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Cellular mechanisms governing cross-presentation of exogenous antigens

Anne L Ackerman & Peter Cresswell

The recent discovery of fusion of endoplasmic reticulum membrane with nascent phagosomes suggests that this peripheral compartment in macrophages and dendritic cells may serve as an organelle optimized for major histocompatibility complex (MHC) class I–restricted cross-presentation of exogenous antigens. The process allows intersection of the endosomal system with the endoplasmic reticulum, the classical site of MHC class I peptide loading, and may reconcile the seemingly conflicting evidence indicating both of these sites are crucial in cross-presentation. Here we discuss the potential mechanisms involved in loading exogenous antigens onto MHC class I molecules and the implications of this new evidence for the *in vivo* function of dendritic cells.

Major histocompatibility complex (MHC) class II molecules on the surface of professional antigen-presenting cells (APCs) typically present only peptides derived from exogenous proteins acquired by endocytosis or from internalized plasma membrane proteins. MHC class I molecules generally present peptide antigens derived from endogenously synthesized proteins. MHC class I is expressed by the vast majority of cells, allowing circulating $CD8^+$ cytotoxic T cells to survey them for possible infection or improper protein expression, such as might be seen during tumorigenesis. For the creation of this surface library of endogenous peptides, cellular proteins are degraded in the cytosol, mainly by the proteasome. Peptides generated by the proteasome are transported by the transporter associated with antigen processing (TAP) into the lumen of endoplasmic reticulum (ER)[1], where an ER aminopeptidase produces the mature MHC class I–binding peptide by preferentially trimming the peptide to an optimal eight or nine amino acids[2,3] (also reviewed in this issue by Rock *et al.*[4] and Kloetzel[5]).

TAP, a heterodimer composed of TAP1 and TAP2 subunits, associates with nascent MHC class I–β_2-microglobulin (β_2M) dimers through interactions in the peptide-loading complex (**Fig. 1**). This complex includes the transmembrane glycoprotein tapasin; calreticulin, a lectin-like chaperone that binds a monoglucosylated *N*-linked glycan on the MHC class I heavy chain[6]; and ERp57, a thiol oxidoreductase noncovalently associated with calreticulin and disulfide-linked to tapasin[7]. In addition to linking TAP to the MHC class I–β_2M dimer, tapasin may also act as a peptide 'editor', allowing MHC class I molecules to exchange peptides and encouraging the binding of higher-affinity peptides[8] or a broader range of peptides[9]. An intact loading complex in the ER is essential for efficient MHC class I–peptide association; cells and mice deficient in β_2M, tapasin, calreticulin or TAP demonstrate defective MHC class I antigen presentation[10–16]. The action of this complex, probably involving the rearrangement of disulfide bonds and editing of MHC class I–associated glycans, facilitates the binding of TAP-delivered peptides to MHC class I–β_2M dimers, producing a mature complex capable of interacting with $CD8^+$ T cells[17].

Before becoming competent effector cells, T cells must first be primed against specific antigens by professional APCs of hematopoietic origin. These cells abundantly express MHC class I and II antigens as well as costimulatory and adhesion molecules. The most potent APC is the dendritic cell (DC), which is thought to be the main 'priming' cell *in vivo*[18]. A chief difficulty is understanding how $CD8^+$ T cell responses are primed to infectious organisms that do not infect APCs[19,20]. $CD8^+$ T cell responses are efficiently generated against pathogens that are restricted to the endocytic pathway and never reach the cytosol[21–24]. Pathogen-infected DCs are also often functionally compromised[25,26]. How can DCs prime cytotoxic T cells against these antigens when they do not express the antigens produced after infection? The classical model describing MHC class I presentation of endogenous antigens seems inadequate to fully explain the priming event.

