHC-II processing was attributed to rapid trafficking of MHC-II from the phagosome to the plasma membrane prior to Ag availability. In *Salmonella*-induced vacuoles, MHC-II and HLA-DM do not appear to interact, and MHC-II molecules do not appear to be loaded with Ags [50]. This may allow *Salmonella* to evade CD4 T cell responses.

Can organisms that interfere with phagosomal maturation affect MHC-II Ag processing and presentation? *M. tuberculosis* survives within host macrophages by inhibiting phagosome maturation; this mechanism may also help the bacteria evade CD4+ T cell responses by reducing proteolytic processing of its Ags for presentation by MHC-II molecules. However, infection of mouse macrophages or DCs *in vitro* with *Mycobacterium bovis* Bacille Calmette-Guérin (BCG) mutants that are unable to escape lysosomal transfer resulted in efficiency of Ag presentation similar to that observed with wild-type mycobacteria [51]. Moreover, *in vivo*, these mutants induced CD4+ Th1 and CD8+ CTL responses in mice against various mycobacterial Ags that were comparable to those induced by their wild-type counterparts. This observation is in agreement with earlier studies in which efficiency of processing of live and dead *M. tuberculosis* (H37Ra) by macrophages were compared [52]. Both the live and dead bacteria showed identical efficiency in processing and presentation to MHC-II-restricted, *M. tuberculosis*-specific T cells, despite the fact that dead bacteria lose capacity to inhibit phagosome maturation. This suggested that efficiency of phagosomal MHC-II processing of *M. tuberculosis* was independent of phagosomal maturation. In other studies, however, *M. tuberculosis* H37Rv and BCG were found to interfere with phagosome maturation and were less efficiently processed than *M. tuberculosis* H37Ra, which produced less inhibition of phagosome maturation, suggesting that inhibition of phagosome maturation corresponded to reduced MHC-II antigen processing [53]. In addition, *M. tuberculosis* H37Rv mutants with partially reduced inhibition of phagosome maturation were more efficient at priming CD4+ T cells compared to wild type H37Rv and BCG [54]. *M. bovis* BCG secreting cathepsin S was found to be more efficiently processed than the wild type strain, and this was found to correlate with the accelerated fusion of its phagosome with lysosomes [55]. Additional studies comparing different strains of mycobacteria are needed to determine whether control of phagosome maturation correlates with efficiency of Ag processing.

Regulation of phagosomal processing by TLR signaling and autophagy

There is controversy as to whether signals from TLRs can regulate phagosome maturation and hence phagosomal processing and presentation. Blander and Medzhitov found that activation of TLRs in macrophages by bacteria but not apoptotic cells regulated both phagocytosis and the kinetics of phagosome maturation [56]. The inducible mode of phagosome maturation, as seen with bacteria, was controlled by TLRs, while the constitutive mode of maturation as seen with apoptotic cells was not. They subsequently demonstrated that apoptotic cells or particulate Ags are only presented efficiently by MHC-II in DCs when they are associated with a TLR ligand [57], suggesting the existence of a regulatory mechanism to prevent recognition of self by the immune system [58,59]. In contrast, Russell and co-workers demonstrated that phagosome maturation was not affected by agonists of TLR2 or TLR4 [60–63].

Macroautophagy (referred to here simply as autophagy) is a cellular process that leads to the engulfment of cytosol and organelles (including phagosomes) in an autophagic vacuole. Lysosomes then fuse with the autophagic vacuole, resulting in degradation of its contents. Autophagy-related proteins have been found in the phagosomal proteome [64]. The presence of TLR2 agonists on phagocytosed beads leads to localization of the autophagosome marker protein, LC3, to the phagosome [65]. LPS signaling recruits LC3 to the phagosome through the MyD88/Trif-dependent signaling pathway [66]. Agonists of other TLRs, particularly TLR7, induce autophagy and decrease survival of intraphagosomal bacteria [67]. Autophagy can induce killing of intracellular pathogens that otherwise resist microbicidal mechanisms [68]. A number of studies have established a role for autophagy in processing of endogenous (cytosolic) Ags for presentation by MHC-II molecules, as reviewed elsewhere [69]. A recent study demonstrated that autophagy occurs constitutively in APCs, contributing to MHC-II peptide presentation [70].

Conclusions

The composition and function of phagosomes appear to be differentially regulated in cells, and this directly impacts antigen processing functions of phagocytic APCs. Relative to DCs, macrophages have greater microbicidal function, and their phagosomes are more acidic and have higher proteolytic activity. These conditions allow for killing of microorganisms as well as robust MHC-II processing, but they may not optimize MHC-I cross processing. Dendritic cells are less microbicidal than macrophages and have evolved mechanisms to limit phagosomal acidification, curtail proteolysis and enhance MHC-I cross processing.

While phagosomes have been demonstrated to mediate formation of peptide:MHC-II complexes, it remains unclear whether regulation of phagosome maturation impacts MHC-II processing and presentation. The exact roles of phagosomes in cross processing remain controversial. Whether the phagosome does or does not mediate formation of peptide:MHC-I complexes with or without the assistance of the ER may depend on the cells being studied, their activation status and the nature of the phagosome cargo.

A number of questions remain regarding the roles of TLRs and autophagy in phagosomal Ag processing. TLR signaling may regulate functions of both phagosomes and autophagosomes. TLR-dependent activation of autophagy and recruitment of autophagy machinery to the phagosome may enhance MHC-II Ag processing. In addition to its role in processing of endogenous Ag for MHC-II presentation, autophagy may hypothetically contribute to MHC-II processing of exogenous or vacuolar Ag, or to MHC-I cross processing, but these hypotheses require future study. With regard to MHC-I cross processing, the autophagosome may contain ER-derived protein translocases or MHC-I

loading machinery that could mediate phagosomal cross processing, especially under conditions of increased autophagy. The role of autophagy in phagosomal Ag processing itself remains speculative until future studies dig into these questions. Bon appetit!

Acknowledgments

The authors' research is supported by National Institute of Health grants A1076792 (to L.R.) and AI035726, AI034343 and AI069085 (to C.V.H.).

Abbreviations

Ag	antigen
APC	antigen presenting cell
DC	dendritic cell
ER	endoplasmic reticulum
GILT	gamma-interferon inducible lysosomal thiol reductase
LLO	Listeriolysin O
LMP	low molecular weight polypeptide
MHC-I	major histocompatibility complex class I
MHC-II	major histocompatibility complex class II
NADPH	nicotinamide adenine dinucleotide phosphate
NOX2	NADPH oxidase 2
TLR	toll-like receptor

References and annotations

Papers of particular interest that were published within the period of review have been highlighted as:

* of special interest

** of outstanding interest

1. Burgdorf S, Kurts C. Endocytosis mechanisms and the cell biology of antigen presentation. *Curr Opin Immunol.* 2008; 20:89–95. [PubMed: 18249105]
2. Savina A, Amigorena S. Phagocytosis and antigen presentation in dendritic cells. *Immunol Rev.* 2007; 219:143–156. [PubMed: 17850487]
3. Kinchen JM, Ravichandran KS. Phagosome maturation: going through the acid test. *Nat Rev Mol Cell Biol.* 2008; 9:781–795. [PubMed: 18813294]
4. Segal AW. How neutrophils kill microbes. *Annu Rev Immunol.* 2005; 23:197–223. [PubMed: 15771570]
5. Trombetta ES, Ebersold M, Garrett W, Pypaert M, Mellman I. Activation of lysosomal function during dendritic cell maturation. *Science.* 2003; 299:1400–1403. [PubMed: 12610307]
6. Trombetta ES, Mellman I. Cell biology of antigen processing in vitro and in vivo. *Annu Rev Immunol.* 2005; 23:975–1028. [PubMed: 15771591]
7. Delamarre L, Pack M, Chang H, Mellman I, Trombetta ES. Differential lysosomal proteolysis in antigen-presenting cells determines antigen fate. *Science.* 2005; 307:1630–1634. [PubMed: 15761154]