In nonhematopoietic cells, the restriction of MHC class I presentation to endogenous antigens is critical; otherwise, healthy cells may become targets for killing by $CD8^+$ T cells. Thus, there is a specialized pathway that allows the acquisition of antigens from infected or abnormal cells by DCs and possibly macrophages, facilitating the generation of an MHC class I–restricted immune response. This pathway, called cross-presentation, allows display of exogenous antigens in the context of MHC class I molecules. Restriction of presentation to a subset of APCs that uses passive acquisition rather than endogenous expression of antigens may prevent the dissemination of pathogens or the spread of cancerous cells through the lymphatic system. Restriction of presentation to a subset of APCs that uses passive acquisition circumvents the need for endogenous expression of antigens by DCs or their direct infection, limiting the potential dissemination of pathogens or the spread of cancerous cells throughout the lymphatic system.

Section of Immunobiology, Howard Hughes Medical Institute, Yale University School of Medicine, P.O. Box 208011, New Haven, Connecticut 06520-8011, USA. Correspondence should be addressed to P.C. (peter.cresswell@yale.edu).

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Discovery of cross-presentation

Cross-presentation was first extensively documented in the mid-1970s in studies by Bevan[27,28]. In one experiment, spleen cells from *H2* homozygous mice (either $H2^b$ or $H2^d$) were injected into mice with both of these *H2* haplotypes ($H2^{b/d}$) on a different genetic background[27]. A portion of the reactive recipient $CD8^+$ T cells were restricted to an *H2* class I allele of the F_1 recipient mice that was absent from the immunizing cells, contradicting the classical idea of allogenic recognition. These T cells could lyse targets from *H2* congenic mice sharing the background of the donor, indicating that the T cells had been primed to a peptide derived from a minor histocompatibility antigen acquired from the donor cells bound to MHC class I molecules derived from the host. Since these initial findings, reports demonstrating that cross-presentation occurs in physiological circumstances other than transplantation, including viral infections, autoimmune diseases such as diabetes, and cancer, have appeared in the literature in ever-increasing numbers.

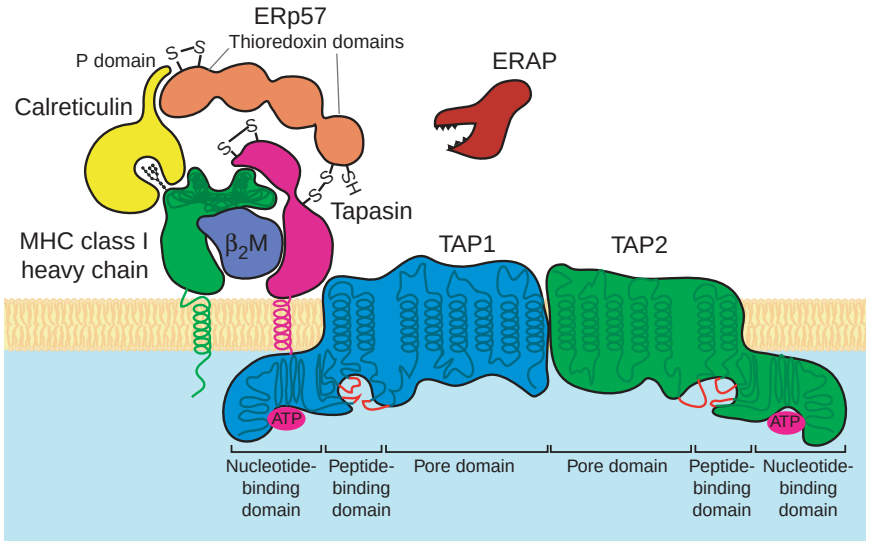

Figure 1 MHC class I peptide-loading complex. A single tapasin molecule is associated with the TAP heterodimer here, although data suggest that there may be four[75]. The peptide-loading complex, plus ER aminopeptidase (ERAP), is found in macrophage and DC phagosomes.

The cross-presenting cell

Despite studies of cross-presentation spanning four decades, the precise identity of the APC responsible has been hotly debated. Although the cross-presenting cell is clearly an APC of hematopoietic origin, cross-presentation activity has been detected in both macrophage and DC populations. Because of the lack of effective markers distinguishing these populations (especially difficulties distinguishing immature dendritic cells from resting macrophages), many initial studies identifying one population or the other as the critical cell *in vivo* may have been complicated by small contaminating fractions of the other cell type. More comprehensive studies extensively examining splenic and lymph node DC subsets in mice have identified $CD8^+$ DCs as the only population capable of cross-presenting ovalbumin and herpes simplex epitopes. Despite the capacity of macrophages and other DC subsets to acquire antigens, the ability to effectively stimulate cytotoxic T lymphocyte responses seems to be restricted to this population[29–31]. As the number of distinguishable DC subsets grows and the number of known DC maturation stimuli mounts, this model may be further refined to define the critical cell subset *in vivo*.