8. Lah TT, Hawley M, Rock KL, Goldberg AL. Gamma-interferon causes a selective induction of the lysosomal proteases, cathepsins B and L, in macrophages. *FEBS Lett.* 1995; 363:85–89. [PubMed: 7729559]
9. Honey K, Rudensky AY. Lysosomal cysteine proteases regulate antigen presentation. *Nat Rev Immunol.* 2003; 3:472–482. [PubMed: 12776207]
10. Yates RM, Hermetter A, Taylor GA, Russell DG. Macrophage activation downregulates the degradative capacity of the phagosome. *Traffic.* 2007; 8:241–250. [PubMed: 17319801]
**Demonstrates that macrophage activation suppresses hydrolytic activity in early phagosomes and enhances degradative capacity of late phagolysosomes.
11. Jutras I, Houde M, Currier N, Boulais J, Duclos S, LaBoissiere S, Bonneil E, Kearney P, Thibault P, Paramithiotis E, et al. Modulation of the phagosome proteome by interferon-gamma. *Mol Cell Proteomics.* 2008; 7:697–715. [PubMed: 18156134] *Demonstrates that in interferon-activated macrophages, early phagosomes show delayed proteolytic activity coupled to increased recruitment of MHC-I peptide-loading complex.
12. Jancic C, Savina A, Wasmeier C, Tolmachova T, El-Benna J, Dang PM, Pascolo S, Gougerot-Pocidalo MA, Raposo G, Seabra MC, et al. Rab27a regulates phagosomal pH and NADPH oxidase recruitment to dendritic cell phagosomes. *Nat Cell Biol.* 2007; 9:367–378. [PubMed: 17351642] **See note to reference 13.
13. Savina A, Jancic C, Hugues S, Guernonprez P, Vargas P, Moura IC, Lennon-Dumenil AM, Seabra MC, Raposo G, Amigorena S. NOX2 controls phagosomal pH to regulate antigen processing during crosspresentation by dendritic cells. *Cell.* 2006; 126:205–218. [PubMed: 16839887]
**References 12 and 13 highlight how Rab27a-positive vesicles traffic NOX2 to the phagosomes and control phagosomal acidification to allow processing of Ags for cross presentation.
14. Mantegazza AR, Savina A, Vermeulen M, Perez L, Geffner J, Hermine O, Rosenzweig SD, Faure F, Amigorena S. NADPH oxidase controls phagosomal pH and antigen cross-presentation in human dendritic cells. *Blood.* 2008
15. Albert ML, Sauter B, Bhardwaj N. Dendritic cells acquire antigen from apoptotic cells and induce class I-restricted CTLs. *Nature.* 1998; 392:86–89. [PubMed: 9510252]
16. Schaible UE, Winau F, Sieling PA, Fischer K, Collins HL, Hagens K, Modlin RL, Brinkmann V, Kaufmann SH. Apoptosis facilitates antigen presentation to T lymphocytes through MHC-I and CD1 in tuberculosis. *Nat. Med.* 2003; 9:1039–1046. [PubMed: 12872166]
17. Albert ML, Jegathesan M, Darnell RB. Dendritic cell maturation is required for the cross-tolerization of CD8+ T cells. *Nat Immunol.* 2001; 2:1010–1017. [PubMed: 11590405]
18. Luckashenak N, Schroeder S, Endt K, Schmidt D, Mahnke K, Bachmann MF, Marconi P, Deeg CA, Brocker T. Constitutive crosspresentation of tissue antigens by dendritic cells controls CD8+ T cell tolerance in vivo. *Immunity.* 2008; 28:521–532. [PubMed: 18387832] **Shows that a DC-specific deficiency in uptake of apoptotic material inhibits cross presentation and prevents activation of autoreactive T cells in vivo.
19. Sigal LJ, Crotty S, Andino R, Rock KL. Cytotoxic T-cell immunity to virus-infected non-haematopoietic cells requires presentation of exogenous antigen. *Nature.* 1999; 398:77–80. [PubMed: 10078533]
20. Kovacsics-Bankowski M, Rock KL. A phagosome-to-cytosol pathway for exogenous antigens presented on MHC class I molecules. *Science.* 1995; 267:243–246. [PubMed: 7809629]
21. Pfeifer JD, Wick MJ, Roberts RL, Findlay K, Normark SJ, Harding CV. Phagocytic processing of bacterial antigens for class I MHC presentation to T cells. *Nature.* 1993; 361:359–362. [PubMed: 7678924]
22. Shen L, Sigal LJ, Boes M, Rock KL. Important role of cathepsin S in generating peptides for TAP-independent MHC class I crosspresentation in vivo. *Immunity.* 2004; 21:155–165. [PubMed: 15308097]
23. Garin J, Diez R, Kieffer S, Dermine JF, Duclos S, Gagnon E, Sadoul R, Rondeau C, Desjardins M. The phagosome proteome: insight into phagosome functions. *J Cell Biol.* 2001; 152:165–180. [PubMed: 11149929]

24. Gagnon E, Duclos S, Rondeau C, Chevet E, Cameron PH, Steele-Mortimer O, Paiement J, Bergeron JJ, Desjardins M. Endoplasmic reticulum-mediated phagocytosis is a mechanism of entry into macrophages. *Cell*. 2002; 110:119–131. [PubMed: 12151002]
25. Hatsuzawa K, Tamura T, Hashimoto H, Yokoya S, Miura M, Nagaya H, Wada I. Involvement of syntaxin 18, an endoplasmic reticulum (ER)-localized SNARE protein, in ER-mediated phagocytosis. *Mol Biol Cell*. 2006; 17:3964–3977. [PubMed: 16790498]
26. Touret N, Paroutis P, Terebiznik M, Harrison RE, Trombetta S, Pypaert M, Chow A, Jiang A, Shaw J, Yip C, et al. Quantitative and dynamic assessment of the contribution of the ER to phagosome formation. *Cell*. 2005; 123:157–170. [PubMed: 16213220]
27. Houde M, Bertholet S, Gagnon E, Brunet S, Goyette G, Laplante A, Princiotta MF, Thibault P, Sacks D, Desjardins M. Phagosomes are competent organelles for antigen cross-presentation. *Nature*. 2003; 425:402–406. [PubMed: 14508490]
28. Guermonprez P, Saveanu L, Kleijmeer M, Davoust J, Van Endert P, Amigorena S. ER-phagosome fusion defines an MHC class I cross-presentation compartment in dendritic cells. *Nature*. 2003; 425:397–402. [PubMed: 14508489]
29. Ackerman AL, Kyritsis C, Tampe R, Cresswell P. Early phagosomes in dendritic cells form a cellular compartment sufficient for cross presentation of exogenous antigens. *Proc. Natl. Acad. Sci. USA*. 2003; 100:12889–12894. [PubMed: 14561893]
30. Howland SW, Wittrup KD. Antigen release kinetics in the phagosome are critical to cross-presentation efficiency. *J Immunol*. 2008; 180:1576–1583. [PubMed: 18209053]
31. Ackerman AL, Giodini A, Cresswell P. A role for the endoplasmic reticulum protein retrotranslocation machinery during crosspresentation by dendritic cells. *Immunity*. 2006; 25:607–617. [PubMed: 17027300] **Highlights how cross processing depends on the ER retrotranslocation mechanisms.
32. Liu Y, Gao X, Masuda E, Redecha PB, Blank MC, Pricop L. Regulated expression of FcγR3 in human dendritic cells controls cross-presentation of antigen-antibody complexes. *J Immunol*. 2006; 177:8440–8447. [PubMed: 17142741]
33. Graham DB, Stephenson LM, Lam SK, Brim K, Lee HM, Bautista J, Gilfillan S, Akilesh S, Fujikawa K, Swat W. An ITAM-signaling pathway controls cross-presentation of particulate but not soluble antigens in dendritic cells. *J Exp Med*. 2007; 204:2889–2897. [PubMed: 17984307]
*Links ITAM-dependent receptors with activation of NOX2 complex and production of reactive oxygen species.
34. Maric M, Arunachalam B, Phan UT, Dong C, Garrett WS, Cannon KS, Alfonso C, Karlsson L, Flavell RA, Cresswell P. Defective Antigen Processing in GILT-Free Mice. *Science*. 2001; 294:1361–1365. [PubMed: 11701933]
35. Singh R, Jamieson A, Cresswell P. GILT is a critical host factor for *Listeria monocytogenes* infection. *Nature*. 2008; 455:1244–1247. [PubMed: 18815593] **Elegantly demonstrates that GILT activation of listeriolysin O is essential for phagosomal escape of *L. monocytogenes* and that GILT-deficient mice are resistant to *L. monocytogenes* infection.
36. Pfeifer JD, Wick MJ, Harding CV, Normark SJ. Processing of defined T-cell epitopes after phagocytosis of intact bacteria by macrophages. *Infect Agents Dis*. 1993; 2:249–254. [PubMed: 7513597]
37. Svensson M, Wick MJ. Classical MHC class I peptide presentation of a bacterial fusion protein by bone marrow-derived dendritic cells. *Eur. J. Immunol*. 1999; 29:180–188. [PubMed: 9933099]
38. Rosales-Reyes R, Alpujch-Aranda C, Ramirez-Aguilar Mde L, Castro-Eguiluz AD, Ortiz-Navarrete V. Survival of *Salmonella enterica* serovar Typhimurium within late endosomal-lysosomal compartments of B lymphocytes is associated with the inability to use the vacuolar alternative major histocompatibility complex class I antigen-processing pathway. *Infect Immun*. 2005; 73:3937–3944. [PubMed: 15972480]
39. Harding CV, Pfeifer JD. Antigen expressed by *Salmonella typhimurium* is processed for class I major histocompatibility complex presentation by macrophages but not infected epithelial cells. *Immunology*. 1994; 83:670–674. [PubMed: 7875747]
40. Qimron U, Madar N, Mittrucker HW, Zilka A, Yosef I, Bloushtain N, Kaufmann SH, Rosenshine I, Apte RN, Porgador A. Identification of *Salmonella typhimurium* genes responsible for