Although cross-presentation *in vivo* has been localized mainly to DCs, multiple types of endocytic cells, including macrophages, B cells, keratinocytes and L cells, can cross-present exogenous antigens *in vitro* with a low degree of efficiency[32,33]. Nonendocytically active cells, however, do not seem to be competent for this process. Although peptides derived from particulate antigens are presented with an efficiency three to four orders of magnitude greater than that of soluble antigens[34,35], proteins internalized through nearly all methods of endocytosis, including macropinocytosis, receptor-mediated endocytosis and phagocytosis, can be cross-presented[33,36,37]. The enhancement of cross-presentation cannot be explained by increased antigen uptake, as presentation can also be enhanced by the addition of unconjugated particles to soluble antigen particles[32]. This suggests that the phagocytic process itself enhances antigen delivery into the MHC class I pathway.

A peptide exchange model for cross-presentation

Internalization of exogenous antigens may allow cell fragments, intracellular pathogens and proteins to be degraded in the endocytic pathway by mechanisms involving reduction, unfolding and lysosomal proteolysis. Such a process could contribute to cross-presentation by facilitating the exchange of previously loaded MHC class I–associated peptides for newly generated peptides derived from exogenous proteins[38]. These previously loaded MHC class I molecules could be derived from the cell surface by endocytosis. MHC class I internalization from the cell surface is regulated, signaled by monoubiquitination of a conserved lysine in the MHC class I cytoplasmic tail[39]. Lysine modification also regulates the inclusion of MHC class I molecules into multivesicular bodies, where they colocalize with MHC class II on the membranes of the internal vesicles[40]. MHC class I can be secreted from this compartment on exosomes[41], which correspond to the internal vesicles and which may deliver MHC molecules to additional APCs and be capable of stimulating T cells themselves.

The endocytic exchange pathway seems to be dependent on lysosomal degradation, given its sensitivity to chloroquine, an inhibitor of endosomal acidification. Although this pathway also seems to be independent of TAP-mediated peptide transport, it is dependent on the egress of properly assembled MHC class I complexes from the ER[42]. Inhibitors of TAP-mediated peptide translocation, however, inevitably reduce the transport of MHC class I molecules out of the ER, limiting their appearance at the cell surface and in endosomes. It is difficult to separate the effect of these inhibitors on cross-presentation from their more general effect on MHC class I trafficking.

Fidelity to classical processing

Although DCs may be capable of using an endocytic exchange mechanism to create MHC class I–peptide complexes, typical somatic cells have only the classical pathway at their disposal. For $CD8^+$ T cells induced by cross-presentation to be functionally useful against pathogen-infected cells, it would seem that the epitope generation mechanisms used should be the same as those in classical MHC class I processing. An endosomal exchange mechanism, in which peptides are generated by different proteases in radically different conditions

from those in the endogenous pathway, therefore seems unlikely to contribute substantially to the CD8 T cell repertoire. Cytoplasmic processing, including proteasomal proteolysis, and ER-based trimming would be expected to be involved to generate the same peptide sequences as those made by nonhematopoietic cells. Although partial proteolysis may occur in the endocytic pathway, extensive experimental evidence suggests that exogenous antigens must reach the cytoplasm to be effectively cross-presented[33]. In DCs, the presentation of exogenous antigens is unaffected by both chloroquine and inhibitors of lysosomal proteolysis[32,43–45]. Exogenous antigen presentation is, however, highly sensitive to specific inhibitors of the proteasome[43,44], indicating cytoplasmic proteolysis are the main form of epitope generation in the cross-presentation pathway.