- interference with peptide presentation on MHC class I molecules: Deltayej Salmonella mutants induce superior CD8+ T-cell responses. *Cell Microbiol.* 2004; 6:1057–1070. [PubMed: 15469434]
41. Canaday DH, Ziebold C, Noss E, Chervenak K, Harding CV, Boom WH. Activation of human CD8+ alpha beta TCR+ cells by *Mycobacterium tuberculosis* via an alternate class I MHC antigen-processing pathway. *J. Immunol.* 1999; 162:372–379. [PubMed: 9886409]
 42. Lewinsohn DM, Alderson MR, Briden AL, Riddell SR, Reed SG, Grabstein KH. Characterization of human CD8+ T cells reactive with *Mycobacterium tuberculosis*-infected antigen-presenting cells. *J. Exp. Med.* 1998; 187:1633–1640. [PubMed: 9584141]
 43. Lewinsohn DM, Grotzke JE, Heinzl AS, Zhu L, Ovendale PJ, Johnson M, Alderson MR. Secreted proteins from *Mycobacterium tuberculosis* gain access to the cytosolic MHC class-I antigen-processing pathway. *J Immunol.* 2006; 177:437–442. [PubMed: 16785540] *Shows that secreted proteins from *M.tuberculosis* egress into cytosol and are degraded by the proteasome into peptides prior to binding MHC-I
 44. Woodworth JS, Fortune SM, Behar SM. Bacterial protein secretion is required for priming of CD8+ T cells specific for the *Mycobacterium tuberculosis* antigen CFP10. *Infect Immun.* 2008; 76:4199–4205. [PubMed: 18591224] *Shows that MHC-I cross processing of *M. tuberculosis* Ags is dependent on their secretion, i.e. secreted Ag is more available for cross processing than Ag that is retained within *M. tuberculosis*.
 45. van der Wel N, Hava D, Houben D, Fluitsma D, van Zon M, Pierson J, Brenner M, Peters PJ. M. tuberculosis and M. leprae translocate from the phagolysosome to the cytosol in myeloid cells. *Cell.* 2007; 129:1287–1298. [PubMed: 17604718]
 46. Ramachandra L, Noss E, Boom WH, Harding CV. Processing of *Mycobacterium tuberculosis* antigen 85B involves intraphagosomal formation of peptide-major histocompatibility complex II complexes and is inhibited by live bacilli that decrease phagosome maturation. *J. Exp. Med.* 2001; 194:1421–1432. [PubMed: 11714749]
 47. Ramachandra L, Song R, Harding CV. Phagosomes are fully competent antigen processing organelles that mediate the formation of peptide:class II MHC complexes. *J. Immunol.* 1999; 162:3263–3272. [PubMed: 10092778]
 48. Torres M, Ramachandra L, Rojas RE, Bobadilla K, Thomas J, Canaday DH, Harding CV, Boom WH. Role of phagosomes and major histocompatibility complex class II (MHC-II) compartment in MHC-II antigen processing of *Mycobacterium tuberculosis* in human macrophages. *Infect Immun.* 2006; 74:1621–1630. [PubMed: 16495533]
 49. Hava DL, van der Wel N, Cohen N, Dascher CC, Houben D, Leon L, Agarwal S, Sugita M, van Zon M, Kent SC, et al. Evasion of peptide, but not lipid antigen presentation, through pathogen-induced dendritic cell maturation. *Proc Natl Acad Sci U S A.* 2008; 105:11281–11286. [PubMed: 18685099] **Highlights how *M.tuberculosis*-driven DC maturation results in loss of MHC-II presentation, whereas mechanisms for CD1 Ag presentation are maintained.
 50. Zwart W, Griekspoor A, Kuijl C, Marsman M, van Rheeën J, Janssen H, Calafat J, van Ham M, Janssen L, van Lith M, et al. Spatial separation of HLA-DM/HLA-DR interactions within MIIC and phagosome-induced immune escape. *Immunity.* 2005; 22:221–233. [PubMed: 15723810]
 51. Majlessi L, Combaluzier B, Albrecht I, Garcia JE, Nouze C, Pieters J, Leclerc C. Inhibition of phagosome maturation by mycobacteria does not interfere with presentation of mycobacterial antigens by MHC molecules. *J Immunol.* 2007; 179:1825–1833. [PubMed: 17641049] *Uses a mycobacterial strain that is unable to escape lysosomal transfer to demonstrate that MHC-II processing and presentation of mycobacteria is modulated independently of phagosome maturation.
 52. Ramachandra L, Smialek JL, Shank SS, Convery M, Boom WH, Harding CV. Phagosomal processing of *Mycobacterium tuberculosis* antigen 85B is modulated independently of mycobacterial viability and phagosome maturation. *Infect Immun.* 2005; 73:1097–1105. [PubMed: 15664953]
 53. Singh CR, Moulton RA, Armitage LY, Bidani A, Snuggs M, Dhandayuthapani S, Hunter RL, Jagannath C. Processing and presentation of a mycobacterial antigen 85B epitope by murine macrophages is dependent on the phagosomal acquisition of vacuolar proton ATPase and in situ activation of cathepsin D. *J Immunol.* 2006; 177:3250–3259. [PubMed: 16920965] *Demonstrates