Topological complications

For exogenous antigens to be processed in the cytoplasm, they must somehow be translocated across a membrane, presumably after endocytosis. Transport of internalized antigens into the cytoplasm from endocytic compartments is probably not due to vesicular disintegration, because large nondegradable particles cannot reach the cytoplasm. The cutoff for this transport process is relatively large: 3-kilodalton (3-kDa) and 10-kDa dextrans reach the cytoplasm efficiently; nondegradable dextrans of 40 kDa are less efficiently translocated; and dextrans 200 kDa and larger are retained in the endocytic compartment[46]. Although it is unclear whether cross-presentation can be effectively mimicked by polysaccharides, protein antigens can also reach the cytoplasm with large domain structures intact. After internalization of horseradish peroxidase by mouse DCs, horseradish peroxidase enzymatic activity could be detected in the cytoplasm. Ovalbumin could also be detected in the cytoplasm, although it was fragmented, indicating that it had undergone partial proteolysis[46].

ER-mediated phagocytosis

'Retrotranslocation' of misfolded proteins into the cytosol for their subsequent degradation is a well established property of the ER. The process is believed to involve the use of the Sec61 pore complex. Although the main function of Sec61 is to insert secretory or transmembrane polypeptides cotranslationally into the ER lumen, it also has the capacity to shuttle intact proteins into the cytosol from the ER[47,48]. Sec61-mediated retrotranslocation removes unstable or accumulating proteins from the ER for proteolysis by the proteasome, a process called ER-associated degradation[49]. The discovery by Desjardins and coworkers, initially by proteomic analysis, that phagocytosis by a mouse macrophage cell line involves the donation of ER membrane to nascent phagosomes[50,51] suggests a potential mechanism by which extracellular proteins could be translocated into the cytosol. During phagocytosis, the ER membrane fuses with the plasma membrane to form the phagocytic cup and initial phagosome[51] (**Fig. 2**). Although maturation of the vesicle into a phagolysosome leads to proteolysis and a decrease in the quantity of ER components, the nascent phagosome in part resembles the ER in composition. During ER-mediated phagocytosis, exogenous antigens would be expected to encounter a compartment containing the Sec61 pore complex and any other ER-based cofactors involved in ER-associated degradation. Ribosomes are excluded from nascent phagosomes[51]; their exclusion might remove competition for Sec61, perhaps leaving it free to function solely in retrotranslocation. ER-mediated phagocytosis may have originated as a mechanism to preserve plasma membrane surface area in highly endocytic cells[52]. In APCs such as DCs and macrophages, the process may have been adapted to facilitate retrotranslocation of internalized proteins into the cytosol and their subsequent processing for cross-presentation.

Although the properties of the Sec61 complex make it a good candidate for involvement in cross-presentation, the evidence for this is circumstantial. The crystal structure of the multimolecular Sec61 complex has been published[53]. In an open conformation, the Sec61 pore is predicted to form an opening of approximately 20 Å in diameter, large enough to transport a translating polypeptide loop. Although it is entirely unknown whether the Sec61 pore is permeable to molecules other than proteins, the 3-kDa and 10-kDa dextrans used to establish the size parameters of transport from the endocytic pathway to the cytosol[46] have Stokes' radii in this range. The 40-kDa dextrans that inefficiently reach the cytoplasm have a Stokes' radius of approximately 40 Å (ref. 54). In the presence of proteasome inhibitors, large proteins, including 40-kDa HLA class I heavy chains, can be detected in the cytosol after retrotranslocation from the ER[55,56], indicating that Sec61 is capable of transporting long polypeptide chains. Evidence suggests that tightly folded protein domains can be retrotranslocated[57], and associated *N*-linked glycans of approximately 3 kDa are believed to be enzymatically removed in the cytosol after translocation. If Sec61 is indeed responsible for mediating cross-presentation, many mechanistic questions remain, including the potential involvement of phagosomal or lysosomal 'unfoldases', thiol reductases and proteases for particularly large protein antigens. Lumenal and cytoplasmic cofactors seem to be required to facilitate retrotranslocation[58].