how phagosome maturation promotes a V-ATPase-dependent phagosomal activation of cathepsin D and enhances MHC-II presentation.

54. Katti MK, Dai G, Armitage LY, Marrero CR, Daniel S, Singh CR, Lindsey DR, Dhandayuthapani S, Hunter RL, Jagannath C. The Delta fbpA mutant derived from *Mycobacterium tuberculosis* H37Rv has an enhanced susceptibility to intracellular antimicrobial oxidative mechanisms, undergoes limited phagosome maturation and activates macrophages and dendritic cells. *Cell Microbiol.* 2008; 10:1286–1303. [PubMed: 18248626]
55. Soualhine H, Deghmane AE, Sun J, Mak K, Talal A, Av-Gay Y, Hmama Z. *Mycobacterium bovis* bacillus Calmette-Guerin secreting active cathepsin S stimulates expression of mature MHC class II molecules and antigen presentation in human macrophages. *J Immunol.* 2007; 179:5137–5145. [PubMed: 17911599]
56. Blander JM, Medzhitov R. Regulation of phagosome maturation by signals from toll-like receptors. *Science.* 2004; 304:1014–1018. [PubMed: 15143282]
57. Blander JM, Medzhitov R. Toll-dependent selection of microbial antigens for presentation by dendritic cells. *Nature.* 2006 **Shows that TLRs regulate phagocytosis at several levels, including uptake and phagosome maturation, and suggests a consequent mechanism for DCs to discriminate recognition of self and non self Ags, driven by the presence of TLR ligands in the phagocytosed cargo.
58. Blander JM, Medzhitov R. On regulation of phagosome maturation and antigen presentation. *Nat Immunol.* 2006; 7:1029–1035. [PubMed: 16985500]
59. Blander JM, Medzhitov R. Reply to "Toll-like receptors and phagosome maturation". *Nat Immunol.* 2007; 8:217–218. [PubMed: 17304226]
60. Yates RM, Russell DG. Phagosome maturation proceeds independently of stimulation of toll-like receptors 2 and 4. *Immunity.* 2005; 23:409–417. [PubMed: 16226506] **Questions the role of TLRs in regulating phagosome maturation.
61. Russell DG. New ways to arrest phagosome maturation. *Nat Cell Biol.* 2007; 9:357–359. [PubMed: 17401383]
62. Russell DG, Yates RM. TLR signalling and phagosome maturation: an alternative viewpoint. *Cell Microbiol.* 2007; 9:849–850. [PubMed: 17346307]
63. Russell DG, Yates RM. Toll-like receptors and phagosome maturation. *Nat Immunol.* 2007; 8:217. author reply 217–218. [PubMed: 17304226]
64. Shui W, Sheu L, Liu J, Smart B, Petzold CJ, Hsieh TY, Pitcher A, Keasling JD, Bertozzi CR. Membrane proteomics of phagosomes suggests a connection to autophagy. *Proc Natl Acad Sci U S A.* 2008; 105:16952–16957. [PubMed: 18971338]