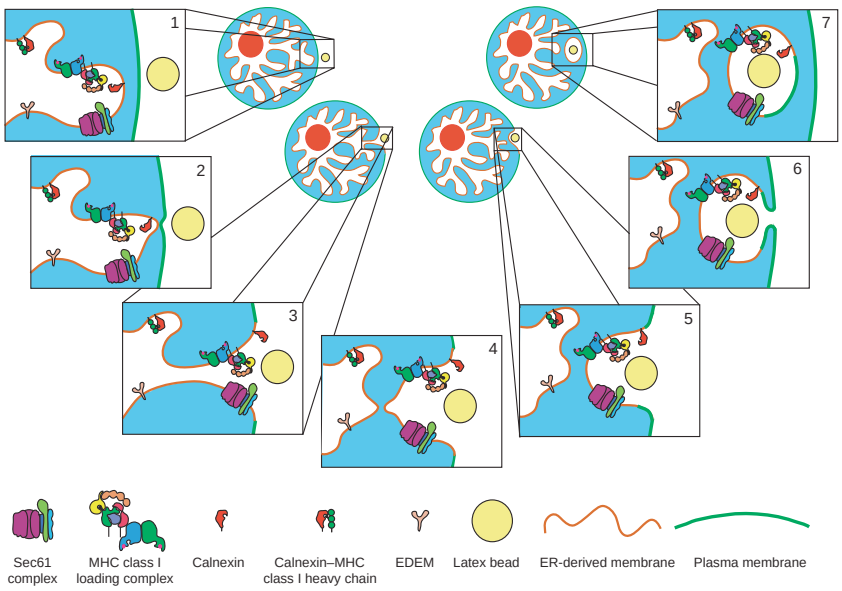

Figure 2 Sec61p, the probable gateway from the ER to the cytosol used in the degradation of misfolded glycoproteins, and the peptide-loading complex are recruited to phagosomes by ER-mediated phagocytosis. Steps 1–7 represent phases in a continuous process of phagocytosis. EDEM, ER degradation enhancer, mannosidase α-like.

After reaching the cytosol, exogenous protein antigens should be free to enter the classical processing pathway. In perhaps the simplest model for cross-presentation, peptides derived from them by proteasomal degradation could enter the lumen of the ER via TAP. The critical function of TAP has been confirmed by the abrogation of cross-presentation in TAP-deficient cells and mice[43,59]. Cross-presentation is sensitive to brefeldin A, an inhibitor of protein egress from the ER[32,44], consistent with the idea that MHC class I molecules acquire peptides derived from exogenous antigens in the ER. Brefeldin A inhibits protein secretion and exocytic trafficking by collapsing the Golgi stacks into the ER.

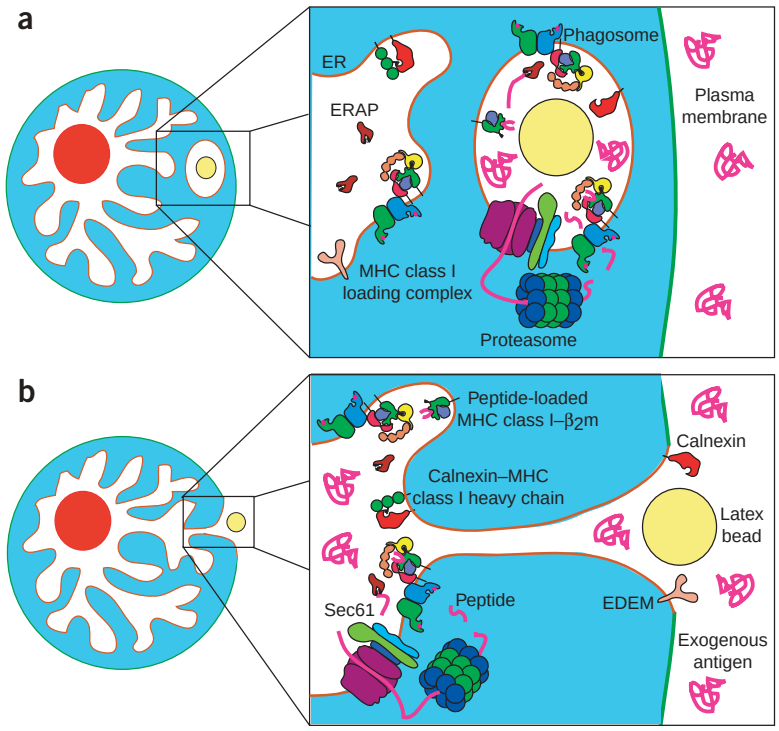

Figure 3 Two models of cross-presentation. (**a**) The phagosome is self-contained, incorporating Sec61, associated proteasomes and the peptide-loading complex. A concerted mechanism functioning in the phagosome is responsible for the generation of MHC class I–peptide complexes from proteins internalized into the same phagosome. (**b**) External proteins have direct access to the ER by transiently available continuities or by a regulated mechanism. In this case, cross-presentation could occur in the ER proper. This mechanism may be more important for antigens internalized by pinocytosis or receptor-mediated endocytosis.

Phagosomes as organelles optimized for cross-presentation

In addition to Sec61, nascent phagosomes also contain additional factors necessary for the loading of MHC class I with peptide[60,61]. TAP is present in early phagosomes, in association with a properly assembled MHC class I loading complex. This complex contains calreticulin, a disulfide-linked ERp57-tapasin conjugate, and endoglycosidase H (endo H)–sensitive MHC class I heavy chains[60,61]. TAP in phagosomes is competent for peptide translocation, and MHC class I molecules in the compartment can bind TAP-translocated peptides[60,61], dissociating from the loading complex after peptide binding[60]. ER aminopeptidase, which trims antigenic peptides, is also present in phagosomes[60]. Thus, phagosomes are capable of creating MHC class I–peptide complexes from translocated peptides. Proteasomes, together with associated ubiquinated proteins, are bound to the cytoplasmic face of phagosomes[62], which suggests that these organelles could be completely self-sufficient for mediating cross-presentation. In this scheme (**Fig. 3a**), the Sec61 pore would retrotranslocate large protein domains derived from exogenous antigens into the cytoplasm. Proteasomes attached to the phagosome cytoplasmic face would then degrade these proteins into peptides that could be redelivered into the phagosomal lumen via TAP. The correctly assembled MHC class I loading complex would then facilitate peptide binding to the MHC class I–β_2M dimer, producing mature MHC class I complexes containing peptides derived from internalized exogenous antigens.

For this hypothetical mechanism to be valid, however, MHC class I–peptide complexes generated in phagosomes would have to return to the cell surface for recognition by $CD8^+$ T cells. Conceivably there is a direct route to the cell surface from phagosomes. MHC class II molecules are transferred from intracellular lysosome-like compartments to the cell surface of DCs after maturation, induced, for example, by lipopolysaccharide[63,64]. Regulation of MHC class I trafficking during DC maturation has also been noted[65,66]. A pool of MHC class I molecules, retained intracellularly in immature DCs, can be rapidly redistributed to the cell surface from endocytic compartments after maturation[66]. Alternatively, MHC class I–peptide complexes could return to the ER from the phagosome and enter the secretory pathway. The ability of brefeldin A to inhibit cross-presentation[32,44] would be consistent with this, although dissenting findings have been presented by Houde and colleagues[62]. At present, however, there is no evidence for either of these pathways and it remains possible that complexes generated in phagosomes are simply degraded.

Desjardins and colleagues followed the fate of calnexin, a transmembrane ER-resident protein with lectin-like activity similar to that of calreticulin, throughout phagosome maturation in a mouse macrophage cell line[51]. Calnexin was progressively degraded over time, correlating with increasing lysosomal content. This result suggests that the ER components remaining in the phagosome are subjected to lysosomal degradation during maturation to a phagolysosome. However, a portion of these components, perhaps selectively the MHC class I molecules that are successfully peptide-loaded, might be reclaimed from the phagosome to the ER. Nearly all MHC class I heavy chain molecules across 15 highly divergent species contain a tyrosine residue in the cytoplasmic tail. Mutation of this residue abrogates cross-presentation by DCs, but not endogenous presentation[67]. At a cellular level, the phenotype correlates with reduced MHC class I in endocytic compartments. MHC class I molecules exit the ER and arrive at the cell surface normally, thus it is not apparent what causes this deficiency. Impaired endocytosis is the most obvious possibility and is consistent with possible involvement of the tyrosine as an endocytic signal, but the precise function of the tyrosine residue is unknown. Enhanced degradation, associated with a defect in retrieval to the ER or in direct transport to the plasma membrane, could also be