65. Sanjuan MA, Dillon CP, Tait SW, Moshiah S, Dorsey F, Connell S, Komatsu M, Tanaka K, Cleveland JL, Withoff S, et al. Toll-like receptor signalling in macrophages links the autophagy pathway to phagocytosis. *Nature.* 2007; 450:1253–1257. [PubMed: 18097414] **Shows that the presence of Toll-like receptor agonists within a phagosome leads to recruitment of autophagy machinery to the phagosome.
66. Shi CS, Kehrl JH. MyD88 and Trif target Beclin 1 to trigger autophagy in macrophages. *J Biol Chem.* 2008
67. Delgado MA, Elmaoued RA, Davis AS, Kyei G, Deretic V. Toll-like receptors control autophagy. *EMBO J.* 2008; 27:1110–1121. [PubMed: 18337753]
68. Gutierrez MG, Master SS, Singh SB, Taylor GA, Colombo MI, Deretic V. Autophagy Is a Defense Mechanism Inhibiting BCG and *Mycobacterium tuberculosis* Survival in Infected Macrophages. *Cell.* 2004; 119:753–766. [PubMed: 15607973]
69. Schmid D, Munz C. Innate and adaptive immunity through autophagy. *Immunity.* 2007; 27:11–21. [PubMed: 17663981]
70. Schmid D, Pypaert M, Munz C. Antigen-Loading Compartments for Major Histocompatibility Complex Class II Molecules Continuously Receive Input from Autophagosomes. *Immunity.* 2007; 26:79–92. [PubMed: 17182262] **Demonstrates that MHC-II molecules colocalize with autophagy machinery and that fusing a protein to LC3 leads to increased MHC-II presentation of peptide from that protein.

Figure 1.

Potential cross processing pathways. In pathway A, ER fuses with the phagosome, introducing molecules that may transport Ag to the cytoplasm and proteasome-generated peptides back into the phagosome for loading onto MHC-I. The phagosome to cytosol model of cross processing (pathway B) involves transfer of Ag across the phagosomal membrane into the cytosol, antigen proteolysis by the proteasome to produce peptides, transport of these peptides via TAP into the ER, and loading of peptides onto MHC-I. In the vacuolar pathway (pathway C), antigenic peptides are generated by vacuolar antigen proteolysis and bind to MHC-I in phagosomes.

Figure 2.

Regulation of phagocytic antigen processing. Rab27a-mediated delivery of NOX2 blunts acidification in early phagosomes and late phagosomes of DCs, but macrophages produce greater acidification of phagolysosomes and activation of proteases. The regulation of acidification and proteolytic activity of phagosomes leads to a model of spatial segregation of MHC-I processing in early phagosomes and MHC-II processing in late phagosomes, as discussed by Burgdorf and Kurts [1]. The role of TLRs in regulating TLR maturation is somewhat controversial, but TLR signaling may drive phagosome maturation, increasing acidification and proteolytic activity. Some pathogens, e.g. *M. tuberculosis* (MTB), inhibit phagosome maturation, but autophagy may overcome this inhibition and drive fusion of autophagosomes containing bacteria with lysosomes.