responsible for reduced MHC class I accumulation in the endosomes. Phagosomal MHC class I molecules derived from the ER should not have been subjected to the modifications in the Golgi that render their *N*-linked glycans resistant to endo H. Therefore, many endo H–sensitive MHC class I molecules should be present at the DC surface if direct phagosome–to–plasma membrane transfer occurs. Such molecules are difficult to find (A.L.A. and P.C., unpublished data). If loading in phagosomes contributes substantially to the surface MHC class I repertoire, then recycling back to the ER followed by transport to the plasma membrane via the Golgi seems the most likely route.

Cross-presentation after endocytosis

Molecular analysis of the function of phagosomes in cross-presentation has relied heavily on the internalization of nondegradable latex beads, which may not precisely mimic the physiological situation *in vivo*. Do phagosomes containing more readily degradable pathogens or apoptotic bodies follow the same time course and maturational pattern? ER proteins can be recruited to phagosomes containing opsonized red blood cells, *Leishmania donovani* or *Salmonella typhimurium*[51], suggesting that similar processes may apply, but the question of involvement of the ER remains for antigens internalized by fluid phase endocytosis or macropinocytosis. An experimental attempt to address this question suggests that regardless of the internalization substrate, the ER may function in the endocytic process. After internalization, both latex bead–conjugated and soluble antibodies to ER molecules were able to interact with ER components, despite the absence of these ER molecules from the cell surface[60]. These results suggest that the process of ER membrane donation may function in both phagocytosis and other forms of endocytosis in DCs. The efficiency of cross-presentation may relate to the amount of ER membrane incorporated into the endocytic vesicle, possibly explaining the increased cross-presentation of soluble antigens noted when phagocytic substrates are included during internalization[32].

Molecular mechanisms

Two models explaining cross-presentation after phagocytosis are consistent with the data presented thus far. In the first model (**Fig. 3a**), all of the steps occur in peripheral phagosomes. Antigen retrotranslocation to the cytosol, proteasomal proteolysis, peptide transport via TAP and loading of peptides into MHC class I–β_2M dimers all occur within and on the face of the phagosome. Consistent with this model, Amigorena and coworkers found that phagosomes containing ovalbumin-coated latex beads also contained H2-K^b molecules associated with an ovalbumin-derived peptide, detectable by a specific monoclonal antibody, whereas phagosomes containing bovine serum albumin–coated beads in the same cell apparently did not[61]. Also consistent with this model are the observations of Desjardins and coworkers that proteasomes and polyubiquitinated proteins, including an internalized protein antigen, are associated with the cytosolic face of the proteasome[62].

In the second model, antigens are internalized into phagosomes, but at some point during this process the ER becomes contiguous with the lumen of the phagosome, allowing soluble antigens to access the lumen of the ER (**Fig. 3b**). Exposure of cells containing phagocytosed particles to bafilomycin, which prevents vacuolar acidification through inhibition of the vacuolar H$^+$-ATPase, showed a potential intermediate in ER-mediated phagocytosis[51]. In the presence of bafilomycin, continuities were found between the lumen of the ER and the cell exterior by electron microscopy. The drug may have 'frozen' the normal process of pinching off the ER membrane extension that allows the ER to reseal and the donated membrane to form the phagocytic cup at the plasma membrane (**Fig. 2**). Repetition of this process, providing a succession of transient continuities, could allow access of antigens acquired by phagocytosis or, if the same transient fusion process occurs during fluid phase uptake, by macropinocytosis, to the ER lumen. Once there, antigens would become substrates for ER-associated degradation, undergoing Sec61-mediated retrotranslocation and proteasomal processing. In this model, both phagosomes and the ER may be competent to mediate the entirety of cross-presentation.

Exposure of DCs to soluble US6, a protein inhibitor of peptide translocation originally identified in human cytomegalovirus that interacts with the lumenal domain of TAP, completely abrogates cross-presentation[60]. This finding both confirms that cross-presentation is dependent on peptide translocation by TAP and demonstrates that the TAP molecules involved in cross-presentation of fluid-phase antigens are accessible from the cell exterior. Treatment of DCs with this inhibitor also substantially reduces cell surface MHC class I molecules. A variety of experiments has shown that inhibition of TAP function causes a reduction in MHC class I surface expression because peptide-free MHC class I molecules are unstable and fail to exit the ER[68,69]. Accommodating this information into the model in **Figure 3a** would indicate that a large proportion of the MHC class I complexes at the cell surface must be loaded with peptides derived from exogenous antigens. The main function of the DC is to initiate an immune response by presenting environmentally acquired antigens rather than those expressed endogenously. This model of MHC class I processing could provide a mechanism by which DCs facilitate immunity to external threats while limiting autoimmunity by excluding endogenous antigens from MHC class I presentation altogether. In DCs *in vivo*, the cross-presentation pathway may prove to be more than an alternate pathway and instead may be the dominant source of loaded MHC class I–peptide complexes. In the model presented in **Figure 3b**, however, the soluble TAP inhibitor could access the lumen of the ER, blocking peptide translocation at that site. In this scenario, peptides from endogenous and exogenous antigens compete for MHC class I binding and cross-presentation is not necessarily the preferred mode of operation for DCs.

Cross-presentation, a highly efficient process

A detailed study of protein synthesis and degradation and endogenous MHC class I presentation using vaccinia virus–encoded antigens as a model system[70] estimated that to create a single MHC class I–peptide complex, between 500 and 3,000 viral translation products must be degraded. Strong evidence suggests that newly synthesized proteins are the main source of endogenously processed peptides presented by MHC class I molecules[71]. If protein antigens are internalized by DCs in the form of apoptotic or necrotic cell fragments, the concentration of antigenic molecules in the phagosome may be similar to the steady-state level in intact, virally infected cells, but synthesis of viral proteins will presumably not continue. Nevertheless, cross-presentation of exogenous antigens is readily detectable, either by flow cytometry using monoclonal antibodies specific for MHC class I–peptide complexes or by T cell responses. Even if the entirety of the phagocytic or endocytic pathway (approximately 3% of the cell volume[72]) is filled with cell fragments, most of the available antigens are likely to be degraded in the phagolysosome rather than reaching the cytoplasm to become proteasomal substrates. Subsequent TAP transport and MHC class I binding would also have their inherent inefficiencies, and these accumulated

losses should make the detection of a cross-presentation signal highly unlikely. Yet in an *in vitro* study examining vaccinia virus–derived antigens, their cross-presentation proved to be highly efficient. DCs endocytosing apoptotic or necrotic fragments from cells infected with recombinant vaccinia virus constructs achieved MHC class I–restricted presentation equivalent to that obtained with a pulse with 10–100 nM peptide[73]. Thus, although *in vivo* experiments have called into question the involvement of cross-presentation in viral infections[74], cross-presentation of viral antigens *in vitro* is unexpectedly potent.

The incongruity between the expected and observed efficiencies of cross-presentation suggests that the molecular mechanisms governing this pathway ensure high processing efficiency. As all of the components necessary for cross-presentation are present in phagosomes, integration of the process in a single compartment may be sufficient to account for this. The presence of phagosome-associated proteasomes suggests that the key steps of cross-presentation (retrotranslocation into the cytoplasm, proteolytic processing, TAP retranslocation into phagosomes and MHC class I loading) may be linked molecularly. Although a noncovalent direct association between TAP and MHC class I via tapasin is well established, linkage of these molecules to Sec61 or the proteasome has not been noted. Nevertheless, direct intramolecular interactions of the essential components, or indirect associations mediated by membrane subdomains or the cytoskeleton, may exist, facilitating cross-presentation of exogenously acquired antigens.

Conclusion

The phenomenon of ER-mediated phagocytosis seems to explain many of the previously ill understood aspects of cross-presentation. The recruitment to the phagosome from the ER of a potential translocator of proteins into the cytosol, as well as the components involved in peptide generation, provides a compelling, if still somewhat circumstantial case for its importance. Future work will undoubtedly demonstrate the molecular details involved, as well as unraveling the mechanisms underlying cross-presentation of soluble protein antigens.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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